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Posters

BIOPHYSICS OF MOVEMENT CONTROL

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Finding food, escaping danger or mating – all these behavior involves movement. Movement in space is crucial for animals. Every movement should follow the laws of physics: body and its parts should accelerate, stop, overcome a friction and keep balance at the same time. Animals travel distances exceeding their body size by a few orders of magnitude. The amplitude of single movement is comparable with the size of animal. The strategy for traveling longer distance is to repeat the same "elementary" single movement: step, swing etc. Even the simple movement is produced by contraction of several muscle groups in a precise order. This requires very fine control involving anticipation and negative feedback. The mechanisms and principles of movement control remains to be elucidated.

The limb or muscle can produce a ballistic high force generating movement. The same limb or muscle can produce very subtle movements as well. How the control of muscles is achieved in a very wide dynamic range?

Muscles of vertebrates are directly controlled by spinal motoneurons. The properties of motonerons are believed to play an important role in control of movement.

In my talk I will discuss how the activation properties of motoneurons, investagated in our group, contributes to the of movement control.

LOCATION OF THE RETINAL OF CHEMICALLY AND GENETICALLY MODIFIED BACTERIORHODOPSIN: FLUORESCENCE RESONANCE ENERGY TRANSFER STUDY

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Bacteriorhodopsin (BR), the retinal-opsin complex present in the purple membrane of *Halobacterium salinarum*, undergoes light-driven cyclic reactions resulting in protons transport across the membrane. To elucidate the molecular mechanism of proton pumping, changes in the location and orientation of retinal during the photocycle [BRground state → intermediates (K→L→M→N→O) → BRground state] must be determined.

It is supposed that the starting retinal configuration and location in BR result from specific retinal-opsin interaction and may influence retinal position in the photocycle intermediates. It is also supposed that this specific interaction is affected by chemical structure of chromophore and opsin functionally important amino acids. The aim of our study was to clarify the influence of the retinal chemical structure and Asp-96 of opsin on the chromophore location in BRground state and in red-shifted, O-like intermediate.

The technique of incorporation of retinal analogues with changed shape or altered electronic properties into the binding site of bR (or mutant bR) was used to strengthen the influence of the protein surrounding. The experiments were performed with wild-type and D96N-mutated bR carrying retinal or 8-, 10-, 14-Fluororetinal. Distances from fluorescent lipid probes RhB18, DPH, DiI, 2-AP, 16-AP to the retinal chromophore of BR incorporated into phospholipid vesicles were measured with fluorescence resonance energy transfer (FRET). Steady-state fluorescence was used to find the closest approach from probes to ground-state retinal. Phase modulation of FRET was used to find distances for retinal in bacteriorhodopsin's red-shifted, O-like intermediates.

It is demonstrated that both modification of retinal chemical structure and genetic modification of opsin (substitution of aspartic acid with asparagine at 96 position) may influence chromophore location in BR. Results show that retinal is buried more deeply in the modified BR than in native BR. The biggest changes in retinal position were observed for 14-Fluororetinal suggesting that environment close to chromophore linkage with protein plays significant role in retinal location in BR. Retinal is buried more deeply in the protein when BR is in its ground state than in red-shifted, O-like intermediates. Presented results support earlier assumptions that the retinal chemical property and the shape of protein pocket influence the location of retinal in BRgroud state and in the intermediates of BR photocycle. The problem whether the starting retinal location in BRgroud state is significant for BR physiological activity will be also discussed.

UTERINE BIOELECTRICAL ACTIVITY – REVIEW OF MEASUREMENTS METHODS

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The study of uterine contractile activity is crucial for both diagnosis, treatment and prevention of premature birth. Efficient assessment of uterine contractions in women is difficult from a technical point of view and still requires

research.

Currently used measurement methods are indirect and do not allow to fully observe the dynamics of muscle contraction of the uterus. Direct measurement of the pressure in the wall of the uterus is unattainable except for special cases. Examination of the uterine electrical activity associated with its mechanical activity by means of implants placed on myometrium is possible only in animals [1]. The mechanisms underlying the premature release of uterine contractions and consequently preterm labor is unknown.

The recording of changes of myometrium electrical potentials is called electrohysterography (EHG - uterine electromyography). There are a few limitations of EHG. There are difficulties to reliably measure muscle activity caused by variable skin resistance and variable distance to muscles (fetal shifts) [2]. However, its great advantage is non-invasiveness and ease of use applying telemetry.

The magnetic field activity corresponds to uterine electrophysiological activity. Unlike uterine EMG signals, MMG signals are detectable outside the boundary of the skin without making electrical contact with the body. Unlike electrical recordings, the magnetic recordings are independent of any kind of reference, thus ensuring that each sensor mainly records localized activity. For the magnetic recordings each sensor mainly records localized activity. This makes measurements with even 151 sensors [3] possible.

EHG has practical clinical importance in diagnosing preterm labour. Our study showed the differences in the bioelectrical uterine activity in patients with symptoms of preterm labor, who delivered within 7 days, and a group of patients who delivered after 7 days [4].

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REFERENCES

[1] Brzozowska E., Oczeretko E., Pawliński B. & Gajewski, Z. (2014). Telemetry Recording of the Electromyographic Activity of Female Reproduction Tract. [In:] Piętka E., Kawa J., Więcławek W. (eds.) Information Technologies in Biomedicine, Springer International Publishing ,pp.101-112.

[2] DAY, S., Important factors in surface EMG measurement. Bortec Biomedical Ltd publishers, 1-17, 2002.

[3] Govindan, R. B., Siegel, E., Mckelvey, S., Murphy, P., Lowery, C. L., & Eswaran, H. (2014). Tracking the changes in synchrony of the electrophysiological activity as the uterus approaches labor using magnetomyographic technique. Reprod Sci, 22, 595-601.

[4] Lemancewicz A. , Borowska M, Paweł Kuć P., Jasińska E., Laudański P., Laudański T. & Oczeretko E. (2016). Early diagnosis of threatened premature labor by electrohysterographic recordings – The use of digital signal processing, Biocybern Biomed Eng, 36, 302-307.

STRUCTURAL DYNAMICS OF PROTEINS INVOLVED IN miRNA MEDIATED GENE SILENCING

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miRNAs are short RNA sequences, that usually silence the expression of mRNAs with almost perfectly complementary miRNA-binding sites in their 3'UTRs. These miRNAs guide Argonaute proteins to targeted mRNA. Argonautes, in turn, interact with GW182 proteins. GW182 recruits the CCR4-NOT deadenylase complex *via* interactions with *i.a.* the scaffolding subunit CNOT1 [1,2]. This brings about inhibition of translation and/or mRNA deadenylation and further decay.

Structural dynamics of the GW182 and CNOT1 proteins as well as interactions with a short peptide coresponding the CNOT1-binding site were studied using hydrogen-deuterium exchange coupled with ion mobility mass spectrometry (HDX-IMS). HDX-MS technique allows studies of solvent accessibility of particular protein regions that depends on structural properties of a protein and on the presence of a binding partner.

We will present the results describing structural properties of the GW182 silencing domain and the CNOT1 fragment interacting with this domain, as well as the results of the HDX-IMS studies of the CNOT1 interactions with a tristetraprolin peptide (TTP), a fragment of a protein involved in suppression of inflammatory processes.

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REFERENCES

[1] Fabian M. R., Cieplak M. K., Frank F., Morita M., Green J., Srikumar T., Nagar B., Yamamoto T., Raught B., Duchaine T. F., Sonenberg N. (2011). miRNA-mediated deadenylation is orchestrated by GW182 through two conserved motifs that interact with CCR4-NOT. *Nat Struct Mol Biol.* **18**, 1211-7.

[2] Braun J. E., Huntzinger E., Fauser M., Izaurralde E. (2011). GW182 proteins directly recruit cytoplasmic deadenylase

complexes to miRNA targets. *Mol. Cell* **44**, 120–33.

COMPARISON OF THE INFLUENCE OF SELECTED BROMINATED FLAME RETARDANTS ON HEMOLYSIS AND ERYPTOSIS IN HUMAN ERYTHROCYTES

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Flame retardants are the compounds of anthropogenic origin that are used to reduce flammability and burning rate of polymeric materials.

Brominated flame retardants (BFRs) are up to 25% of the market of flame retardants. Due to the fact that the main component of these substances is bromine, and there are no limitations according to their structure, this group covers above 80 various chemicals.

BFRs can be divided into two groups containing three commercial mixtures, such as additive compounds, which include pentabromodiphenyl ethers (PBDE) and hexabromocyclododecane (HBCD) as well as reactive compounds, which include tetrabromobisphenol A (TBBPA). The compounds from the first group are mixed with other components of the polymeric material during, before or after polyreactions, which enables migration of these substances into the environment. Reactive compounds are bound into the polymer chain during polymerization reaction, which limits the migration of these substances into the environment [1]. Among the group of brominated flame retardants, tetrabromobisphenol A is produced in the highest amounts [2].

The aim of this study was to evaluate the effect of the most commonly used brominated flame retardants (BFRs), i.e. terabromobisphenol A (TBBPA), tetrabromobisphenol S (TBBPS), pentabromophenol (PBP), 2,4-dibromophenol (2,4-DBP) and 2,4,6-tribromophenol (2,4,6-TBP) on the level of hemolysis and eryptosis in human red blood cells. In order to analyze hemolysis, the erythrocytes were incubated with BFRs in the concentrations ranging from 0.01 to 500 μg/ml for 24 h, 48 h or 72 h. Eryptotic changes were assessed in red blood cells incubated with BFRs in the concentrations range from 1 to 250 μg/ml for 48 h (the concentrations of bisphenols and incubation time were selected on the basis of the results obtained for hemolytic changes). Eryptosis (phosphatidylserine translocation) was analyzed by flow cytometry using annexin V conjugated with fluorescein isothiocyanate. The results showed that BFRs revealed cytotoxic and eryptotic potential in red blood cells. It was also observed that TBBPA exhibited the strongest, while TBBPS the lowest changes in the parameter studied. The observed changes were caused by BFRs in the concentrations, which may only affect human body as a result of acute poisoning with these substances.

REFERENCES

[1] Eljarrat E., Barceló D. Brominated flame retardants. Springer Science & Business Media. 2011:vol.16;

[2] Covaci A., Voorspoels S., Abdallah M. A. E., Geens T., Harrad S., Law R. J.: Analytical and environmental aspects of the flame retardant tetrabromobisphenol-A and its derivatives. J Chromatogr A. 2009;1216(3):346–363;

STUDIES OF THE THERMOTROPIC PHASE PROPERTIES OF BINARY MIXTURE OF DPPC AND NEW ACETYLENIC DERIVATE OF BETULIN

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Betulin is one of the organic compounds from the group of triterpenes pentacyclic lupane type. Betulin has a broad spectrum of biological activities such as immunostimulatory, antioxidant, anti-inflammatory. However, the most important is its anticancer effects. The discovery of the selective influence of betulin on cancer cells without damaging healthy cells, contributed to begin research on modifying the structure of betulin to obtain compounds with more favorable pharmacological properties [1].

Differential scanning calorimetry was used to investigate the thermotropic phase properties of binary mixture of lipid and betulin derivative containing pharmacophore bearing an acetylenic function. These compounds have been synthesized at the Department of Organic Chemistry, Medical University of Silesia in Katowice [1-2]. All new compounds, as well as betulin, were tested in vitro for their antiproliferative activity. Most of the compounds showed better cytotoxicity than betulin and cisplatin used as reference agent [1].

In this paper we analyzed influence of betulin and its acetylenic derivate on the fluidity of liposome lipid bilayers being synthetic analogues of natural membranes. Incorporation of betulin into the lipid bilayers produces broadening of the phase transition with a decrease main transition temperature (T_m) of DPPC. In the case of betulin derivative were observed even greater decrease in temperature T_m and the disappearance of the pretransition peak (T_p).

The degree of biological membranes fluidity plays a key role in the course of most physiological processes. Also, in many disease states, e.g cancers, membrane fluidity is disturbed. These studies may contribute to a better understanding of the mechanism of action of betulin and its derivatives.

REFERENCES

[1] S. Boryczka, E. Bębenek, J. Wietrzyk, K. Kępińska, M. Jastrzębska, J. Kusz, M. Nowak, Synthesis, structure and cytotoxic activity of new acetylenic derivatives of betulin, Molecules 18 (2013) 4526-4543.

[2] E. Bębenek, E. Chrobak, J. Wietrzyk, M. Kadela, A. Chrobak, J. Kusz ,M. Książek, M. Jastrzębska, S. Boryczka, Synthesis, structure and cytotoxic activity of acetylenic derivatives of betulonic and betulinic acids. Journal of Molecular Structure 1106 (2016) 210-219

ROSE BENGAL-PHOSPHORUS DENDRIMER COMPLEX FOR ENHANCED PHOTOTOXICITY AGAINST BASAL CELL CARCINOMA CELL LINES

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Dendrimers have proven to act as efficient platforms for different molecules such as anticancer drugs, imaging agents or nucleic acids. Their potential is strictly based on their unique, dendritic architecture, which provides high loading capacity and possibility of both covalent and non-covalent interactions with active molecules. Some studies have been also focused on dendrimers as carriers of photosensitizers used in photodynamic therapy (PDT). The need for improvement of PDT is related to specific limitations of photosensitizers. Among others, PDT is imited due to poor selectivity, hydrophobicity of photosensitizers and prolonged light sensitivity. Those issues may be resolved by using a drug delivery system for photosensitizers based on dendrimers.

The aim of our study was to evaluate whether a phosphorus dendrimer has a potential as a carrier of a photosensitizer (rose bengal). Spectrofluorimetric studies revealed that dendrimer was able to interact with rose bengal molecules and that the rose bengal-dendrimer complex was formed via electrostatic interactions. A stoichiometry of the complex was determined and a 5:1 (rose bengal:dendrimer) molar ratio was chosen for further studies. In order to evaluate the influence of the dendrimer on singlet oxygen production by irradiating rose bengal the Singlet Oxygen Sensor Green was used. In comparison with a free photosensitizer, the complex showed remarkably high singlet oxygen production. We chose three basal cell carcinoma cell lines as suitable biological models for studying a photodynamic effect in vitro. The rose bengal-dendrimer complex showed higher cellular uptake compared to free rose bengal and next, it showed an enhanced phototoxicity against all three basal cell carcinoma cell lines. Hence, we demonstrated that the cationic phosphorus dendrimer of third generation was an excellent candidate for improving photodynamic therapy using rose bengal.

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AGGREGATION OF ENCHANCED GREEN FLUORESCENT PROTEIN (EGFP) DURING FOLDING MONITORED BY ANALYTICAL ULTRACENTRIFUGATION

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Matured monomeric GFP possess unique chromophore created after proper folding, which excited at 489 nm emits green fluorescence. The protein is non-toxic and does not participate in the biochemical reaction in cells. Because of that GFP and its mutants become well know biological markers used in molecular biology, biochemistry and medicine [1]. After maturation GFP is very stable, but during folding (also when it is fused and co-expressed together with other proteins) is prone to aggregation.

We have observed the aggregation accompanying the folding of EGFP (enchanced green fluorescent protein, mutant of GFP) purified form inclusion bodies [2] in buffer with 6M Guanidinium Hydrochloride (GdnHCl). The folding was initiated by dilution of the sample in buffers with lower concentration of denaturant. During reduction of GdnHCl concentration we have observed the aggregation (accompanied folding) of the protein on different stage of folding. By the help of analytical ultracentrifugation we have monitored the amount and size of EGFP aggregates as a function of time and GdnHCl concentration. The absorption detection at 280 nm allows to observe monomeric and aggregated EGFP, the absorption at 488 nm allows to observe the molecules with matured chromophore and the more sensitive fluorescence detection allows to detect the molecules with functional chromophore.

