PHYSICOCHEMICAL STUDIES OF INTERACTIONS BETWEEN MAIN COMPOUND OF *Oenothera gigas* TANNINS AND LIPOSOMES

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Tannins are ones of plant metabolites with wide biological activity including antioxidant, antitumor, anti-inflammatory and antibacterial properties. Despite many different experiments focused on their biological activities the physicochemical mechanisms responsible for interactions between tannins and biopolymers are still unclear. There are only a few papers concerned tannins-lipids interactions. At present studies the interaction between 1-O-galloyl-4,6-hexahydroxydiphenoyl-β-D-glucose (OGβDG; main compound of *Oenothera* plant tannins) and 3 types of liposomes (with different lipidograms) was analyzed. The main goals of experiments were to verify how different lipid compositions influence liposomes-OGβDG interactions and what is biophysical and physicochemical nature of this reactions. Liposomes (100 nm diameter) were prepared using extrusion methods and composed from lecithin, DMPC and DPPC phospholipids in three different weight ratios. The hydrolysable tannin OGβDG possessing 11 –OH groups, 3 aromatic rings and glucose ring was dissolved in water. Spectrofluorimetric measurements (using TMA-DPH and DPH dyes) were used to analyze the interaction of OGβDG with liposomes outer and inner monolayer. The measurement parameter was fluorescence anisotropy. Zeta-size and Zeta potential measurements were applied to estimate the changes in liposomes diameter and charge induced by OGβDG. Fourier Transform Infra-Red (FTIR) analysis was used to study what chemical groups are engaged in liposomes-OGβDG interactions. Anisotropy analysis showed that OGβDG enhanced rigidity of inner monolayer and decreased rigidity of outer monolayer in liposomes. This observation demonstrated that OGβDG enters the liposomes and penetrate into structures of fat globules. Zeta-size and Zeta potential analysis revealed increase of liposomes diameter and decrease of zeta potential.

Changes in FTIR spectra show that CH_2 alkanes groups (from phospholipids chains) and hydroxyl –OH groups (from OGβDG molecules) are responsible for liposome-OGβDG interactions. Obtained results demonstrate that OGβDG strongly interacts with lipids but should note that effects depend on the composition of used liposomes.

REGULATION OF TRANSCRIPTION: NEGATIVE AND POSITIVE FEEDBACK LOOPS COUPLED BY A COMMON PROMOTER

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We consider a hypothetical system of two genes, whose transcription is controlled by a common promoter. According to our assumption, transcription of the both genes takes place when the promoter is free of the repressor and bonds the inductor (~Rep^Ind). Protein precursors of the repressor and inductor are encoded in the two genes. Transient transformations of these two proteins lead to products which can function as transcription factors. We are modeling this system by a set of ordinary differential equations. The two equations, referring to mRNA synthesis, are nonlinear. Values of derivatives of the nonlinear terms in equilibrium, which are dependent on system's parameters, determine qualitative features of the evolution. Characteristic equation and conditions of saddle-node bifurcation have been obtained in general form. Hopf bifurcation and the possibility of oscillatory solutions have been derived in some special cases. The oscillatory solutions exist if the difference between Hill coefficients of repression and induction is sufficiently high. Highly cooperative repression promotes oscillations. In contrast, highly cooperative induction suppresses them. The oscillation are impossible, if the time of turnover in the loop of induction is too much shorter than that in the loop of repression.

MULTIFUNCTIONAL $NaYF_4$: Er^{3+}, Yb^{3+}, Gd^{3+} NANOPARTICLES UP-CONVERING INFRARED LIGHT TO VISIBLE AND ULTRAVIOLET RADIATION FOR USE IN CANCER IMAGING AND PHOTODYNAMIC ANTICANCER THERAPY

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Multifunctional NaYF4 nanoparticles doped with Er^{3+}, Yb^{3+}, Gd^{3+} ions with optical and magnetic properties were synthesized. These nanoparticles absorb infrared (IR) light and as a result of up-conversion, emit visible (VIS) and ultraviolet (UV) light. This allows for their application in biology and medicine as fluorescent markers (VIS) and as a potential agent for anticancer photodynamic therapy, through the production

of reactive oxygen species (ROS), under the influence of ultraviolet (UV) light.