The results show that in the high concentration of GdnHCl (3-6 M) protein remains unfolded and the aggregation is not observed. In the middle (1-2 M)

concentration, EGFP aggregates are slowly created. The aggregation accelerates in low concentration of denaturant. The aggregates became huge and fast sedimenting, especially in 0.1M GdnHCl. The fluorescence detection shows that fluoresce mainly stable monomers, while the conglomerates of EGFP molecules are non-fluorescent.

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REFERENCES

[1] Enterina J.R., Wu L., & Campbell R.E. (2015) Emerging fluorescent protein technologies. *Curr Opin Chem Biol.* **27**, 10-17.
[2] Krasowska J., Olasek M., Bzowska A., Clark P.L. & Wielgus-Kutrowska B. (2010) The comparison of aggregation and folding of enhanced green fluorescent protein (EGFP) by spectroscopic studies. *Spectroscopy* **24**, 343-348.

MOLECULAR BEACONS DEDICATED TO pH SENSING

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Traditional molecular beacons (MBs) are hairpin-shaped dual-labeled oligonucleotide probes that emit fluorescence upon hybridization to an RNA or DNA target sequences. Their loops serve as recognition elements and consist of 15 to 25 nucleotides, whereas their stems are only 5 to 7 nucleotides long. In design proposed by Tyagi and Kramer, the stem termini are labeled with reporter groups (a quencher and a fluorophore) that are responsible for analytical signal production [1]. Other approaches of MBs contain systems functionalized by one or multiple fluorophores located anywhere in the sequence and give analytical signal based on FRET as well as on excimer fluorescence [2].

The principal goal of our research is the development of the fluorescent pH sensors based on MBs containing cytosine-rich sequences. The sequences including tracts of cytosine are able to switch from a random coil to a folded i-motif in response to pH decreasing [3]. Therefore, we inserted the $C_4GC_4GC_4GC_4TA$ fragment from RET proto-oncogene [4] into a loop of pyrene functionalized MB; as consequence our system exhibited excimer fluorescence ($\lambda_{max.}$ ~ 480 nm) at acidic pH, whereas pH increasing caused i-motif unfolding followed by stem destabilization and pyrene separation ($\lambda_{max.}$ ~ 400 nm) [5]. Later, we have investigated the influence of 6 base pairs stem composition onto pH working range of 37-mer MBs with i-motif in the loop [data not published].

In presented approach, we have modified our MBs by replacement of 2-deoxycytidine in a loop with its fluorescent analogue tC (1,3-diaza-2-oxophenothiazine) or tC° (1,3-diaza-2-oxophenoxazine) [6]. We performed the spectral characterization of 37-mer MBs in the various pH solution by using UV-*vis*, CD and fluorescence measurements. The pH-induced i-motif formation in the loop of MBs resulted in fluorescence quenching of tC or tC° fluorophore, which is in agreement with our previous studies [7]. Suitability of these sensors for monitoring pH changes was also demonstrated.

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REFERENCES

[1] Tyagi, S.; Kramer, F. R. Nat. Biotech. 1996, 14, 303-308.
[2] Tan, W.; Wang, K. Curr. Opin. Chem. Biol. 2004, 8(5), 547-553.
[3] Brooks, T. A.; Kendrick, S.; Hurley, L. FEBS J. 2010, 277 (17), 3459-3469.
[4] Guo, K.; Pourpak, A.; Beetz-Rogers, K.; Gokhale, V.; Sun, D.; Hurley, L.H. J. Am. Chem. Soc 2007, 129, 10220-10228.
[5] Dembska, A.; Juskowiak, B. Spectrochim. Acta A: Mol. Biomol. Spectrosc. 2015, 150, 928-933.
[6] Preus, S.; Kilsa, K.; Wilhelmsson, L.M.; Albinsson, B. Phys. Chem. Chem. Phys. 2010, 12, 8881–8892.
[7] Bielecka, P.; Juskowiak, B. Molecules 2015, 20, 18511-18525.

THERMOTOLERANCE OF HUMAN ERYTHROCYTES INDUCED BY NEAR INFRARED RADIATION (NIR)

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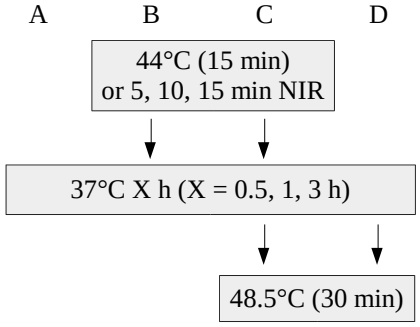
Thermotolerance is defined as a phenomenon involving the acquisition of resistance to elevated temperature for cell, tissue or organism, which is caused as a result of the pretreatment thermal shock, physical or chemical agents. It is a temporary phenomenon, and the period of cell resistance to elevated temperatures depends on the cell type, temperature and duration of the first thermal shock,

and the time interval between the first and second thermal shock.

In erythrocytes – cells without intracellular structures – a key role in the acquisition of resistance to elevated temperatures plays the cell membrane.

The viability of erythrocytes critical temperature is 46°C. Above this temperature are observed irreversible changes in the erythrocyte proteins which lead to cell death.

In this study development of thermotolerance induced by fractioned heating or by NIR was determined by hemolysis of the erythrocytes, the osmotic resistance and ATPase activity.



Experimental conditions

Pre-incubation of erythrocytes at 44°C results in the acquisition of resistance to second thermal shock (48.5°C). Maximum this phenomenon is observed after 3 hours of incubation at 37°C between thermal shocks, and disappeared after 7 hours.

Erythrocytes treated NIR were resistant to the second heat shock (48.5°C). In this study used three exposure times: 5, 10 and 15 minutes. Maximum of thermotolerance was dependent on time exposure to NIR. At the longest time exposure (15 minutes) thermotolerance effect reaches a maximum after 1 hour incubation at 37°C prior to the second thermal shock (48.5°C) and for the shortest time exposure (5 min) effect reaches a maximum after 3 hours.

Based on the obtained results it can be concluded that the thermotolerance phenomenon in erythrocytes depends on the energy supplied during the first heat shock or exposure to NIR.

THERMAL STABILITY OF URINARY STONES IN RELATION TO CHEMICAL COMPOSITION AND MECHANICAL STRENGTH

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In spite of numerous studies relating to mechanical strength of urinary calculus, a need to continuous improvement of extracorporeal lithotripsy makes the issue of physical properties of urinary stones still interesting. In particular, new perspectives offered by laser lithotripsy show a necessity to learn more about thermal stability of the urinary calculi. The main objective of this work was to examine the relationship between the chemical composition of kidney stones and their physical stability in terms of mechanical and thermodynamic properties.

Fragments of freshly removed urinary stones from patients operated due to urolithiasis were subject to standard chemical analyses. Other fragments of the same stones were subjected to Raman spectroscopy, compression to failure and differential scanning calorimetry (DSC) in temperatures from 60°C to 350°C.

Basing on Raman spectra, main mineral components of the calculi were identified and the high percentage of two-component stones was proved. Calcium oxalate (COM-monohydrate and COD-dihydrate) was found to be the predominant component in 68% of the calculi examined, hydroxyapatite (HAP) in 18%, uric acid (UA) in 9%, and struvite (MAPH) in 5%. The strength was of COM calculi was significantly higher than others.

Processes of thermal decomposition were observed on DSC thermograms in all urinary stones even though HAP and UA crystals melt in temperatures higher than the range applied in the experiment. A peak temperature of the main thermal process correlated with the mechanical strength of the stones. The peak temperatures for three main mineral types of the calculi (COM, HAP and UA) were significantly different. Nonetheless, the thermograms revealed a complex structure of disintegration of calculi which was not directly related to their mineral composition. So, some of thermal processes taking place temperatures up to 250°C were attributed to disintegration of glycosaminoglycans and other organic components of the stones. The more so that particular glycosaminoglycans were identified in the Raman spectra of the calculi under investigation.

In conclusion, the DSC analysis allowed for obtaining temperature limits of disintegration of particular types of urinary stones, and showed that physical stability of the caluli was affected not only by their mineral composition, but also, to a great extent, by presence of glycosaminoglycans that "glue" the crystals of mineral together.

ELECTRON PARAMAGNETIC RESONANCE (EPR) SPECTROSCOPY STUDIES OF THE MYCOTOXIN-STIMULATION OF RADICAL SPECIES IN WHEAT

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One of the main active mycotoxin produced by pathogens of the Fusarium groups is zearalenone (ZEA) which is

responsible for damages of plant cells. Protective mechanisms against free oxygen radicals generated in stress include the possibility of their "deactivation" by organic molecules acting as specific electron traps [1]. Difference in such radicals generation may serve as indicator of stress intensity and genotype-tolerance to stress action. In presented studies the ZEA influence on the creation of organic radicals and changes in the behaviour of metal species were investigated in wheat genotypes (tolerant and sensitive) by EPR method. Moreover, the effects of selenium ions (Se) and 24-epibrassinolid (BR), as potential protective substances against ZEA, were analyzed in terms of radicals generation.

In investigated samples (control and ZEA, BR and Se treated), EPR spectra (recorded at 77 K) revealed the presence of signals originating from Fe(III), Mn(II) and organic radicals. The signal with g = 2.00, exhibiting HFS structure (A = 9.2 mT), was ascribed to Mn species, whereas broad signal with g in the range 2.40 – 2.60 was attributed to Fe(III) bonded to proteins, probably situated in the ferritin protein shell. The line of small intensity, at g = 4.30, was connected with non hem high spin Fe(III). Analysis of Mn(II) signal indicated that it had originated from dipole-dipole interacting manganese ions in protein structures and from isolated Mn(II) aqua complexes. Upon interaction with ZEA and ZEA+BR, the intensities of Fe(III) and Mn(II) signals decreased, whereas the addition of Se to samples containing ZEA inhibited this decrease. In comparison with transition metal ions spectra, the intensity of organic radical signals was very low, however, contrary to metal ion signals, it increased in spectra of samples containing ZEA and ZEA+BR and decreased upon addition of Se to samples comprising ZEA. The influence of above mentioned compounds on the intensity of EPR signals showed that Se action was more effective than BR against ZEA toxicity.

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REFERENCES

[1] Łabanowska M., Filek M., Kurdziel M., Bidzinska E., Miszalski Z., Hartikainen H. (2013). EPR spectroscopy as a tool for investigation of differences in radical status in wheat plants of various tolerances to osmotic stress induced by NaCl and PEG-treatment. *J. Plant Physiol.*, **170**,136-145

RELATIONSHIP BETWEEN CELLULAR STRUCTURE AND BLACKSPOT SUSCEPTIBILITY OF POTATO TUBER PARENCHYMA TISSUE AFTER LONG TERM OF STORAGE

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Mechanical injuries are the main cause of damage and loss of quality of plant raw materials. Potatoes are susceptible to external and internal pressures, which cause bruising and fracture in soft tissues. The paper shows research concerning the relationship between geometrical parameters of the microstructure of potato tuber tissue and susceptibility of potatoes to blackspot damage after six months of storage. Twenty eight potato varieties from 2011 and 2012 crops in Poland were used in the experiment. The tubers had a similar size and shape [1]. Each tuber was undergone mechanical effects by Constant Height Multiple Impacts technique [2]. A confocal microscope was used to study the microstructure of soft tissue in potato tubers and microscope images of the perimedullary zone of the tissue were analyzed [3]. Cylinder shape samples of 1 mm thickness and 10 mm diameter were taken from the outer core of a potato tuber [4]. Obtained microscope images were put to analysis. The blackspot index (BIP), which describes potato susceptibility to blackspot damage, was calculated in accordance with the methodology described by Baumgartner *et al.*, [5]. As a result of the experiment parameters of a size flat section were assigned area A and perimeter P for all researches and susceptible to damage's blackspot.

The study shows that there is a correlation between the size of perimedullary tissue cells in potato tubers and blackspot damage. Potato tubers with smaller cells of the perimedullary zone show higher susceptibility to blackspot damage, described by the blackspot index BIP, in comparison with potatoes with larger cells which were stored for six months.

REFERENCES

[1] Gancarz M., Konstankiewicz K. (2007). Application of computer image analysis for fast determination of size and shape of potato tubers (in Polish). *Acta Agrophysica*, **10** (1), 47-57.

[2] Bajema R.W., Hyde G.M. (1998). Instrumented pendulum for impact characterization of whole fruit and vegetable speci mens. *Trans. ASAE*, **41**(5): 1399–1405.

[3] Konstankiewicz K., Czachor H., Gancarz M., Król A., Pawlak K., Zdunek A. (2002). Cell structural parameters of potato tuber tissue. *Int. Agrophysics*, **16**, 2, 119-127.

[4] Gancarz M., Konstankiewicz K., Zgórska K. (2014). Cells orientation of potato tuber parenchyma tissue. *Int. Agrophysics*, **28**, *1, 15-22.*

[5] Baumgartner M., Keller E.R., Schwendimann F. (1983). Versuch einer Charakteriesierung von blaustabilität und blaulabilität bei der kartoffel durch knolleneigenschaften. *Potato Res*. **26**, 15-30.

CROSS-LINKING OF PECTINS (SODIUM CARBONATE FRACTION) BY DIVALENT METAL IONS: A CASE STUDY ON ZINC IONS

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Pectins belong to group of polysaccharides localized in the middle lamella and the primary cell walls of higher plants [1]. One of the most important property of pectins is binding ability of divalent metal ions, for example calcium ions. Mechanism of gel formation is explained with "egg-box" model [2]. Formation of junction zones is related to the interactions between calcium ions and non-esterified galacturonic acid residues [3]. Stability and strength of junction zones depend on number and distribution of adjacent non-esterified galacturonic acid units. It can be assumed that other divalent metal ions bind to galacturonic acid residues of pectin chain according to "egg-box" model. The aim of this study was to evaluate an assembly mechanism (gelling) of the sodium carbonate extracted pectins (DASP) with metal ions, in this case zinc ions were considered. The mechanism were studied with rheological methods and FT-IR methods. On the basis of rheology measurements an increase of viscosity of pectin solutions with addition of zinc ions compared to pectin solution without these ions (the control) was observed. These solutions are pseudoplastic fluids. Analysis of the FT-IR spectra showed differences in the intensity and positions of bands in zinc ions-pectins system in comparison with the control. These results may indicate effective cross-linking of pectins by zinc ions that suggests assembly mechanism similar to the egg-box model. This result will be verified through computational modeling (density functional theory and molecular dynamics).

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REFERENCES

[1] Schols, H. A., & Voragen, A. G. J. (2002). The chemical structure of pectins. In: Seymour, G. B., & Knox, J. P. (Eds.). Pectins and their Manipulation. Blackwell Publishing, Oxford, 1–29.

[2] Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K., Funami, T., & Williams, P. A. (2008). Binding behavior of calcium to polyuronates: Comparison of pectin with alginate. Carbohydrate Polymers, 72, 334–341.

[3] Ngouémazong, D. E., Tengweh, F. F., Fraeye, I., Duvetter, T., Cardinaels, R., Van Loey, A., Moldenaers,P.,& Hendrickx, M. (2012). Effect of de-methylesterification on network development and nature of Ca2+-pectin gels: Towards understanding structure-function relations of pectin. Food Hydrocolloids, 26, 89–98.

THE COMPARISON OF REVERAROL AND PICEATANNOL EFFECT ON NEUROBLASTOMA CELLS

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Polyphenols are known as a "multiple effects compounds" with limitless possibilities. Especially the principal representative of polyphenols - resveratrol has become the subject of intensive research over the past two decades owing to its outstanding pharmacological properties [1]. However its specify problem with rapid metabolism and low bioavailability limits its application. Therefore it's highly important to examine its metabolites effect. In laboratory assays as well as *in vivo*, differences in hydrophilicity/lipophilicity caused by metabolic modifications such as the addition of hydroxyl group or glucuronyl or sulfate would be predicted to have a huge impact on biochemical activities, inter alia due to differences in the ability to enter cells by permeating the cellular membrane. One of the major product of resveratrol metabolism formed in liver *via* cytochrome P450 enzymes is piceatannol (3,5,3',4'-tetrahydroxystilbene).

Extending knowledge about its properties opens up new possibilities for the prevention and treatment of a many diseases. In order to recommend these natural compounds as a drugs it's necessary to validate it's diverging roles in different cell types. Therefore the aim of this research was to determinate the effect of piceatannol and resveratrol on neuroblastoma cells. Furthermore, it's extremely important to assess its role in counteracting oxidative stress. As it has been established, oxidative stress has been implicated in a number of neurodegenerative diseases The most frequently formed reactive oxygen species (ROS) are superoxide and hydroxyl radicals and hydrogen peroxide, all of which are generated in many redox processes in the human body. Therefore, we also evaluate the effects of piceatannol and resveratrol on cells under oxidative stress conditions induced by H_2O_2. The influence on cell viability and cellular apoptosis was determined in Neuro-2a cells exposed to a various range of piceatannol or resveratrol concentrations (2.5-50 μM). Cells were subjected to polyphenols action for 6 hours and afterwards incubated with hydrogen peroxide (25 μM) for 24 hours.

Picetannol exerts cytotoxic effects to Neuro-2a cells to a greater extent than resveratrol [2]. Contrary, under oxidative stress conditions, piceatannol protects cells from H_2O_2-inudced injury, resveratrol intensifies impairment.

REFERENCES

[1] Gerszon J. Rodacka A. Puchała M. (2014). Antioxidant properties of resveratrol and its protective effects in neurodegenerative diseases. *Advances in Cell Biology*. 4

(2), 97-117.
[2] Gerszon J. Rodacka A. (2016). Determination of Trans-resveratrol Action on Two Different Types of Neuronal Cells, Neuroblastoma and Hippocampal Cells. *Czech Journal Food Science, 34, (2): 118–126.*

PREPARATION AND STUDY OF THE RELEASE OF SILVER NANOPARTICLES FROM DIFFERENT TYPES OF SKINCARE FORMULATIONS

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In recent years, many researchers focus their attention on the synthesis and the application of nanoparticles of noble metals [1]. Metal nanoparticles have outstanding electrical, optical or magnetic properties compared to their counterparts on a molecular scale [2]. Specially, silver nanoparticles show interesting properties, therefore they are widely used in medicine and pharmacy. In the nanometer scale silver has a very good biocidal properties.

Silver nanoparticles are widely used in cosmetics and textiles, because of their antiseptic properties [3]. The introduction of silver nanoparticles to cosmetic products raises a lot of controversy and questions due to their capability to penetrate or permeate the skin, which in turn may lead to penetration into the bloodstream and their accumulation in organs. Therefore, issues related to the transport of silver nanoparticles through the skin is an important part of the research of modern cosmetic products. Nowadays, the penetration behaviour of an active ingredient can be evaluated by in vitro, ex vivo, and in vivo methods. Most of the data on percutaneous penetration have been gained with in vitro or ex vivo studies by experiments using a Franz diffusion chamber [4].

In this work we show preparation of small silver nanoparticles and the study of release from O/W and W/O emulsions using Franz diffusion chamber. First time as a membrane an "artificial skin" which consists of liposome and cellulose acetate was used. Moreover, we investigated the bactericidal properties of silver nanoparticles, which can be used as a potential preservative in cosmetic products.

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REFERENCES

[1] El-Kheshen, A.A., El-Rab, S.F. (2012). Effect of reducing and protecting agents on size of silver nanoparticles and their anti-bacterial activity. *Der Pharma Chemica* **4**, 53-65.
[2] Gryczynski, I., Matveeva, E.G., Sarkar, P., Bahril, S., Borejdo, J., Mandecki, W., Akopowa, I., Gryczynski, Z. (2008). Metal enhanced fluorescence on silicon wafer substrates. *Chem. Phys. Letters* **462**, 327.
[3] Larse, F.F., D'Agostin, F., Crosera, M., Adami, G., Renzi, N., Bovenzi, M., Maina, G. (2009). Human skin penetration of silver nanoparticles through intact and damaged skin. *Toxicology* **255**, 33-37.
[4] Franz, T.J. (1975). Percutaneous absorption. On the relevance of in vitro data. *J. Invest. Dermatol*., **64**, 190-195.

APPLICATION OF GIANT UNILAMELLAR VESICLES IN STUDIES OF LOCALIZATION, ORIENTATION AND MOLECULAR ORGANIZATION OF BIOMOLECULES IN LIPID MEMBRANES

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An idea of determination of orientation of molecules bound to a single lipid bilayer membrane, based on a confocal fluorescence microscopy, is presented along with the examples of its realization. The system was tested with application of well-known fluorescence marker Nile blue. A single liposome was imaged with application of two fluorescence detection channels, with separated polarizations, parallel and perpendicular with respect to the polarization of the excitation laser beam. A simple analysis of images, in terms of a linear dichroism formalism, enabled calculation of orientation of a transition dipole of the chromophore with respect to the axis normal to the plane of the membrane. Biomolecules with polyene chromophores were incorporated to giant unilamellar vesicles. Combined analysis of fluorescence intensity, lifetimes and anisotropy enabled to gain insight into molecular organization, orientation and localization of amphotericin B and carotenoids in a single lipid bilayer.

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CHILDREN WITH HCMF AUDITORY SYSTEM EVALUATION – CASE STUDY

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Hemicraniofacial microsomia (HCMF) and Goldenhar syndrome are prenatal branchial arch disorders affecting aural, oral and mandibular development. HCMF is phenotypically heterogenous and usually unilateral. It is characterised by deformities of the outer, middle and even inner ear, microtia or even anotia, preauricular skin tags and preauricular pits, ocular malformations such as epibulbar dermoids and lipodermoids, upper lid coloboma, facial nerve palsy or paresis, mandible and face development disorders.

High prevalence of hearing loss in patients with Goldenhar syndrome and HCFM was documented. Either, conductive, sensorineural or mixed hearing losses was documented. Conductive component can be caused by auricle deformities or absence, narrow, blocked or absent ear canal or middle ear abnormalities. Sensorineural component can be result of vestibule and cochlea underdevelopment or malformations.

Detailed audiological evaluation of patients with Goldenhar syndrome and HCFM, and in consequence proper rehabilitation, selection and fitting of hearing aids and consulting either patients hearing disorders or sensory and motor development auditory training. Specific diagnosis and early training determine normal psychosocial development of children with HCMF.

The purpose of the present study was the assessment of particular levels of the auditory pathway for both, deformed and normally developed ear. Examinations such as pure tone audiometry for both, air and bone conduction, auditory brainstem responses (BERA) for bone conducted stimuli were measured. Tympanometry, DPOAE, TEOAE and SOAE for normally developed ear was also carried out.

RADIOACTIVITY OF NATURAL MEDICINAL PREPARATIONS WITH PEAT MUD AVAILABLE IN RETAIL TRADE USED EXTERNALLY

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Despite a great medical progress within the last decades, the treatment of many disorders is completed with health resort therapies. The latter include a variety of compresses or substances added to baths. No reports on the attempt to evaluate skin exposure to ionizing radioactivity related to the use of such preparations has been found in literature. There are suggestions, that small doses of radioactivity change homeostasis within the skin. Also, according to certain reports, equivalent doses of radon to the skin during therapeutic cycle of thermal baths are within the range of 0.12 – 0.33 mSv. At the market there are preparations with peat mud, therapeutic peloid, which has been used in the therapy of numerous disorders for ages.

The aim of the work is to identify and measure the activity of radioactive isotopes present in preparations with peat mud and to estimate the doses obtained during the therapy.

The examination included 22 preparations with peat mud. The activity measurement of the samples was carried out with the method of gamma spectrometry using spectrometric set by CANBERRA with 34.8% coaxial germanium detector and computer system of collecting and analyzing spectra, Genie 2000.

The total number of isotopes in particular preparations was various. The median of the total activity was 24.8Bq/kg. Total maximal isotope activity of 146 Bq/kg was observed in the Iwonicka Peat Mud Cube. Also, considerable amounts of isotopes were observed in Kołobrzeska Peat Mud Paste - 112 Bq/kg.

The effective dose obtained from different groups of peat mud preparations was established. The agents for rubbing the body or for the compresses contain large amounts of peat mud – within the range of 80-90% of the total mass. The effective dose for the whole body with the use of those preparations from β emitters on the depth of 70 μm and gamma is about 5 μSv. This value is rather small compared to the effective dose obtained by a statistical Polish citizen from natural sources which amounts to 2.43 mSv.

METHYL ESTER OF SINAPIC ACID AS THE FLUORESCENT PROBE FOR PROTEIN AT ALKALINE PH

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The main objective of this work was to investigate the interaction between bovine serum albumin (BSA) and methyl ester of sinapic acid (MESA) at alkaline pH 10.5 . From our research, it is known that MESA form complexes over range of pH 5-11. Evidences of the formation of the protein-ligand molecular complexes are new absorption bands in the range of 400-450 nm and additional fluorescence bands with higher intensity and quantum efficiency than ligands without presence of protein.

Spectrophotometer Carry 5000 (Agilent) and fluorometer Eclipse (Agilent) was used in the study. As the protein bovine serum albumin was used and as the ligand methyl

ester of sinapic acid was used which was synthesized in our lab.

Since increase and blue shift of fluorescence was observed, MESA can be used in other research as the fluorescent probe of hydrophobic sites in protein. Such behavior was also observed in non-polar solvents.

By using Scatchard model, stoichiometry of the complex, the number of binding sites and the association constant of the protein-ligand complex at a pH 10.5 for the different temperatures has been determined. Using these data binding energy and change of entropy has been calculated from Van't Hoff equation.

THEORETICAL AND EXPERIMENTAL ESTIMATION OF DIPOLE MOMENTS OF METHYL ESTER OF SINAPIC ACID

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Sinapic acid is compound which is widespread in many plants. Recently there were reports that methyl ester of sinapic acid (MESA) can act as antioxidant. This phenolic ester can act either in water or non-polar environment. The aim of this work was to investigate physico-chemical properties of MESA in many non-water environments - polar and non-polar.

MESA was synthesized and its structure was checked by ^{1}H NMR technique. Fluorescence and absorption spectra of MESA were measured in hexan, cyclohexane, chloroform, ethanol, methanol, acetonitrile and dimethyl sulfoxide. Based on the spectra's, Stokes shift has been calculated. These data has been used to calculate dipole moments in excited states from Bilot-Kawski method.

To obtain dipole moments in ground states ab initio calculation was done. All calculations were carried out by Gaussian03 package with higher level DFT-BhandHLYP (or Time Dependent-DFT for excited states) method and aug-cc-pVDZ basis set. The structures were optimized in the ground state and vibrational analysis were performed to prove that stationary point were reached.

The optimized-ground-state geometries were used as the initial one for electronic excitation computations. Vertical excitation energies were calculated with respect to the solvent response.

Dipole moments in excited states has been calculated both from experimental data and from theoretical calculation. Discrepancy between these results has been observed. These difference can be explained as results of assumption in Bilot-Kawski method that dipole moments in ground and excited states are parallel to each other. Calculation without this assumption has been made. Achieved results was much more in line with theoretical results.

INTERACTION OF CYANIDIN AND ITS GLYCOSIDES WITH LIPID MEMBRANE: STRUCTURE-ACTIVITY RELATIONSHIP

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Cyanidins are plant pigments widely distributed in fruits, vegetables and grains, and therefore abundant in human diet. They possess a wide range of biological activity, i.e. antioxidant, anticancer and anti-inflammatory. However, their interaction with the lipid membrane, responsible for the proper functioning of the cells, is still unknown. Therefore, the aim of this work is to determine the effect of cyanidins on physical properties of the lipid membrane.

The impact of cyanidin and its 5 glycosides, containing different number and type of sugars in their structure, on the properties of hydrophobic part of the membrane was determined by using steady state and time-resolved fluorimetric methods. The effect of the compounds on the properties of hydrophilic part of the membrane was determined by using an infrared spectroscopy method. Additionally, the abilities of the compounds to aggregate lipid membranes and change the Zeta potential were determined by using the dynamic and electrophoretic light scattering methods.

The results indicate that cyanidin, as an aglycone, causes great changes in both the hydrophobic and hydrophilic part of the membrane. It decreases the membrane fluidity, causing an increase in order of lipid acyl chains and limiting their mobility. Cyanidin glycosides at low concentration (25 μM) do not induce changes in the hydrophobic part of the membrane, but at a higher concentration of 100 μM they decrease membrane fluidity. All the compounds also induce changes in the region of the polar heads of lipids, altering their orientation. The changes induced by glycosides depend on the number and position of the sugar substituent as fallows: cyanidin monosides are more active than biosides and cyanidin monosaccharides are more active than disaccharides. Additionally, the studies have shown that cyanidin strongly aggregates liposomes regardless of their net charge and decreases the membrane Zeta potential. In the case of its glycosides neither aggregation of liposomes nor change in Zeta potential were observed.

The changes induced by the compounds in the physical properties of the lipid membrane indicate that cyanidin glycosides are located mainly in the hydrophilic part of the lipid membrane, whilst cyanidin can penetrate deeper to the transition area of the membrane.

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THE EFFECT OF VARIABLE MAGNETIC FIELD ON THE GERMINATION AND EARLY GROWTH OF WHEAT SEEDS (*TRITICUM* L.)

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The magnetic fields have a great impact on processes within plants. Nowadays, one of the priorities is the search for ecological and economical ways to accelerate plant growth, or improving yields. We decided to carry out experiments involving wheat (*Triticum* L.) as it is commonly used cereal plant (3rd place in the world in the production of cereals). Wheat seeds were subjected to an alternating magnetic field amplitude intensity of 3 mT, frequency of 50 Hz and an exposure time which was followed by 5, 10, 15 and 20 min and then sown and cultured for 8 consecutive days. Every day counted the number of new seeds germinated and the last day of the experiment the plants were measured in terms of the length of the root and shoot. On this basis, all samples were characterized in terms of average root length, average stem length, the average length of seedlings, germination ability, Germination Rate Index (GRI) and vigor index. For the shoot length we obtained 62-68% increase, for root length – 35-44% increase, for seedling length – 47-55% increase, for GRI – 12-20% increase, for germination ability – 3-5% increase, for vigor index – 52-60% increase in performance compared to the control sample (not subjected to an electromagnetic field). The results clearly show that even relatively low variable solenoid field and used a short exposure time, has a positive effect on germination and early growth of wheat seedlings. For each test parameter was achieved better results compared to the control sample, but it is difficult to identify any particular relationship. Anyhow, this correlation does not seem to be linear. The most promising results were obtained on the exposure time of 5 and 20 minutes, but 10 and 15 minutes also performed superior to than the control.

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EFFECT OF DNAZYME MODIFICATION ON PEROXIDASE ACTIVITY

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Peroxidase-mimicking DNAzymes attracted a great attention in bioanalysis thanks to many advantages over protein enzymes like thermal stability, easy and low-cost synthesis and purification [1,2]. Despite many advantages, these systems possess some drawbacks connected to hemin (DNAzyme cofactor) which is present also in blind probe. Other disadvantage is low activity of some G-quadruplex-forming sequences like telomeric sequence $(GGGTTA)_n$). To overcome these disadvantages we decided to evaluate peroxidase activity of modified DNAzymes.

Peroxidase-mimicking DNAzymes are formed by the complex between hemin and G-quadruplex (G4). These two components interacts with each other through end-stacking mode. This type of interaction is possible since G-quadruplex is formed by planar G-tetrads stacked on each other.

In presented research we examined covalent attachment of hemin molecule to DNA oligonucleotide as well as employment of DNA analogue: 2’-OMe-RNA. The first approach enable reduction of a background signal. The second approach utilize DNA analogue which allows on creation of more stable G-quadruplex structures. Literature also indicate that 2’-OMe-RNA ribozymes forms DNAzymes with higher activity [3]. The activity of DNAzymes was monitored using two fluorogenic substrates: Amplex Red and 4-(N-Methylhydrazino)-7-nitro-2,1,3-benzooxadiazole (MNBDH).