Nanoparticles containing Gd^{3+} ions exhibit paramagnetic properties that allow cancer cells imaging using magnetic resonance imaging (MRI). Another field of the nanoparticles application is a selective killing of cancer cells in living organisms by hyperthermia. Superparamagnetic materials, placed in an external high-frequency magnetic field, heat up to the temperature at which protein denaturation occurs, thus eventual cancer cells death.

NaYF4: Er^{3+}, Yb^{3+} nanoparticles, after functionalization by polyvinylpyrrolidone (PVP), were introduced into the HeLa cancer cells. Location of the nanoparticles was determined in the cells as a function of incubation time, concentration of nanoparticles and presence of transfection agent Lipofectamine 2000.

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PHOTO-OXIDATION OF *cis*-PARINARIC ACID

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Non-oxidized conjugated fatty acids are found in minor concentrations in vegetable oils and improve their value due to their health-promoting impact on the human body. This paper analyzes whether oxidation with triplet oxygen was the only process occurring in the samples of *cis*-parinaric acid (CP) exposed to UV radiation at 250-300 nm. The study was carried out using samples of CP dissolved in n-hexane and containing oxygen and those de-oxygenized with argon. The efficacy of de-oxygenation was approximately 79%. The samples were radiated and the absorption and fluorescence spectra were simultaneously measured. The spectra of both de-oxygenized and non- de-oxygenized samples changed as a result of radiation: the specific CP acid bands disappeared. Although the decays were similar, they differed in the rate of changes. The rate constants for the processes were calculated based on both absorption spectra and on fluorescence excitation spectra. The calculations included the amount of absorbed photons. The calculations demonstrated that the decays were two-exponential (consistent with first-order reactions) and the rate constants for the argonized samples were smaller by approximately 50%. As the fading of spectra was two-exponential and de-oxygenation insufficiently inhibited this process, it was concluded that *cis*-parinaric acid also underwent photo-degradation, most probably under the influence of UV radiation.

THE EFFECT OF DIAMOND NANOPARTICLES AND THEIR FORM AFTER CHEMICAL MODIFICATION ON THE ANTIOXIDANT SYSTEM IN LUNG CANCER CELLS.

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Nanotechnology is one of the fastest growing scientific areas in the contemporary world. Particles received at the nanoscale have completely different optical, structural and electrical properties compared to materials in the macroscale, that is why they are used in biology, biotechnology, cosmetology and pharmacology. Nanodiamonds with sizes below 10 nm can easily uptake through cell membranes and may cause changes in the cell. One of the mechanisms of their action may be associated with the induction of oxidative stress which we observed by the increased production of free radicals. That's why cells have the antioxidant system with the protective function. The antioxidant enzymes in cells are catalase, superoxide dismutase and glutathione peroxidase.

In this study we used lung cancer cells line A549, which were incubated with diamond nanoparticles (D - nanodiamond and D+OH – nanodiamonds after the chemical modification with connected hydroxyl groups on the surface of nanodiamond) for 24, 48 and 72 hours, at concentrations 0 - 100 □g/ml. We observed changes in the antioxidant activity of the enzymes because of the dependence on the type and concentration of nanoparticles and time incubation.

LHCIIb IN LIPID BILAYER ENERGY MINIMIZATION AND MOLECULAR DOCKING OF ASCORBIC ACID

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The light harvesting complex II (LHCII) is the most abundant membrane protein present in the biosphere. The X-Ray crystallographic structure has been resolved for a number of species. However, so far no attempt has been made to describe LHCII in the natural environment within a lipid membrane. With the use of the program YASARA a MGDG/DGDG lipid bilayer was created using a modified version of the default macro. A LHCIIb trimer was placed in the lipid bilayer and the system energy was minimized. The resulting protein and crystallographic structure was compared. Molecular docking of ascorbic acid to the energy minimized LHCIIb structure from the lipid bilayer was performed using Molegro Virtual Docker v5.5. The docking results suggest three probable spaces of interaction between ascorbic acid and LHCIIb located near the monomer-monomer contact sit

TIME RESOLVED FLUORESCENCE SPECTROSCOPY OF ANTIBIOTIC AMPHOTERICIN B

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Amphotericin B (AmB) is a popular drug from the group of polyenes, applied in the treatment of mycosis. It is envisaged that as a metabolite of *Streptomyces nodosus* recognizes and destroys lipid membranes of the fungi. Unfortunately, its activity is also characterized by high toxicity, which identify. Because biological activity of AmB depends on the molecular organisation of AmB system, understanding of molecular mechanisms that govern the organisation of AmB is important, not only for the understanding of different biological effects, but also for minimizing the toxic side effects of the drug.