Covalent attachment of hemin not only allowed on reduction of blind probe signal but also resulted in substantial higher activity of DNAzyme based on telomeric sequence. Whereas application of 2’-OMe-RNA resulted in increase of activity of all studied DNAzymes with the highest increase for telomeric sequence.

The presented modifications allowed on increase and control of catalytic activity of DNAzymes. On the one hand they allow to increase the activity of systems even those which when unmodified exhibits very low activity. On the other hand application of DNA analogues is beneficial from the point of further application in living cells, where unprotected foreign DNA is destroyed by deoxyribonuclease. Both modifications open the new possibilities in development of DNA probes for bioanalytical application.

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REFERENCES

[1] Travascio P., Li Y., Sen D.(1998). DNA-enhanced peroxidase activity of a DNA aptamer-hemin complex. *Chem. Biol.* 5, 505-517.
[2] Kosman J., Juskowiak B.(2011). Peroxidase-mimicking DNAzymes for biosensing applications: a review. *Anal. Chim. Acta* 707, 7-17.
[3] Li C., Zhu L., Zhu Z., Fu H., Jenkins G., Wang C., Zou Y., Lu X., Jang C.J.(2012) Backbone modification promotes peroxidase activity of G-quadruplex-based DNAzyme. *Chem. Comm.* 48, 8347-8349.

STRUCTURE AND ROLE OF PECTINS (SODIUM CARBONATE FRACTION) FOR CELL WALLS MECHANICS

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Pectins are the most complex and diverse biopolymers in plant cell walls. Recently, it was found that covalently linked pectins in cell wall, that might be extracted with sodium carbonate (DASP) from fresh fruits and vegetables may form a regular network on freshly cleaved mica [1]. The goal of this work was to evaluate the structure and the role of the covalently linked pectin for cell walls mechanics. Cell walls from pear ('Xenia' and 'Conference') were used. DASP pectins were extracted after *in vitro* pectinase treatment of cell walls. The mechanical properties of cell walls were evaluated with atomic force microscope [2].

The height of the DASP molecules on mica was between 0.2-1.8 nm. An average height was greater for 'Xenia' (0.82 nm) than for 'Conference' (0.66 nm). Too dense structure did not allowed to evaluate the branching index for 'Xenia', nevertheless for 'Conference' this parameter was 8.2 per 1 micrometer. AFM images revealed a clear change of the DASP structure after treatment with pectinase. In both cultivars, pectinases caused loosening of the regular structure that was characteristic for the control sample. Thickness of the DASP molecules decreased as well. The average height decreased to 0.25 nm ('Conference') and to 0.41 nm ('Xenia'). Moreover for 'Conference' the branching index decreased to 7.8. Further increase of the pectinase activity caused total depolimerization of the regular structure to a spot-like and oval shape structure.

The Young's modulus of cell walls before pectin extraction was 2.5 and 3.8 MPa for 'Conference' and 'Xenia' respectively. After extraction of the DASP, cell walls of both cultivars reached very similar stiffness of about 0.8-1 MPa. 'Xenia' had thicker, more dense, branched and less susceptible for pectinase pectin molecules that corresponds to stiffer cell walls and harder texture of fruit as well. These results suggests that particularly the sodium carbonate fraction is responsible for the difference in mechanical properties between these two cultivars.

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REFERENCES

[1] Cybulska J., Zdunek A. & Kozioł A. (2015). The self-assembled network and physiological degradation of pectins in carrot cell walls. Food Hydrocolloids 43,pp. 41-50.
[2] Zdunek A., Kozioł A., Cybulska J., Lekka M. & Pieczywek P.M. (2016). The stiffening of the cell walls observed during physiological softening of pears. Planta 243(2),pp. 519-529.

THE EFFECT OF NANOPARTICLES $C_{60}(OH)_X$, X>30 ON HUMAN BLOOD CELLS

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Due to their properties, fullerenols can be used in radiobiology, chemotherapy, or in the treatment of neurodegenerative diseases. There are many reports that fullerenol protects cells against oxidative stress generated by ionizing radiation and/or anticancer drugs. The fullerenol molecule can be also excited with visible or ultraviolet light creating a reactive molecule that easily interacts with oxygen or biomolecules and has applications in photosensitization (Grebowski, Kazmierska & Krokosz, 2013).

We demonstrated that highly hydroxylated fullerenol $C_{60}(OH)_x$, x>30 (up to 150 mg/L) is non-toxic to human erythrocytes, however, can adsorb to plasma membrane proteins, especially to ion-dependent ATPases decreasing their activity and to band 3 protein (Krokosz & Grebowski, 2016).

Fullerenol protected lymphocytes against X-ray induced loss of cell viability after 48-hour post-radiation incubation, however, it enhanced human peripheral blood lymphocytes granularity after 24-hour incubation without causing cytotoxicity (Nowak, Krokosz, Rodacka & Puchala, 2014).

In this work the influence of fullerenol $C_{60}(OH)_x$, x>30 up to 150 mg/L on erythrocyte membrane parameters under ionizing-radiation generated oxidative stress as well as the effects of fullerenol on peripheral blood mononuclear cells (PBMC) were assessed. Fullerenol added 1 hour before irradiation protected erythrocyte membrane –SH groups against radiation oxidation and preserved the conformation of membrane proteins as determined by spin labeling method, however, the effect

was post-radiation time dependent.

In case of PBMC, the cytofluorometric measurements indicated that the fullerenol had a little effect on the mitochondrial potential. Moreover, at a concentration of 150 mg/mL a few necrotic cells were visible assessed by propidium iodide (PI) and calcein-AM double staining.

Our studies confirm a little toxicity of fullerenol against blood cells and its antioxidative properties.

REFERENCES

[1] Grebowski J., Kazmierska P. & Krokosz A. (2013). Fullerenols as a New Therapeutic Approach in Nanomedicine. *BioMed Res. Internat.*, **2013**, 751913(9 pages).

[2] Krokosz A. & Grebowski J. (2016). Activity of membrane ATPases in human erythrocytes under the influence of highly hydroxylated fullerenol. [In:] Chakraborti S., Dhalla N.S. (eds.) *Regulation of Membrane Na^+-K^+ ATPase*, Springer International Publishing Switzerland, pp. 159-172.

[3] Nowak K., Krokosz A., Rodacka A. & Puchala M. (2014). Study on the effect of polyhydroxylated fullerene, $C_{60}(OH)_{36}$, on X-ray irradiated human peripheral blood mononuclear cells. *Radiat. Phys. Chem.*, **97**, 325–331.

ACTIVITY OF HUMAN CYTOSOLIC NUCLEOTIDASE, cN-IIIB TOWARDS NUCLEOSIDE MONOPHOSPAHTES

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Human cytosolic 5' nucleotidase, cN-IIIB, belongs to the family of eight enzymes catalyzing the hydrolytic defosforylation of nucleoside monophosphates to nucleosides and orthophosphate. As a one of catabolitic enzymes, it contributes to the regulation of nucleotide levels in living cells, however, its exact role in the cell has not been established so far. Due to the distinctive activity towards m^7GMP, it has been assumed that cN-IIIB participates in mRNA decay, and protects cells against undesired salvage of m^7GMP and its incorporation into nucleic acids. In our study, we used a library of fifty nucleoside monophosphates to investigate the substrate specificity and inhibition of cN-IIIB enzyme by means of HPLC. This will allow to understand the basis of cN-IIIB selectivity for substrates as well as to design unnatural inhibitors of the protein. The selected compounds could be used in the future as molecular probes to monitor the enzyme activity in cells.

APPLICATION OF SOME SPECTROSCOPIC METHODS TO PHOTOPHYSICAL PROPERTIES INVESTIGATIONS OF BIOMEDICAL OBJECTS

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One of the most interesting and controversial group of the molecules of the last decades are hydroxy derivatives of stilbene. Their chemotherapeutic and chemopreventive properties have been a subject of increasing interest lately. Their photophysical properties are widely described in many theoretical and experimental reports. Huge increase of the number of reports on the existence of the stilbene-like molecules in plants, fruits, food products and beverages is the proof on very high interest of biophysicist, physicist, chemist and biomedical scientists.

Because of the chemical structure of these molecules, they are very attractive objects for investigation of photoisomerization, intramolecular charge transfer, ultrafast radiationless and solvent relaxation, as well. Among the experimental methods to investigate such processes are emission anisotropy spectra and time-resolved emission spectra measurements. Both methods are complementary and allow to study behavior of the molecules in different environment (liquids, solid state, thin layer) and conditions with picosecond time resolution and subnanometers resolution in the wavelength scale.

Revitalized setup of the system for emission anisotropy spectra measurements (methodology – [1]) with credibility tests are described. Comparison to previous results [2] are made and some new results are presented. Time-resolved emission spectra [3] for the same samples are also presented, with the interpretation of deactivation pathways presented for samples in various conditions. Ultrafast deactivation obviously seen in the blue region of emission spectra can be interpreted as simultaneous intramolecular charge transfer and solvent relaxation, as well.

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REFERENCES

[1] Kubicki A. (1989), A Universal Photon-Counting Measuring System for Polarized Spectroscopy, Exp. Tech. Physik, 37, 329.

[2] Borowiak M., Grobelna B., Synak A., Bojarski P.,Kubicki A. A. (2013) Time-resolved emission spectra of 4-dimethylamino-4'-cyano-stilbene and resveratrol in high viscosity solvents and silica matrices,Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 115, 111.

[3] Kubicki A. A., Bojarski P., Grinberg M., Sadownik M., Kukliński B. (2006), Time resolved streak camera system

with solid state laser and optical parametric generator in different spectroscopic applications Opt. Comm., 263, 275.

THE EFFECT OF BISPHENOL A AND ITS SELECTED ANALOGS ON CELL MEMBRANE FLUIDITY OF HUMAN ERYTHROCYTES

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Bisphenols are chemical substances used in massive amounts in the production of epoxy resins, polycarbonates and thermal paper. Bisphenol A (BPA) is the most representative bisphenol with an annual production of above 6 million tons. Moreover, a significant increase in the production of BPA analogs such as bisphenol F (BPF), bisphenol S (BPS) and bisphenol AF (BPAF) has been noted, which is mainly associated with a ban on BPA in some countries (including the European Union, since 2011) in various products including products for infants, packaging and bottles.

The widespread of BPA and its analogs in the environment and human surrounding (food, drinking water, dust) leads to significant exposure of human to these substances. The occurrence of BPA and its analogs in human organism has been documented in numerous research works.

BPA is the best-studied compound among bisphenols. It exhibits estrogenic activity, and is potentially carcinogenic for humans. The results of toxicological studies have indicated that other bisphenols may also exhibit a similar or even stronger toxicity than BPA, but the number of research works in this area is too small to provide correct conclusions at this moment.

Red blood cells are the most plentiful cells of the circulatory system. The erythrocytes are directly exposed to xenobiotics like bisphenols that enter to the body because these cells participate in transport of various chemicals. As enucleated cells, they are suitable model to assess the effect of xenobiotics on changes in cell membrane properties. Up to now, no work has been undertaken in order to analyze the effect of bisphenols on erythrocyte membrane, thus, in this study the effect of BPA and its analogs on changes in red blood cells membrane fluidity was studied.

The erythrocytes were incubated with BPA or its analog in the concentrations ranging from 0.5 to 250 μg/mL for 4 or 24 h. After incubation, the excess of the compounds was discarded, the erythrocytes were labeled with 5-doxylstearic acid (5-DSA) or 16-doxylstearic acid (16-DSA), and then changes in cell membrane fluidity were analyzed by electron paramagnetic resonance (EPR) spectroscopy.

For the erythrocytes labeled with 5-DSA, order parameter S was determined for which no changes for any bisphenol were noted. The compounds examined disturbed hydrophobic regions of erythrocytes membrane labeled with 16-DSA (for this analysis, relaxation times of tau B and tau C were determined). The strongest changes were noticed in red blood cells incubated with BPA, BPF and BPAF that altered erythrocyte membrane fluidity from the concentrations of 5 μg/mL and 0.5 μg/mL, respectively after 24 h of incubation. The most probably, BPA and its analogs due to their significant lipophilicity were located in hydrophobic layer of the erythrocyte membrane. Changes in the parameter studied were noted for bisphenols in the concentrations that may influence human body as a result of occupational exposure (BPA, BPAF) or subacute poisoning (BPF, BPS) with these substances.

VESTIBULAR EVOKED MYOGENIC POTENTIALS (VEMP), MEASURED FOR 0,5, 1, 2KHZ TONE BURST AND CLICKSTIMULI, CHARACTERISTICS ANALYSIS

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Balance control system includes integrated vestibular, visual and somatosensory inputs. Vestibular sensory system contains five receptors: two macular (saccule and utricule) and three canalar (lateral, posterior and superior). This mechanism is responsible for upright posture control and vision stabilization. The vestibular evoked myogenic potentials test (VEMP) is more and more popular examination, which gives us information about the otolith function – saccule and utricule, inferior vestibular nerve, and central connections. VEMP examination is very useful in superior semicircular canal dehiscence, Ménière disease, vestibular neuritis, otosclerosisor and Multiple Sclerosis diagnosis. Myogenic potentials evoked by high intensity tone burst stimulus can be recorded from contracted sternocleidomastoid (cVEMP) or from extraocular muscles (oVEMP). Parameters like presence or absence of VEMP response (P1 and N1 waves), P1-N1 amplitude ratio, threshold, tuning curves across frequencies and difference between amplitudes for both ears are analyzed. Consistent muscle contraction during examination is very important. Departments and research facilities are encouraged to develop their own normative data depending on equipment settings.

The aim of this study was to compare the vestibular evoked myogenic responses registered for 500, 1000 and 2000 tone burst and click stimuli. VEMP examination was assessed using ICS Chartr EP 2000 system and insert headphones. Measurement was carried out in Faraday cage to eliminate disturbances related to external electromagnetic field.

DYNAMIC POSTUROGRAPHY IN STUDIES ON ACOUSTIC DISTURBANCES OF POSTURAL STABILITY

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One of the clinical tests evaluating the motor skills is posturography. Posturography examinations divided into static posturography and dynamic posturography. During static posturography patient stays on the stable platform while during dynamic one platform is unstable. In both, static posturography and dynamic posturography, patients are examined either with eyes opened or eyes closed. The methods permits the evaluation of the balance control system including vestibular, visual and somatosensory mechanisms. The high intensity acoustic stimuli can induce the vestibular signs and symptoms as vertigo, nystagmus, oscillopsia and electromyographic potentials, what have been studied and discussed in literature [1-2].

The aim of this study was to determine the 4kHz and 65dB stimulus effect on postural stability of healthy subjects. Up to now, the influence of normal conversational pressure level sound was not described in the available literature. The most sensitive to acoustic stimuli posturography parameter was also searched.

Measurements were carried out using Multitest Equilibre platform produced by FRAMIRAL. The platform allows to carried both, static posturography and dynamic posturography. Such parameters as velocity and surface were recorded. During researches patients firstly made test without acoustic stimulus.Next, procedure was repeated with 4 kHz, 65 dB acoustic stimulus. Data were collected from 30 subjects, aged 20 to 35 years (10 males and 20 females) and none of them had hearing loss. Hearing threshold for each patient was measured with pure tone audiometry before posturography examination. The exclusion criteria were: medications taken in regular basis and history of vertigo or balance disorders.

The statistically significant difference between some parameters measured in the presence of 4000 Hz, 65 dB acoustic stimulus and without additional disturbances was observed. The research was approved by the Bioethical Committee Research.

REFERENCES

[1] Selmani, Z., Ishizaki, H., &Pyykkö, I. (2004). Can low frequency sound stimulation during posturography help diagnosing possible perilymphatic fistula in patients with sensorineural hearing loss and/or vertigo?. European Archives of Oto-Rhino-Laryngology and Head & Neck, 261(3), 129-132

[2] Alessandrini, M., Lanciani, R., Bruno, E., Napolitano, B., & Di Girolamo, S. (2006). Posturography frequency analysis of sound-evoked body sway in normal subjects. European Archives of Oto-Rhino-Laryngology and Head & Neck, 263(3), 248-252

BIOPHYSICS OF BRAIN: PERSPECTIVES OF BRAIN-COMPUTER INTERFACE IN BIOMEDICAL ENGINEERING

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The beginning of the XXI century in science and technology has resulted in tremendous development of diagnostic and therapeutic methods, based on digital techniques using feedback from the brain-computer. Studies provided by neurophysiologists, which indicated the presence of typical potential activities of the brain, enabled the construction of BCI interfaces (Brain Computer Interface). This technology [1,2], by analyzing of EEG signals, makes it possible to control correctly prepared applications running on PCs, smartphones and tablets. In practice, it enables the control of consumer and professional electronic devices by people with the most serious neurological deficits. Therefore, the potential of BCI technology for use in biocybernetics and biomedical engineering is not to be underestimated. The development of BCI technology clearly divides into two fundamental groups . The first one leads to the use of feedback between the patient (subject) and the appropriate PC application and is used mainly for neuropsychological and psychiatric therapies and meditation or mental techniques. The second group is the use of BCI interface to control machines. It covers a broad application in biomedical engineering (neuroproteasis, exoskeletons, bionic support systems) and special support of patients with serious neurological deficits (eg. severe paralysis). Both groups fundamentally alter the position of the patient, open up new possibilities, which until recently were described as Science Fiction. EEG devices available on the market can be divided into two groups of BCI. The first one is a set of "headphones" of EEG which can cooperate with ready-made software packages for the PC and mobile devices. Their main addressee are people using EEG biofeedback techniques. These systems are characterized by simple, one or several electrode headsets put on the forehead, with the reference electrode in the form of a clip which placed on the earlobe (ie. kits of NeuroSky and Emotiv Insight classic). The second group involves much more advanced kits which can fully map functions of brain currents. They consist of a helmet containing a dozen or several dozen salt electrodes, coupled with the BCI interface. With software such sets (eg. Emotiv EPOC and the EPOC +) it impossible to do scientific work, design-built biomedical and cyber devices for industrial and medical applications.

REFERENCES

[1] Nicolas-Alonso L.F. & Gomez-Gil J. (2012). Brain computer interfaces, a review. *Sensors (Basel)*, **12**(2), 1211-1279.

[2] Shih J.J., Krusienski D.J., Wolpaw J.,R. (2012). Brain-computer interfaces in medicine. *Mayo Clinic Proceedings*. **87**(3), 268-279.

ENCAPSULATED DOXORUBICIN ARMED WITH gH625 PEPTIDE DOES NOT DAMAGE NORMAL ENDOTHELIAL CELLS AS FREE DOXORUBICIN

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Doxorubicin (DOX) is one of the oldest and most widely used anthracyclines employed in the chemotherapy of a broad spectrum of solid tumors and hematopoietic and lymphoid malignancies. Chemotherapy based on DOX although very effective causes a number of adverse effects. The most dangerous among them is cardiomyopathy, leading to congestive heart failure. One of the approaches aimed at reducing DOX side effects is the development of liposomal formulations of DOX, which limit the drug cardiotoxicity and facilitate its release in the close vicinity of the tumor. This strategy works well in clinical practice. The problem remains, however, with the transport of this form of drug through the blood-brain barrier (BBB).

Cationic cell-penetrating peptides are a group of short peptides able to cross the lipid bilayer using a mechanism mediated by endocytosis. This kind of molecules are well suited for development as drug delivery vehicles. Peptide gH625, derived from glycoprotein H of herpes simplex virus type 1, can enter cells efficiently. Nanoparticles armed with gH625 are able to cross the BBB in *in vitro* model.

In the study the toxicity of free DOX, DOX encapsulated in liposomal carrier and DOX encapsulated in liposomal carrier armed with gH625 peptide towards normal HMEC-1 cells derived from dermal microvascular endothelium was evaluated on the basis of their ability to induce the collapse of mitochondrial transmembrane potential ($\Delta\Psi$m) as an early indicator of apoptosis. The cells were treated with five different concentrations of DOX – IC10 (0.35 nM), 0.5 IC50 (0.30 nM), IC50 (0.61 nM), 2IC50 (1.22 nM) and IC90 (1.04 nM) for 3h. Then the drug was removed and changes in $\Delta\Psi_m$ were assessed with the fluorescent probe JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazolo-carbo cyanine iodide). Fluorescence of JC-1 monomers and dimmers was measured over 0-60 min period after the treatment in order to estimate the kinetics of $\Delta\Psi_m$ changes caused by the investigated compounds.

Free DOX causes depolarization of mitochondrial membrane, regardless of the drug concentration. After 60 min of the treatment the treated cells recovered their $\Delta\Psi_m$, which returned to the level characteristic of untreated cells. Encapsulated DOX did not affect the mitochondrial membrane potential. The encapsulated DOX armed with gH625 peptide cause transient mitochondrial membrane hyperpolarization, and this effect became more pronounced with an increasing drug concentration.

These results suggest that liposomal encapsulation of DOX can protect normal endothelial cells against mitochondrial damage and collapse of $\Delta\Psi_m$ induced by free DOX.

FIVE – MEMBERED NITROXYL DERIVATIVES ENHANCE PACLITAXEL ANTICANCER ACTIVITY IN HUMAN BREAST CANCER CELLS

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Aim of study. Nitroxides Pirolin (2,2,5,5-tetra-methyl-3-carbamoyl pyrroline-1-oxyl, PL) and Pirolid (2,2,5,5-tetramethyl-3-carbamoylpyrrolidine-1-oxyl, PD) are five-membered, cell-permeable stable radical antioxidants with a broad spectrum of activity. In light of our previous SAR studies on six-membered nitroxides, acting as less toxic anticancer agents and antioxidants, we aimed at investigating the effect of five-membered PL and PD on the anticancer activity of paclitaxel (PTX) toward human breast cancer cells (MCF-7 cell line) with an emphasis on DNA damage and microtubule disruption induced by this drug. The exact mechanism of PTX cytotoxicity against tumor cells is still under extensive study. It is commonly accepted that the drug interacts with microtubules and induces apoptosis in various tumor cells.

Material and Methods. MCF-7 breast cancer cells were incubated with the IC_{50} concentration of paclitaxel (0.4 μmol/l) for 2 h and with 50 μmol/l concentration of nitroxides for 3 h. When combination of both compounds was used the cells were pretreated with Pirolin or Pirolid for 1 h before the addition of PTX. After the treatment investigated compounds were removed and the cells were immediately subjected to further analysis or after 24-72 h culture in drug-free medium. DNA damage was analyzed using single cell electrophoresis (comet assay). Inhibition of microtubule depolymerization was investigated using an immunohistochemistry method with monoclonal antibody against β-tubulin.

Results. Paclitaxel caused both DNA damage and microtubule disruption. Preincubation with nitroxyl derivatives enhanced antitumor effect of PTX – accelerated appearance of DNA damage and increased their level. The nitroxides did not interfere with the main cellular mechanism of PTX anticancer activity – stabilization of the microtubule polymerization and their protection from disassembly.

Conclusions. Five-membered nitroxyl derivatives Pirolin and Pirolid seem to be promising compounds with antitumor properties, which do not exhibit cytotoxic properties by themselves, but enhance the anticancer activity of paclitaxel toward human breast cancer cells.

INTERCONNECTION BETWEEN APOPTOSIS AND AUTOPHAGY IN HUMAN BREAST CANCER CELLS TREATED WITH PIROLIN AND DOXORUBICIN

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Introduction. Interconnection between apoptosis and autophagy and their significance in the cancer cell death are still under extensive investigation. Autophagy (or autophagocytosis) is an evolutionarily conserved catabolic process that occurs in the cells under stress conditions and allows the orderly degradation and recycling of cellular components Basically, during autophagy cell components are closed within a double-membraned vesicle (autophagosome), which content after fusion with a lysosome is subjected to complete degradation and then recycled. One of the cellular components degraded by autophagy are mitochondria, damaged by induction of apoptosis. Such mitochondria release cytochrome c, which is involved in the propagation of apoptotic cell death.

Aim of study. The aim of the study was to investigate the ability of nitroxyl derivative Pirolin (nontoxic, cell-permeable, low molecular weight and stable free radical) and anticancer drug doxorubicin (DOX) to induce autophagy and cytochrome c release in human breast cancer cells. The nitroxide and anticancer drug were used alone and in combination.

Methods. MDA-MB-231 human breast cancer cells were incubated with IC_{50} concentration of DOX (6 μmol/l) for 2 h and with 50 μmol/l concentration of nitroxide for 3 h. When combination of both compounds was used the cells were pretreated with Pirolin for 1 h before the addition of DOX. The cells were subjected to analysis immediately after the treatment with investigated compounds or after 24-72 h culture in drug-free medium. Induction of autophagy was analyzed on the basis of autophagosome formation assessed by fluorescence microscopic examination of cells stained with acridine orange. Cytochrome c release was analyzed by ELISA method.

Results. Pirolin by itself did not induce formation of autophagosomes in cancer cells, but accelerated the induction of this process in cells treated with DOX. Nitroxide did not cause the efflux of cytochrome c from mitochondria and did not affect significantly the efflux of cytochrome c in cells treated with DOX.

Conclusions. Proline accelerates and enhanced the induction of autophagy by DOX but did not affect apoptosis induced of by this drug in human breast cancer cells.

SPECTROSCOPIC STUDIES OF THE SOLVENT EFFECTS OF SELECTED COUMARIN DERIVATIVES AND THEIR COMPLEXES WITH d-BLOCK METAL IONS

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Different types of coumarin derivatives are well-known compounds with antitumour, antibacterial, or antifungal activity. Both natural and synthetic derivatives of these compounds have been successfully used in in food and cosmetic industry for years. Also, selected coumarin derivatives are used as laser dyes or highly sensitive molecular probes.

A large number of publications have evidenced that the biological activity of these organic compounds is considerably enhanced in their complexes with metal ions. Investigations of complexes of some derivatives of the analysed compounds showed their high antifungal activity. Some complexes of these compounds (e.g. Schiff bases) with Cu(II) exhibit excellent activity against *Candida*. Furthermore, some Cu(II) complexes showed substantially greater activity than that of free ligands, and their IC50 values were comparable to those of commercial Amphothericin B and Ketoconazole.

Despite their high activity in vitro, the low solubility of the analysed compounds and their complexes with metal ions in water or other organic solvents is often a serious drawback limiting considerably their commercial use. It is therefore important to understand the mechanisms of action of these compounds at the molecular level in order to develop a novel series with enhanced solubility and, hence, potential applicability. In an attempt to develop a new compound series, complexes of the aforementioned ligands with Cu(II) and Pd(II), which is well known for its catalytic properties, were prepared. The free ligand (L1), which was derived from 2,3,4-trihydroxybenzaldehyde and 7-amino-4-methylcoumarin together with its corresponding Pd(II) and Cu(II) complex (L1-Pd, L1-Cu), served as a model compound in this study.

The structures and purity of the L1, L1-Pd, and L1-Cu were confirmed using ^{1}H-NMR, FTIR, and AAS spectroscopic methods and elemental analysis. The photophysical properties of all compounds were investigated using UV-vis,

fluorescence (steady state), and Time Resolved Fluorescence (TRF) spectroscopy. Spectroscopic analyses were performed in selected organic solvents.

HORSETAIL (EQUISETUM ARVENSE) EXTRACT'S PHYTOCHEMICALS IN INTERACTION WITH THE BIOLOGICAL MEMBRANE

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The risk of civilizational diseases induced search for new substances, both those of protective action that could diminish the risk, and those that help healing the diseases. Numerous studies have shown that natural, plant-derived compounds are characterized by good bioavailability and have no side effects. One of such substances is *Equisetum arvense* (common horsetail) extract, which due to its high content of polyphenolic substances exhibits strong anti-inflammatory, antibacterial, hepatoprotective, diuretic and antioxidant activity [1]. Its health promoting properties encouraged the authors to undertake biophysical studies on the effect of the extract at the molecular and cell level.

The study aimed to determine the polyphenolic content of the extract, its antioxidant activity, and examine its effect on hemolysis and osmotic resistance of the red blood cell. The purpose of the experiments was to investigate the mechanism of the interaction of horsetail extracts with erythrocyte and DMPC lipid membrane. The content of polyphenolic compounds in the extract was determined by using the chromatographic HPLC-DAD and total phenolic content method. Antioxidant activity was examined with regard to erythrocyte membranes, using spectrophotometric methods, with UVC radiation as the oxidizing agent. With a spectrophotometric method we also determined the hemolytic activity of the extract polyphenols and their effect on osmotic resistance of erythrocytes. Spectrofluorimetrically, changes in packing order and fluidity of the erythrocyte membrane was determined. Using FTIR spectroscopy, changes in the hydration of the lipid membrane was examined. The horsetail extracts showed antioxidant activity in relation to the erythrocyte membrane. The findings indicate that the polyphenolic compounds contained in horsetail extract, due to their composition, do not destroy the biological membrane. Phytochemicals of the extract when interacting with the surface polar part of the erythrocyte membrane protect it from oxidation.

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REFERENCES

[1] Gründemann C., Lengen K., Sauer B., Garcia-Käufer M., Zehl M., & Huber M. (2014). Equisetum arvense (common horsetail) modulates the function of inflammatory immunocompetent cells. BMC Complementary and Alternative Medicine, 14:283

VOLUME, PRESSURE AND VISCOSITY MEASUREMENTS AS A USEFUL TOOL FOR EXPLANATION OF BAKING EXPANSION PROCESS

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Changes in volume, pressure, and viscosity of wheat dough leavened by baking powder during model baking were analysed. The volume changes showed two baking stages, i.e. dough expansion and crumb shrinking. Through the analysis of pressure and viscosity extremes, the baking expansion stage was divided into five phases.

The relaxation phase (R) is the first baking phase during which a decline in pressure and viscosity of dough are observed concurrently. The low pressure of leavening gases relative to the high dough viscosity results in low dough expansion (~2%).

In the softening phase (S), there is a substantial decline in viscosity accompanied by a gradual pressure rise. The overlapping of these two opposite transformations leads to a dynamic increase in the dough expansion (~54%).

The gelatinisation phase (G) is characterised by a significant increase in dough viscosity resulting from the starch gelatinisation and protein aggregation process. It results in a gradual slowing-down of the expansion rate, however, the contribution of this phase to the dough expansion is still significant (~22%).

The opening phase (O) begins at the time of a rapid pressure decrease as a result of forming openings in the bubble walls. However, despite its substantial decrease, the gas pressure is still sufficiently high to overcome the crumb shrinking force and maintain further expansion (~18%).

The boiling phase (B) initiates when the temperature in the peripheral loaf zone reaches the water boiling point. Released water vapour gradually raises dough pressure, which prevents collapse of the crumb and results in a slight volume increase (~4%). Losses in the water content are one of the basic transformations leading to final thermosetting of the crumb cellular structure and to completion of the baking expansion.

5'CAP-BINDING SITE MUTATIONS IN EIF4E ABOLISH POSITIVE EFFECT OF THE CAP ON THE EIF4E – 4E-BP1 INTERACTION

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Specific recognition of the mRNAs 5' terminal cap structure by the eukaryotic initiation factor eIF4E is the first, rate-limiting, step in the cap-dependent translation. Small 4E-binding proteins, 4E-BP1, 4E-BP2, and 4E-BP3, inhibit the translation initiation by competing with eIF4G initiation factor for the same binding site and by blocking the assembly of the translation machinery [1].