Knowledge of the dynamics of excited states, which is associated with molecular organisation of fluorophores, is a key factor in understanding aggregation processes.

Realization of this goal is based on the **Time Resolved Fluorescence Spectroscopy** which measures very precisely fluorescence life-time and as a result easily detects different molecular organisation forms of the drug. Fluorescence lifetime is a handy parameter in distinguishing various types of structures (monomer, dimer, aggregate). Fluorescence lifetime - associated spectra of amphotericin B emission was presented and discussed.

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STUDY OF RELS AND FRET IN Cyt c AND MITOCHONDRIA MODIFIED SPHERICAL AuNP

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The oxygen deprivation to the brain, heart, and peripheral tissues is one of the most important causes of serious illnesses (stroke, angina pectoris, heart attack, obesity, diabetes, cancer, autism, Alzheimer's disease), disability, and mortality. Injuries caused by oxygen deficit and reperfusion are deleterious. Hence, it is important to gain better understanding of the cellular events during hypoxia and uncover the mechanisms of cellular defenses against two opposite conditions: oxygen deficit and oxidative stress. During hypoxia, the mitochondrial matrix is swelling and cytochrome c (Cyt c) is released to cytosol, marking the beginning of cell apoptosis. In this work, we have investigated electrostatic interactions of mitochondria with gold nanoparticles (AuNP) using resonance elastic light scattering spectroscopy (RELS). The responses of the novel mitochondrial system to various drugs influencing the potassium ion-channel opening have been investigated. The interactions of hemoprotein Cyt c with nanoparticles have also been investigated using RELS and fluorescence spectroscopy (FL) techniques.

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CIRCUMNUTATION TRACKER – NEW SOFTWARE FOR ANALYSIS OF CIRCUMNUTATIONS

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Circumnutations are endogenous, helical movements of growing plant organs with an ultradian rhythm. Time-lapse video is the basic method for investigations of circumnutations. *Circumnutation Tracker* (*CT*) is novel software for analysis of time-lapse recordings in the Win environment. Standard parameters, i.e. the period, length, rate, shape, angle, and direction of circumnutations are determined automatically, which reduces duration of the analysis. The function and capabilities of *CT* were evaluated by analysis of circumnutations of *Arabidopsis thaliana* inflorescence stems. The inflorescence stem growing under constant light was found to exhibit a strong ca. 90-min. rhythm and several-hour long fluctuations of circumnutation length. Varied shapes of individual circumnutations with sequences arranged in a characteristic rosette-like pattern were recorded. Additionally, the circumnutation direction changed from clockwise into anti-clockwise and vice versa. The results obtained show usefulness of the new *CT* software for measurements of *Arabidopsis thaliana* circumnutations. We expect that *CT* will be a convenient tool for investigations of circumnutations in various plant species as well as growth, gravitropism, biological clock, and membrane transport, i.e. processes involved in the mechanism of circumnutation.

EGGSHELLS OF GREY HERON (*Ardea cinerea*) AS A TOOL FOR BIOINDICATION OF RIVER VALLEY

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One of the mechanisms for the elimination of substances toxic to the bird's embryo is the process of their deposition in the eggshell. The main factor justifying the usage of the *Ardea cinerea* eggshells for the bio-indication purpose is their diet which consists of different water organisms such as fish, amphibians, mammals and invertebrates. Additionally, high population of birds in a unit area, mobility, longevity, group preying and nesting makes this species a useful marker for environmental monitoring.

The survey was focused specifically on 5 Heron colonies located adjacent to the largest rivers in the Lublin area.

The main contaminants present in such areas are the remains of agricultural activity and the pollutants common to industry deposited into the rivers.

Concentrations of heavy metals in the Grey Heron eggshells were estimated by means of ICP-OES (i.e. inductively coupled plasma optical emission spectrometry) technique.

An important part of the conducted experiments was the determination of toxic element concentrations such as Chromium (Cr), Lead (Pb) and Cadmium (Cd). Their concentrations showed the following sequence: Cr > Pb > Cd. Our survey confirmed this pattern in 4 out of 5 Grey Heron colonies examined.