The affinity of the 4E-BP1 inhibitory protein for wild type (WT) eIF4E increases about 10-fold in the presence of m^7GTP, $K_{as} \sim 6 \cdot 10^6$ M^{-1} for *apo*- and $50 \cdot 10^6$ M^{-1} for cap-saturated eIF4E [2]. On the contrary, the association constant for the binding of m^7GTP to eIF4E ($K_{as} \sim 100 \cdot 10^6$ M^{-1}) decreases twice upon prior incubation of WT eIF4E with 4E-BP1.

Here, we compare the interactions of 4E-BP1 and of a cap analogue m^7GTP with WT eIF4E and its two mutants, W102A and W56A/W102A, using analytical ultracentrifugation and fluorescence titration. The mutations in the cap binding pocket weaken the affinity of eIF4E for m^7GTP at least three orders of magnitude, both for the *apo*- and for 4E-BP1-saturated eIF4E. Unlike in the WT eIF4E, the interactions of mutated eIF4E with 4E-BP1 are not enhanced by m^7GTP.

Our studies show that correct orientation of m^7GTP in the eIF4E binding slot is necessary for cooperativity between the cap and 4E-BP/eIF4G binding sites of eIF4E.

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REFERENCES

[1] Sonenberg, N & Hinnebusch, A.G. (2009). Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* **136**, 731–745.

[2] Modrak-Wojcik A., Gorka M., Niedzwiecka K., Zdanowski K., Zuberek J., Niedzwiecka A. & Stolarski R. (2013). Eukaryotic translation initiation is controlled by cooperativity effects within ternary complexes of 4E-BP1, eIF4E, and the mRNA 5' cap. *FEBS Letters* **587**, 3928–3934.

COMPARISON OF GLASS TRANSITION TEMPERATURE OF BOVINE SERUM ALBUMIN RESULTING FROM DIRECT AND INDIRECT METHOD.

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The results of viscosity determinations on aqueous solutions of bovine serum albumin (BSA) at a wide range of concentrations and at temperatures ranging from 5°C to 45°C are presented. Viscosity-temperature dependence of the BSA solutions is analyzed based on the formula resulting from the Avramov's model. One of the parameters in the three parameters Avramov's equation is the glass transition temperature T_g. It turns out that the T_g of BSA solutions increases monotonically with increasing concentration. The glass transition temperature of a solution depends both on T_g for a dissolved dry protein $T_{g,p}$ and water $T_{g,w}$. To obtain the glass transition temperature of the dry BSA, a modified Gordon-Taylor equation is used. This equation describes the dependence of T_g of a solution on concentration, and $T_{g,p}$ and a parameter "k" depending on the strength of the protein-solvent interaction are the fitting parameters. Thus determined the glass-transition temperature for the dry BSA is equal to (255 ± 12) K and the parameter k = (0.855 ± 0.169). Very recently it has been demonstrated experimentally that the glass transition phenomenon actually occurs in dry BSA [1]. The authors measured the mean-square atomic displacement $\langle x^2 \rangle$ in lyophilized BSA powder by using the incoherent inelastic neutron scattering method. Anomalous temperature behavior of both the mean-square atomic displacement and vibrational and relaxational dynamics, revealed that the glass-like transition occurs in the dry BSA in the vicinity of 250 K. As seen, the thus directly obtained glass transition temperature of dry BSA is consistent - within experimental error – with the value deduced indirectly from the method proposed in the present work.

REFERENCES

[1] Frontzek A.V., Strokov S.V., Embs J.P., Lushnikov S.G. (2014). Does a dry protein undergo a glass transition?. J. Phys. Chem. B **118**, 2796-2802.

ROTATIONAL CORRELATION TIME FOR SOME MAMMALIAN SERUM ALBUMINS IN DILUTE SOLUTIONS DEDUCED FROM THE MAXWELL EFFECT.

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Rotational correlation time is commonly used as a source of information about dynamic behavior of proteins in solution. It can be determined both for the side chains of the protein and for the protein as a whole. In the latter case, the rotational correlation time is called the overall motion correlation time. It may be experimentally obtained by using the Maxwell effect consisting in simulated birefringence in liquids or solutions induced by the mechanical force like shear stress in a streamline flow. For protein in diluted solutions it can be calculated if the intrinsic viscosity, molecular mass and the axial ratio of the protein is known. The intrinsic viscosity has been measured using an Ubbelohde-type capillary microviscometer immersed in a water-bath controlled thermostatically for several mammalian serum albumins. Measurements were carried out in a temperature range between 5^{o}C and 45^{o}C. The thus obtained numerical values of the overall motion correlation time are in the following range: from 134 ns (5^{o}C) to 39.9 ns (45^{o}C) for bovine serum albumin, from 107 ns (5^{o}C) to 34.6 ns (45^{o}C) for rabbit serum albumin, from 101 ns (5^{o}C) to 32.4 ns (45^{o}C) for ovine serum albumin, from 90.1 ns (5^{o}C) to 28.2 ns (45^{o}C) for equine serum albumin and from 89.3 ns (5^{o}C) to 28.8 ns (45^{o}C) for porcine serum albumin. The pH values of the examined solutions were: at the isoelectric point for bovine serum albumin solutions and outside of the isoelectric point for the other albumins solutions. The above results suggest that the overall motion correlation time of a protein reaches a maximum value in the solution at isoelectric point.

DIETARY FIBRE-INDUCED CHANGES IN THE STRUCTURE AND THERMAL PROPERTIES OF GLUTEN PROTEINS STUDIED BY FT-RAMAN SPECTROSCOPY AND THERMOGRAVIMETRY

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Interactions between gluten proteins and dietary fibre supplements at the stage of bread dough formation are crucial in the baking industry. The dietary fibre additives are regarded as a source of polysaccharides and antioxidants, which have positive effects on human health. The fibre enrichment of bread causes significant reduction in its quality, which is connected with changes in the structure of gluten proteins. Changes in the structure of gluten proteins and their thermal properties induced by seven commercial dietary fibres were studied by FT-Raman spectroscopy and thermogravimetry (TGA), respectively. For this aim the bread dough at 500 FU consistency was made of blend of the wheat starch and wheat gluten as well as the fibre, the content of which ranged from 3 to 18% w/w. The obtained results revealed that all dietary fibres apart from oat caused similar changes in the secondary structure of gluten proteins. The most noticeable changes were observed in the regions connected with hydrogen bonded β-sheets (1614 and 1684 cm-1) and β-turns (1640 and 1657 cm-1). Other changes observed in the gluten structure, concerning other β-structures, conformation of disulphide bridges, and aromatic amino acids microenvironment, depend on the fibres chemical composition. The results concerning structural changes suggested that the observed formation of hydrogen bonds in the β-structures can be connected with aggregation or abnormal folding. This hypothesis were confirmed by thermogravimetric results. Changes in the weight loss indicated formation of a more complex and strong gluten network.

THE INFLUENCE OF HUMAN INDIVIDUAL FEATURES ON POSTURAL STABILITY

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Balance disorders affecting 20% to 30% of adults and 8% to 18% of children increase risk of falls and are associated with raised morbidity and mortality. The prevalence of balance disorders tends to increase with age and age-related degenerative processes. Identification of factors associated with worse body balance control in young asymptomatic adults plays an important role in prevention of falls in the elderly.

Gender, height, BMI index and lateral dominance may affect the complicated connection between sensory system and motor control system [1-3].

Assessment of possible relationships between these factors and human balance system can allow to distinguish between individual determinants and disease symptoms. Useful tool to determine these symptoms is Computerized Dynamic Posturography (CDP) which allows an objective and quantitative evaluation of sensory inputs to balance control and coordination of postural responses [4].

The aim of this study was to determine the differences while maintaining body balance based on the gender, height, BMI index and laterality profile of young, healthy people using Computerized Dynamic Posturography.

The study approved by The University Ethics Committee was conducted in the Department of Biophysics of Medical University of Lublin in the group

of 202 healthy, asymptomatic subjects (101 women and 101 man) aged 20 – 26 years. Body balance control was evaluated using the EquiTest dynamic posturography produced by NeuroCom International Inc.. During sensory organization test (SOT) equilibrium score, motor strategy, centre of gravity alignment was evaluated in six sensory conditions. Also the usefulness of visual, vestibular and somatosensory system and visual preference were measured. Motor control test (MCT) analysed the latency and amplitude of postural response, and adaptation test (ADT) determined the sway energy.

REFERENCES

[1] Oliveira C.B., Medeiros I.R.T., Greters M.G., et al. (2011). Abnormal sensory integration affects balance control in hemiparetic patients within the first year after stroke. *Clinics*, Sao Paulo, 66:2043-2048.
[2] Wu E., Ji L., Jin D., Pai Y. (2007). Minimal step length necessary for recovery of forward balance loss with a single step. *J Biomech*, 40: 1559-1566.
[3] Olchowik G., Tomaszewski M., Olejarz P., et al. (2015). The human balance system and gender. *Acta Bioengin Biomech, 17: 69-74.*
[4] Nashner L.M. (1993b). Computerized Dynamic Posturography. [In:] Jacobson G.P., Newman C.W., Kartush J.M., ed. *Handbook of balance function testing*. St Louis: Mosby Year-Book: 280-307

STEADY-STATE AND TIME-RESOLVED AUTOFLUORESCENCE SPECTROSCOPY OF HUMAN BRAIN GLIOMA AND MENINGIOMA TUMORS

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Brain tumors are the abnormal growths of cells in the central nervous system (CNS). The most common primary brain tumors are gliomas and meningiomas, apart from the other ones (e.g., pituitary adenomas and craniopharyngiomas). The Glioblastoma multiforme (GM), one of the gliomas cases, characterizes by especially aggressive course and unfavorable prognosis. For this reason, this brain cancer subtype has been subjected to very systematic investigations for a long time now.

In this communication we want to demonstrate the application of DUV- and UV-excitation steady-state and time-resolved autofluorescence spectroscopy to gliomas and meningiomas. The main information content of both spectroscopic techniques, towards their prospective clinical applications, is exemplified and discussed. We display a particular value of the spectrally- and time-resolved autofluorescence studies at DUV- and UV-excitations, indicating that, very likely, this technique may occur to be an effective *in vivo* diagnostic method enabling to differentiate between the brain cancer types and subtypes. In the clinical practice, the brain cancer types and subtypes become known from the routine histopathology.

THE INTERACTIONS BETWEEN ACRIDINE ORANGE AND SELECTED FLAVONOIDS

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Polyphenols are one of the most frequently consumed alkaloids worldwide. In most studies of natural antimutagenic and anticarcinogenic compounds, their activity is ascribed to their antioxidant properties. However, apart from free radical capture, many of such substances can intercept aromatic compounds which intercalate DNA.

This study compares the intercepting properties of few polyphenolic derivatives (catechin, epigallocatechin gallate, quercetin, rutin and resveratrol). We checked these interactions via spectroscopic titrations for each compound with a commonly used cellular mutagen – acridine orange (AO). We wanted to know if there's any possibility polyphenolic compounds to prevent the intercalation process, which takes a cytotoxic and cytostatic effects.

The association constants K_a were determined by a method based on Evstigneev's equations [1]. To define if our polyphenols are capable to de-intercalate an acridine orange from the DNA structure, we measured changes in absorption (Cary 5000, Varian), fluorescence (Cary Eclipse, Varian) and fluorescence lifetimes (FluoTime 200, Pico Quant) spectra of the mixtures containing DNA and AO during titration with polyphenols.

The data indicate, that chosen polyphenolic derivatives are able to bind some amounts of AO, which confirms them to act as an intercepting molecules. For example, in order to bind 50% acridine orange at 1μM concentration in a complex, a twenty-fold excess of resveratrol is needed [2]. Performed studies seem to be interesting for a pharmacological and medical branch. Moreover, they could be used by food manufacturers to enrich their products with such ingredients.

REFERENCES

[1] Evstigneev V. P., Mosunov A.A., Buchelnikov A.S., Hernandez Santiago A.A., Evstigneev M.P. (2011). Complete solution of the problem of one-dimensional non-covalent non-cooperative self-assembly in two-component systems. J. Chem. Phys., 134, 194902.
[2] Osowski A., Pietrzak M., Wieczorek Z., Wieczorek J. (2010). Natural compounds in the human diet and their ability to bind mutagens prevents DNA-mutagen intercalation. J. Toxicol.

Environ. Health A., 73, (17-18): 1141-9.

THE SIMPLE COMPETITION MODEL IN A THREE COMPONENT SYSTEM: HYPERICINE – ACRIDINE ORANGE – DNA

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The simple competition model [1,2] is based on competition between molecules of DNA and the interceptor for resources of DNA intercalator. The more intercalator molecules bind to the interceptor, the less DNA adducts are formed. The model uses the association constants values of intercalator – DNA and intercalator – interceptor interactions.

It was investigated how hypericin – a natural compound synthesized by plants of the *Hypericaceae* family – proves itself in the role of an interceptor molecule.

Hypericin has many medical applications. It is widely used as a herbal anti-depressant and as a cholagogic and digestion-enhancing agent. Hypericin is a potential drug in photodynamic therapy too. It may, however, affect the metabolism of other orally-administered drugs. The mechanisms of these interactions may involve the formation of stacking complexes with other flat, aromatic molecules [3].

Hypericin is insoluble in water. Buffers with DMSO contents of 20%, 30%, 40% and 50% were used to enable dissolution of hypericin in water. The association constants of hypericin and a model intercalator – acridine orange – were determined in respective buffers using spectroscopic methods. Values of the association constants depend on DMSO concentration in a mixture. The association constant of acridine orange – DNA interaction was determined earlier (Pietrzak at al. 2006).

The application of the simple competition model made it possible to estimate how the presence of hypericin affects the concentration of the DNA – acridine orange complex in a three-component system: acridine orange – DNA – hypericin. To reduce the concentrations of the DNA – acridine orange complex by 50%, about seven-fold excess of hypericin relative to the acridine orange concentration is required.

REFERENCES

[1] Xie H. P., Jiang J. H., Chu X., Cui H., Wu H. L., Shen G. L., Yu R. Q. (2002). Competitive interaction of the antitumor drug daunorubicin and the fluorescence probe ethidium bromide with DNA as studied by resolving trilinear fluorescence data: the use of PARAFAC and its modificatin. *Anal. Bioanal. Chem*., **373**: 159-162.

[2] Pietrzak M., Wieczorek Z., Wieczorek J., Darzynkiewicz Z. (2006). The "interceptor" properties of chlorophyllin measured within the three-component system: intercalator - DNA- chlorophyllin. *Biophys. Chem*., **123**, 11-19.

[3] Pietrzak M., Maciejczyk M., Szabelski M., Kasparek A., Wieczorek Z. (2014). Self-association of hypericin analyzed by light absorption and fluorescence spectroscopy and molecular dynamics simulations. *Chem. Phys. Letters*, **601**: 39-44.

VISCOSITY OF SUPERCOOLED LIQUIDS CAN BE MEASURED BY MEANS OF FLUORESCENCE CORRELATION SPECTROSCOPY

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During the past 25 years there has been a remarkable growth in the development of various experimental techniques connecting fluorescence phenomena with optical microscopy. One method of particular importance is Fluorescence Correlation Spectroscopy (FCS). With this technique the self-diffusion coefficient of fluorophore molecule can be measured with high spatial resolution offered by confocal microscope. The ability to investigate molecular concentration, mobility and interactions on the sub-cellular level made FCS a powerful tool used mostly in monitoring of biochemical activity occurring in biological environments.

The scientific potential of FCS goes well beyond the established biophysical applications. The possibility of performing direct measurement of the self-diffusion coefficient, together with application of theoretical Stokes-Einstein relation, gives a tool for obtaining information on the size of fluorescent tracer particle, as well as the local physicochemical properties of liquid, namely its temperature and viscosity.

In this work we show how FCS technique can be used to determine the temperature dependence of viscosity of the molecular glass-forming liquid ortho-terphenyl (OTP). The measurements were performed in broad temperature range starting from high temperature conditions well above liquids melting point, down to super-cooled metastable state of the system. In the temperature range covered, the viscosity of OTP changed by 3 orders of magnitude showing characteristic departure from simple arrhenian dependence.

The presented work demonstrate how the utilization of optical technique (as FCS) provides viscosity data that not only agree very well with the published ones, but also can fill the gaps in existing literature results from standard viscosimetric methods.

THEORETICAL STUDY OF TAUTOMERIC EQUILIBRIA OF AMINO FORMS OF 8-AZAPURINES

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Tautomeric equilibria of amino forms of 2,6–diamino-8-azapurine and 8-aza-iso-Guanine (efficient fluorescence probe) molecules is revealed by Density Functional Theory computations both in the gas phase and in water.

The most populated tautomer of 2,6–diamino-8-azapurine, in agreement with available experimental data [1,2], is protonated at position N(9). The lowest free energy tautomer of 8-aza-iso-Guanine is protonated at positions N(3) and N(8), both in the gas phase and in water. For biologically more important tautomer, protonated at position N(9), the probability of protonation at position N(3) is slightly higher than protonation at position N(1). This result, observed also for isoGua [3], shows reversed probability of protonation at position N(3) and N(1) compared to results obtained for Guanine. This subtle effect may be responsible for experimentally observed decreased specificity of base-pairing observed in the expanded genetic code and it should also be expected for expanded genetic code in which isoGua is replaced by the fluorescence probe - 8-aza-iso-Guanine.

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REFERENCES

[1] Mędza G., "Spectroscopic Properties and Tautomerization of Chosen 8-Azapurines", Ph.D. Thesis University of Warmia and Mazury, Olsztyn 2014.
[2] Wierzchowski J., Antosiewicz J. M. & Shugar D. (2014). 8-Azapurines as isosteric purine fluorescent probes for nucleic acid and enzymatic research. *Molecular BioSystems*, **10**, 2756-2774.
[3] Blas J. R., Luque F. J., Orozco M. (2004). Unique Tautomeric Properties of Isoguanine. *J. Am. Chem. Soc.*, **126**, 154-164.

EPOTHILONE B INDUCES HUMAN OVARIAN CANCER OV-90 CELL APOPTOSIS

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Ovarian cancer is the leading cause of death from gynecologic. The common form of cancer of the ovary is epithelial ovarian cancer (EOC). Ovarian serous carcinomas (OSCs) as epithelial subtypes, correspond to 75% of all cases. This EOC cell line (OV-90), derived from the cellular fraction of ascites from a chemotherapy-native patient, has been well characterized by morphological, immunohistochemical, cytogenetic, and molecular analyses of gene expression profiles. We evaluated molecular events associated with apoptosis induced by Epothilone B (EpoB, Patupilone) and paclitaxel (PTX) in OV-90 cells. Epothilones are compounds of natural origin with mechanisms of action similar to taxanes, but with more potent antiproliferative activity. The mode of cell death was assessed colorimetrically, fluorimetrically, cytometrically through assessing the activation of caspase-9, -8 and -3. We measured markers of apoptosis, like phosphatidylserine externalization and morphological changes. EpoB and PTX mediate activation of both initiator caspases-8 and -9, leading to the appearance of caspase-3. We have found, that antitumor efficacy of this new drug is related to its apoptosis-inducing ability. The greatest changes in morphology of cells were noted after treatment with EpoB (after 48 h, 32% of apoptotic cells). A lower level of apoptotic cells was determined for PTX (27%, 48 h). In summary, we report that Epothilone B induces apoptosis in OV-90 cells via caspase 8-dependent pathway.

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SPECTROSCOPIC STUDIES OF CHLOROPHYLL IN YELLOW LUPIN GROWING IN SOIL CONTAMINATED WITH CIPROFLOXACIN

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Antibiotics, including diverse fluoroquinolones are a group of frequently detected environmental contaminants [1,2]. Ciprofloxacin (CIP, 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-yl-quinoline-3-carboxylic acid, $C_{17}H_{18}FN_3O_3$) belongs to the second generation fluoroquinolones. It is widely used in medicine and veterinary medicine [3]. The aim of the study was to demonstrate the effect of increasing doses of ciprofloxacin and the time of exposure of plants to contaminated soil on chlorophyll degradation.

The spectroscopic studies of the absorption and fluorescence spectra of chlorophyll extracts derived from yellow lupin (*Lupinus luteus* L.) plants growing on a substrate polluted with increasing concentrations of 3; 9; 15; 30; 90 mg ciprofloxacin/kg soil. The chlorophyll extraction with methanol was carried out three, five, seven and ten days after antibiotic was used. Distinct changes in the chlorophyll absorption spectra indicated that the degradation of chlorophyll occurred. Absorption levels dropped with increasing concentration of antibiotic in the substrate, and with the time of plant exposure to

antibiotic. At the antibiotic concentration of C =3 mg / kg of soil chlorophyll absorbance decreased from A = 1.0 to 0.75 and A= 0.6 after three and ten days of exposure, respectively. When the concentration of antibiotic was 90 mg / kg of soil absorbance value decreased to 0.55 and 0.35 after three and ten days, respectively. Reduction in the chlorophyll concentration in the plants was observed from 7.34×10^{-5} M for the control to 2.6×10^{-5} M (65%) in plants subjected for ten days to ciprofloxacin, 90 mg / kg of soil. The shift of fluorescence spectra and decrease in their intensity with increasing CIP concentration confirmed degradation of chlorophyll occurring in plants.

The reaction kinetics of the chlorophyll degradation was examined. It was shown that it is the second order reaction. Rate constants chlorophyll degradation were determined: k = 870 M^{-1} day^{-1} for using CIP at 3 mg / kg, and k = 2490 M^{-1} day^{-1} for CIP dose of 90 mg / kg of soil.

REFERENCES

[1] Speltini A., Sturini M., Maraschi F., Profumo A. (2010). Fluoroquinolone antibiotics in environmental waters: sample preparation and determination. *J. Sep. Sci*., 33, 1115–1131.

[2] Speltini A., Sturini M., Maraschi F., Profumo A., Albini A. (2011). Analytical methods for the determination of fluoroquinolones in solid environmental matrices. *Trends Anal. Chem*., 30, 1337–1350.

[3] Picó Y., Andreu V. (2007). Fluoroquinolones in soil—risks and challenges. *Anal. Bioanal. Chem.*, 387, 1287–1299.

DRUG AND GENE DELIVERY INTO CELLS USING CELL ELECTROPORATION

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Cell exposure to external electric fields can lead to increase of membrane permeability which is related to formation of transient hydrophilic pores. The phenomenon is known as cell electroporation. The number and size of the pores can be controlled by electrical parameters of electric field pulses. Under well controlled conditions electroporated cell can restore membrane barrier function and therefore electroporation can be compatible with high percentage of cell survival. Cell electroporation allows intracellular delivery of various hydrophilic non-permeant molecules. Our research has revealed that electroporation can be exploited for facilitated anticancer drug bleomycin delivery and enhance cellular cytotoxicity of bleomycin up to 700 times. These achievements have paved the way for preclinical tumor therapy studies, which revealed that electroporation reduces tumor growth and tumor size when combined with chemotherapy. This new approach is called antitumor electrochemotherapy. Currently antitumor electrochemotherapy is implemented in more than 100 oncology center in Europe.

In parallel with development of anticancer therapy, significant progress has been achieved in developing electroporation for intracellular delivery of nucleic acids. Attempts has been devoted both in characterizing mechanisms of gene electrotransfer and in optimizing the protocols in many preclinical trials. Recently this has led to initiating clinical trials of gene electrotransfer to treat metastatic melanomas. Further progress of the method in various clinical trials requires better understanding of mechanisms of gene electrotransfer. Our current study aims to elucidate these mechanisms for further development of the method for gene therapy.

BIOPHYSICAL MECHANISMS AND FACTORS RESPONSIBLE FOR PROTEIN-TANNIN INTERACTIONS

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Tannins belong to plant polyphenols with strong antioxidant, antitumor, anti-inflammatory, antibacterial activity. They can strongly interact with proteins, lipids, polysaccharides or alkaloids [1].

The tannins interaction and binding with proteins depend on many various factors and have different mechanisms. One of the most important is protein and tannins structure. Globular tertiary structure of proteins gives lower ability to bind tannins in comparison with much more flexible proteins with extended random coil structure. The interaction depends on amino acid composition too. Proteins rich in proline have greater affinity to tannins. Haslam [1] demonstrated that not only the number of hydroxyl groups but the bulk, flexibility and hydrophobicity of tannins play important role in protein tannin interactions .

In our studies we investigated the interaction between proteins and tannins isolated from Central Asia plants in order to clarify how the unique structure of these compounds affect these impacts .In our experiments we demonstrated that two tannins: bihexahydroxydiphenoyl-trigalloylglucose (BDTG) and 1-O-galloyl-4,6-hexahydroxydiphenoyl-β-d-glucose (OGβDG), isolated from *Geranium sanguineum* and *Oenothera gigas* leaves possess different affinity to human serum albumin (HSA). Smaller OGβDG stronger quenched albumin fluorescence in comparison with larger BDTG indicating that the chemical structure and size strongly influence tannin-protein interactions [2]. Both tannins quenched fluorescence via the “sphere of action” mechanism (when the quencher-fluorophore complex is immediately

quenched, without the formation of a ground-state complex [3]).

Our other research revealed that 3,6-bis-O-di-O-galloyl-1,2,4-tri-O-galloyl-β-D-glucose ($C_{55}H_{40}O_{34}$) from *Rhus typhina* has very strong affinity to alfa-synuclein (protein that plays a crucial role in etiology of Parkinson disease [4] and inhibited α-syn aggregation as was shown by circular dichroism method. Strong interaction between $C_{55}H_{40}O_{34}$ and alfa-synuclein is probably the result of linear protein structure allowing much better binding of tannin in comparison with globular structures.

Our latest investigations shown that tannins isolated from *Euphorbia E. Turcestanica* (1,2-di-O-galloyl-4,6-valoneoyl-β-D-glucose, 2-O-galloyl-4,6-valoneoyl-β-D-glucose and 3-O-galloyl-1,2-valoneoyl-β-D-glucose) possess valoneoyl groups that makes them stiffer in comparison with rather flexible gallotannins quenched HSA fluorescence via static mechanism.

Obtained results allow to conclude that not only bulk, flexibility and hydrophobicity of tannins but also position of hydroxyls groups determines the strength of tannins-proteins interactions.

REFERENCES

[1] Haslam E.(2007). Vegetable tannins – Lessons of a phytochemical lifetime *Phytochemistry* **68,** 2713–2721.
[2] Sekowski S., Ionov M., Kaszuba M., Mavlyanov M., Bryszewska M., Zamaraeva M.(2014). Biophysical studies of interaction between hydrolysable tannins isolated from Oenothera gigas and Geranium sanguineum with human serum albumin *Colloids and Surfaces B: Biointerfaces* **123**, 623–628.
[3] Soares S., Mateus N, De Freitas V.(2007). Interaction of Different Polyphenols with Bovine Serum Albumin (BSA) and Human Salivary α-Amylase (HSA) by Fluorescence Quenching *J. Agric. Food Chem.* **55**, 6726-6735
[4] Recchia A., Debetto P., Negro A., Guidolin D., Skaper S.D., Pietro Giusti P.(2004). α-Synuclein and Parkinson's disease. *FASEB* **18,** 617-626

SENSITIVE DETECTION OF FLAVONES ON PLASMONIC PLATFORMS

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Metal nanoparticles have numerous potential applications ranging from biological to chemical sensing. In particular, the use of the phenomenon of the Metal Enhanced Fluorescence (MEF), which offers a way to increase brightness and photostability at the same time was investigated [1]. The ability to increase intensity of fluorescence is strongly dependent on the size and shape of the nanoparticles. In this work we present the enhancement of the emission for new plasmonic platform for three different compounds which belong to group of hydroxyflavones. Hydroxyflavones (hydroxy-2-phenyl-4*H*-chromen-4-ones) have been one of the most important compounds for investigations and applications of ESIPT [2]. In this study plasmon resonance effect on the ESIPT systems is investigated for the first time.

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REFERENCES

[1] Sørensen T. J., Laursen B. W., Luchowski R., Shtoyko T., Akopova I., Gryczynski Z., and Gryczynski I. (2009) Enhanced Fluorescence Emission of Me-ADOTA by Self-Assembled Silver Nanoparticles on a Gold Film, *Chem. Phys. Lett.* **476**, 46–50.
[2] Bader A. N., Pivovarenko V. G., Demchenko A. P., Ariese F., Gooijer C. (2004) Excited State and Ground State Proton Transfer Rates of 3-Hydroxyflavone and Its Derivatives Studied by Shpol'skii Spectroscopy: The Influence of Redistribution of Electron Density. *J. Phys. Chem. B,* **108**, 10589-10595.

EFFECT OF DIBUTYL PHTHALATE ON THE FORMATION OF OXIDATIVE STRESS AND DNA DAMAGE IN BLOOD MONONUCLEAR CELLS

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Phthalates are derivatives of phthalic acid and its corresponding salts. Phthalates are used in plasticizers, where they are added to the products to give the material flexibility, transparency and durability. Thus, they have been determmed in many plastic products, cosmetics, perfumes and drugs.

The widespread occurrence of phthalates in the environment endanger human health. Phthalates including dibutyl phthalate (DBP) disrupt hormonal balance and function of the liver and kidneys. They also negatively effect on nervous and immune system, and cause adverse effects on reproduction. Phthalates have also been shown to cause allergies, asthma and tumor development. Moreover, toxic effects of phthalates may contribute to oxidative modification of lipids, proteins and DNA.

DBP has been classified as the substance highly dangerous to the environment and human. For this reason it was banned in toys and cosmetics intended for children.

Nevertheless, there is no appropriate limitation for this compound for use of the products in adults.

The purpose of this study was to determine the effect of DBP on peripheral blood mononuclear cells (PBMCs). PBMC is a good experimental model as this cell type is significantly exposed to xenobiotics and it plays a crucial role in the immune system.

In this work cell viability, ROS level (using fluorescent labels) and oxidative DNA damage (using comet assay) in human PBMCs incubated with DBP (5 - 1000μg/ml) for 12 h were assessed.

The study showed that DBP at 250 μg/ml caused a decrease in cell viability by 23%. It was also noticed that DBP from the concentration of 100 μg/ml and 50 μg/ml caused an increase in ROS level (by 15%) and lipid peroxidation (by 20%), respectively. Moreover, DBP was shown to oxidative DNA damage in the incubated cells. The emergence ROS can lead to oxidative DNA damage in human PBMCs

THE INFUENCE OF SILVER NANOPARTICLES ON PROPERTIES OF VITAMIN E LOCATED IN DPPC LIPOSOMES

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Metal nanoparticles (especially silver and gold) are considered as perspective material for antibacterial and anticancer purposes in medicine, cosmetology, food packaging etc. From literature it is known, that hydrophobic functionalized silver or gold nanoparticles can be introduced successfully into lipid bilayer and change its properties.

The DL-□-tocopherol is known as most important ingredient of vitamin E and recognized as antioxidant and anticancer agent. The antioxidant performance of tocopherol decreases as the result of UV illumination.

In this study we have examined systems consisting of DL-□-tocopherol embedded into bilayer membrane of liposomes with added decanethiol functionalized silver nanoparticles. The aim was to determine how the interactions with silver nanoparticles immobilized within membrane bilayer alter the tocopherol and membrane properties. Special emphasis was put on the relation between photodegradation of tocopherol and changes in temperature of membrane phase transition.

As a model of cell membrane we have used DPPC liposomes (100 nm in diameter). The incorporation of tocopherol and Ag nanoparticles has been performed in organic solutions of DPPC in chloroform and tocopherol and Ag in hexane. After preparation the mixtures of above compounds in organic solvents, the solvents were evaporated and formed film has been hydrated with phosphate buffer at pH 7,4 under mixing.

Systems consisting of DL-a-tocopherol and DL-a-tocopherol/Ag in DPPC liposomes have been illuminated with UV light (290 nm) and changes of spectral properties of tocopherol have been measured using absorption and fluorescence spectroscopy. Simultaneously, the temperature of crystal/fluid transition of membrane for investigated systems has also been estimated applying measurements of fluorescence anisotropy of DPH.

In conclusions, from the results of our experiments follows that presence of Ag nanoparticles in the liposome membrane does not significantly affect the photostability of tocopherol. Neither increase nor decrease in photostability of tocopherol has been detected as a result of interactions with nanoparticles. However, small shift in phase transition temperature of DPPC bilayer, in presence of tocopherol and Ag nanoparticles in the membrane have been recorded. In the case of Ag loaded membrane the decrease in phase transition temperature of DPPC compared to unmodified liposomes and with embedded tocohpherol has been observed.

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STEADY STATE AND TIME-RESOLVED FLUORESCENCE STUDY OF METHYL ESTER OF SINAPIC ACID IN WATER ENVIRONMENT

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Sinapic acid is widespread compound in plant foods. Recently, methyl ester of sinapic acid (MESA) has attracted attention due to their antioxidant activity. This phenolic ester can act either in water or nonpolar environment. In alkaline solutions proton from OH group connected to benzene ring, can dissociate. The aim of this work was to investigate physicochemical properties of MESA in buffer solutions ranged from 5.9 to 10.7. Acid-base equilibrium of this group has been established based on absorption spectra. In alkaline solution this compound was not stable; therefore spectrum of completely ionized OH group of MESA was calculated. pKa of this group was 8.6 ± 0.3. Fluorescence spectra of each form of MESA were measured using appropriate wavelength of excitation. We estimated quantum yield (QY) values at different pH using standard compound of quinine sulfate in 0.1N H_2SO_4 (QY_S=0.54). For not dissociated form of MESA, QY = 1.8 x 10^{-3} and for dissociated form QY = 6.2 x 10^{-4}. Fluorescence lifetimes were measured using spectrometer with TCSPC module and MCP PMT detector. Source of excitation were PLS diode (340 nm) and laser diode (375 nm). The observation was set at 460 nm and 500 nm wavelength, at the

maximum of fluorescence spectrum of each form of MESA. Decays were reconvoluted with the excitation pulse resulting in lifetimes of 10.3 ± 1.3 ps and 3.0 ± 1.6 ps for not dissociated and dissociated form of MESA respectively. Although quantum efficiencies are low and lifetimes are very short, MESA fluorescence can be easily detected and studied.

ENZYMATIC RIBOZYLATION OF TRI-CYCLIC NITROGEN BASES USING PURINE-NUCLEOSIDE PHOSPHORYLASE AS A CATALYST

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We have examined enzymatic ribosylation of tri-cyclic nitrogen bases, including 1,N^6-ethenoadenine and *lin*-benzohypoxanthine using various forms of purine-nucleoside phosphorylase (PNP) as catalysts and α-ribose-1-phosphate (r1P) as a second substrate.

We have found that 1,N^6-ethenoadenine (εAde) is rapidly ribosylated by the *E. coli* PNP (wild form) in phosphate-free media, with α-ribose-1-phosphate (r1P) as a second substrate (ribose donor). The main product of this reaction is the highly fluorescent etheno-adenosine (εAdo), although small amounts of the less intensely fluorescent N7-riboside can also be fund. The K_m for this reaction was found ~20 μM.

Mutation at the 204 residue (Asp→Asn) of the *E. coli* PNP is known to nearly abolish all the activity except the phosphorolysis of N7-methylguanosine [1]. Surprisingly, this mutation has only little influence on the ribosylation of εAde, and its reversal (phosphorolysis of εAdo). The mutated (Asn243Asp) calf enzyme is quite effective in the ribosylation of εAde, although the product of this reaction was certainly not the typical N9-riboside.

Another tri-cyclic base which was examined as a potential PNP substrate was *lin*-benzohypoxanthine, introduced by Leonard [2,3]. Although it was not a substrate for the calf enzyme, a fairly rapid reaction was observed when the wild-type *E. coli* enzyme was used.

Recently we have shown that both the *E. coli* and calf PNP, as well as their mutants, can be used as catalyst in the syntheses of many typical and non-typical ribosides, with possible analytical applications [4]. This work demonstrates that this is also possible for some tri-cyclic bases as well.

REFERENCES

[1] Mikleušević, G., Štefanić, Z., Narczyk, M., Wielgus-Kutrowska, B., Bzowska, A., Luić, M. (2011). Validation of the catalytic mechanism of Escherichia coli purine nucleoside phosphorylase by structural and kinetic studies, *Biochimie* **93**, 1610-1622.
[2] Leonard, N.J. (1984). Etheno-substituted nucleotides and coenzymes: fluorescence and biological activity. *CRC Critical Revs Biochem.*, **15**, 125-199.
[3] Leonard, N.J. (1985). Adenytates: bound and unbound, *Biopolymers*, **24**, 9-28.
[4] Stachelska-Wierzchowska, A., Wierzchowski, J., Bzowska, A., Wielgus-Kutrowska, B. (2016) *Molecules*, **21**, doi:10.3390/molecules21010044.

SELECTIVE RIBOSYLATION OF FLUORESCENT NUCLEOBASE ANALOGS USING PNP AS A CATALYST

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Enzymatic ribosylation of fluorescent 8-azaguanine and 2,6-diamino-8-azapurine, with purine-nucleoside phosphorylase (PNP) as a catalyst, leads to N9, N8, and N7-Ribosides [1].

We have observed modulation of the final proportion of the products, which was effect of point mutations in the enzyme active site. Wild-type of the calf PNP gives N7- and N8-ribosides, while the mutated form of PNP (the N243D mutant) led to the ribosyl substitution at N9- and N7-positions. The N243D mutant allows synthesis of the fluorescent N7-β-D-ribosyl-8-azaguanine.

The N7- and N8-ribosides of the 8-azapurines can be are analytically useful, for example N7-β-D-ribosyl -2,6-diamino-8-azapurine is a good fluorogenic substrate for mammalian forms of PNP and can be used as a specific, fluorogenic substrate for detection of PNP activity in human blood, while the N8-riboside is highly selective to the *E. coli* enzyme [2].

REFERENCES

[1] Stachelska-Wierzchowska A., Wierzchowski J., Wielgus-Kutrowska B. and Mikleušević G. (2013).Enzymatic synthesis of highly fluorescent 8-azapurine ribosides using purine-nucleoside phosphorylase reverse reaction: variable ribosylation sites. *Molecules* **18**, 12587-12598.
[2] Stachelska-Wierzchowska, A., Wierzchowski, J., Bzowska, A., Wielgus-Kutrowska, B. (2016). Site-Selective Ribosylation of Fluorescent Nucleobase Analogs Using Purine-Nucleoside Phosphorylase as a Catalyst: Effects of Point Mutations *Molecules*, 21, doi:10.3390/molecules21010044.

INTERACTION OF ACYLATED AND NON-ACYLATED ANTHOCYANINS WITH CELL-MIMIC MEMBRANES AND HUMAN ALBUMIN

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Anthocyanins exhibit numerous pharma- cological activities such as anticancer, ant-atherosclerotic, antioxidant, anti-inflammatory properties, etc. [1].

The aim of the work was to determine the effect of acylated and non-acylated anthocyanins on cell-mimic membranes that reflect the membrane lipid composition of tumor cells. In addition, the anthocyanin derivatives capacity to make bonds with the main blood transportation protein – the human serum albumin and its antioxidant activity was studied. Using fluorescent probes, the effect of anthocyanin derivatives on the properties of the hydrophilic and hydrophobic regions of mimetic membranes was determined. Antioxidant activity was tested with fluorimetric methods with free radicals induced by AAPH compound. Determination of binding of anthocyanins with albumin was carried out by following the quenching of albumin fluorescence and using the Stern-Volmer equation.

The results of the study have shown that acylated and non-acylated anthocyanins have high biological activity. It has been demonstrated that, all the compounds cause a decrease in the packing order of the hydrophilic region of the tumor-cell mimic membrane. Acylated anthocyanins also showed greatest affinity to the membrane and caused a small decrease of fluidity within its hydrophobic interior. It can be concluded that acetylation at carbon 3 of the glycosyl moiety can be considered the primary determinant for the significant lipid membrane interaction. The results also shows that studied anthocyanin derivatives can bind to human serum albumin and quench its fluorescence. The process of binding of all the compounds to albumin is a static quenching mechanism and the main forces are the van der Waals and hydrogen bonding forces. The determined parameters of the binding of anthocyanins to albumin are essential in the description and understanding of the pharmacokinetics of these substances in the human blood. The results of this research suggest a close relationship between antioxidant activity, that depends on the molecular structure of anthocyanin derivatives, and structural changes in the membranes tested, as well as at a possible mechanism of their anticancer activity.

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REFERENCE

[1] Clifford M.N. (2000). Anthocyanins – nature, occurrence and dietary burden. *J. Sci. Food Agric.*, **80,** 1063-1072.

THE ROLE OF MELATONIN AND RESVERATROL IN THE RADIATION INDUCED STRUCTURAL AND FUNCTIONAL CHANGES OF RABBIT GAPDH AND LDH

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The abundance of proteins in the cell makes them a primary target to redox modifications during oxidative stress. Most of such modifications have a deleterious effect to protein structure and function and so oxidatively modified proteins are present in several disorders including age-related diseases.

Protein especially sensitive to reactive oxygen species (ROS) modifications is glyceraldehyde 3-phosphate dehydrogenase (GAPDH). This glycolytic enzyme has plenty of unrelated functions including its role in apoptotic death of neurons during oxidative stress conditions. Moreover, oxidatively altered, aggregated GAPDH is frequently found as a component of amyloids in Parkinson's and Alzheimer's diseases. One of the strategies to prevent unwanted redox modifications is employing antioxidants scavenging ROS and aiding self-defense antioxidant properties of the cell. Resveratrol and melatonin are naturally occurring direct free radicals scavengers and compounds that activate several cell antioxidant enzymes.

Here, we discuss the effect of radiation induced oxidative stress on the structure and function of two glycolytic proteins: GAPDH and lactic dehydrogenase (LDH). Protein solutions were irradiated with X-rays in the atmosphere of air with the dose rate 21 Gy/min determined using Fricke method of dosimetry. Radiations doses applied to protein samples ranging from (10.5-105) Gy for GAPDH and (52.5-630) Gy for LDH.

Secondly, we verified functional changes of both enzymes upon its interactions with radiation generated ROS and in the presence of resveratrol and melatonin respectively. We observed higher inactivation rate of GAPDH compare to LDH and the general protective effect of both used antioxidants in studied conditions. To investigate structural changes of the irradiated proteins we applied high-performance liquid chromatography (HPLC). Study of the obtained chromatograms revealed that oxidative stress destroys quaternary structure of both proteins in the dose depended manner. Furthermore, we were able to investigate the degree of binding both resveratrol and melatonin to native and oxidatively modified GAPDH and LDH. Finally, we investigate hydrophobicity changes of GAPDH and LDH exposed to X-radiation in the presence and absence of melatonin and resveratrol using fluorescence probe bis-ANS.

HYBRID DENDRIMERS AND ANTI-APOPTOTIC siRNAs – COMPLEX FORMATION

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RNA interference is a selective cellular mechanism of gene silencing. In this case the degradation of the mRNA is caused by the small interfering RNA – siRNA. However, silencing of a gene requires the delivery of siRNA to cells in an active form. For this purpose, the effective, non-toxic and selective carrier that can protect the siRNA particle is needed. Under investigation as nucleic acid delivery vectors are e.g. cationic dendrimers [1].

The cationic carbosilane-viologen-phosphorus dendrimers generation 1st and 2nd were synthesized using novel "onion peel" approach. Those hybrid dendrimers have two kinds of cationic groups: those located at the branches due to viologen quaternized units and those related to the ammonium groups at the surface of carbosilane wedges [2].

The aim of the study was to check out whether 2 generations of carbosilane-viologen-phosphorus dendrimers can form the complexes with anticancer siRNAs: siBcl-xL, siBcl-2, siMcl-1 and scrambled sequence. The complexes were characterized using fluorescence quenching, zeta potential and circular dichroism methods.

The results have shown that formation of complexes occurs between all of anticancer siRNAs and carbosilane-viologen-phosphorus dendrimers both generations. However the complexes have been formed at different dendrimer/siRNA molar ratio. Furthermore, the obtained results reveled that in presence of heparin the siRNA is disassociated from dendrimers.

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REFERENCES

[1] Dzmitruk V., Szulc A., Shcharbin D., Janaszewska A., Shcharbina N., Lazniewska J., Novopashina D., Buyanova M., Ionov M., Klajnert-Maculewicz B., Gómez-Ramirez R., Mignani S., Majoral J. P., Muñoz-Fernández M. A., Bryszewska M. "Anticancer siRNA cocktails as a novel tool to treat cancer cells. Part (B). Efficiency of pharmacological action". Int J Pharm. 2015 May 15;485(1-2):288-94. doi: 10.1016/j.ijpharm.2015.03.034. Epub 2015 Mar 18.

[2] Moreno S., Szwed A., El Brahmi N., Milowska K., Kurowska J., Fuentes-Paniagua E., Pedziwiatr-Werbicka E., Gabryelak T., Katir N., de la Mata J., Muñoz-Fernández M. A., Gomez-Ramirez R., Caminade A. M., Majoral J. P., Bryszewska M. 'Synthesis, Characterization and Biological Properties of New Hybrid Carbosilane-Viologen-Phosphorus Dendrimers'. RSC Adv., 2015,5, 25942-25958, doi: 10.1039/C5RA00960J

PLASMA PROTEIN LEVEL AFTER PROTEASOME ACTIVATION DUE TO THERMAL INJURY.

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Severe injury, including thermal injury, causes catabolic response which is characterized by whole-body protein loss associated with changes in protein metabolism in multiple organs and tissues. Intracellular protein degradation is regulated by multiple proteolytic mechanisms, among which an important role plays proteasome-dependent proteolysis. Proteasome is a multicatalytic proteinase complex localized in cytosol and nucleus of all eukaryotic cells which is responsible for degradation of misfolded, denaturated, damaged, oxidized or improperly translated proteins. Controlled protein degradation by the proteasome is an important and efficient way to remove nonfunctional proteins arising in the course of the burn disease.

The aim of this study was to investigate changes in the proteasome activity and total protein level in the plasma of children with moderate and major burns during their hospitalization, and also to evaluate the proteasome role in the protein degradation.

The study was performed on 35 children managed at the Department of Pediatric Surgery with moderate and major burns (according to the pediatric injury severity score used by American Burns Association) and on 20 healthy volunteers as controls, sex and age-matched. Proteasome activity in the plasma of children after burn injury was assessed using Suc-Leu-Leu-Val-Tyr-AMC peptide substrate. Plasma protein level were determined by standard biochemical laboratory procedures. Proteasome activity and protein concentration in the plasma was measured 2-6 h, 12-16 h, three, five and seven days after injury.

Significantly higher proteasome activity in the plasma of burned children than in controls was detected between 2-6 h, 12-16 h, on the third and fifth day after the injury. The highest level was reached 12-16 h after trauma. There was also a downward trend in the total protein concentration between 2-6 and 12-16 h after burn and the upward trend between 12-16 h and the seventh day of hospitalization. The lowest value of the total protein level

in children plasma was noticed 12-16 h after burn injury, so in the same time when proteasome activity was the highest.

Thermal tissue injury initiates proteasome activation which are involved in damaged protein degradation. The most intense proteasome activation between 12-16 h is accompanied by the largest decrease in the plasma protein level. This indicates that proteasome play an important role in the protein degradation during inflammatory response to thermal injury.