One of the examined elements having a negative influence on the bird's reproductive process is Strontium (Sr). *Aredea cinerea* eggshells contained 150.72 μg/g dry weight (dw) of Sr on average. The maximal level of Sr was found in the eggshell originating from the heronary localized in Chodlik near Opole Lubelskie (above 255 μg/g). The concentration(s) of the elements determined in eggshells were compared with the respective concentrations of elements from the sediments in the rivers closest to the birds' preying areas. Interestingly, it was found that Strontium (Sr) and Aluminium (Al) concentrations in eggshells are many times higher than the respective concentrations found in river sediments. The highest differences were observed among the Grey Heron colony in Wólka Michowska, where a 4-fold concentration increase of Sr and 48-fold higher concentration of Al were found in the Grey Heron eggshells.

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PLASMONIC-BASED INSTRUMENT RESPONSE FUNCTION FOR TIME-RESOLVED FLUORESCENCE

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We investigated plasmonic platforms to target ultrashort fluorescence and accurate instrumental response function in a time-domain spectroscopy and microscopy. The interaction of metallic nanoparticles with nearby fluorophores resulted in the increase of the dye fluorescence quantum yield, photostability and decrease of the lifetime parameter. The properties of platforms were applied to achieve a picosecond fluorescence lifetime (21 ps) of erythrosine B, used later

as a better choice for deconvolution of fluorescence decays measured with "color" sensitive photodetectors. The response functions were monitored on two photo-detectors; microchannel plate photomultiplier and single photon avalanche photodiode as a Rayleigh scattering and ultra-short fluorescence. We demonstrated that use of the plasmonic base fluorescence standard as an instrumental response function results in the absence of systematic error in lifetime measurements and analysis.

STUDY ON SPATIAL DISTRIBUTION OF POLYSACCHARIDES IN PLANT CELL WALL BY RAMAN MICROSCOPE

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The plant cell wall is kind of the cellular skeleton that controls cell shape and determines the relationship between turgor pressure and cell volume. The cell wall is composite of many different natural polymers, mainly cellulose, xyloglucan, pectins and also lignin for secondary cell wall which forms after cell growth. Proteins, lipids, enzymes, aromatic compounds and water are another components of this part of plant cell.

It is thought that percentage of components of plant cell wall has an important influence on mechanical properties of fruits and vegetables. Therefore research on content and spatial distribution of each component of these part of cells are extremely important in studies of quality of fruits and vegetables. So far many analytical and microscopic methods of investigation of plant cell wall was developed. Nevertheless, none of this methods gives data relating to accurate distribution and amount of individual substances in microscale. Raman microscopy can resolve this problem without necessity of staining section of plant tissues.

Briefly, Raman microscope is connection of microscope and Raman spectroscopy. It allows to collect spectra at each points of sample. In this way map of spatial distribution of sample's components can be obtained.

In this work we would like to discuss the methodology of measurement using Raman microscopy and present Raman images obtained for cell walls of several plant tissues. Examples of spatial distributions of main cell wall compounds will be depicted (CH-streching region ~ 2800 cm^{-1}) Due to chosing specific band location, we were able to localize and indentify pectins (856 cm^{-1} α-glycosidic bonds in pectin), hemicellulose (1735 cm^{-1}) or lignin (1600 cm^{-1} phenyl groups in lignin).

ACIVITY OF NEWLY SYNTHESIZED PHENOTHIAZINE DERIVATIVES AS ANTIPROLIFERATIVE AND MDR REVERSING AGENTS IN COLON CANCER CELLS

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Multidrug resistance (MDR) of cancer cells which is one of the form of resistance to chemotherapy, has been extensively studied for more than 30 years. For MDR phenotype are responsible plasma membrane proteins that belong to a large superfamily of the proteins called the ATP-binding cassette (ABC) transporters, particularly:P-glycoprotein (P-gp),multidrug-resistance associated protein 1 (MRP1) and breast cancer resistance protein (BCRP).Compounds that can reverse multidrug resistance may influence on MDR transporters by action at different molecular levels – the protein,mRNA or DNA level.In our studies we have tested the ability of the newly synthesized phenotiazine derivatives to inhibit the growth of human adenocarcinoma cancer cells as well as a possibility of these compounds to reduce drug resistance of LoVo/Dx cells.It occurred that phenothiazines act as antiproliferative agents and cell growth inhibition was observed both in drug sensitive (LoVo) and doxorubicin-resistant (LoVo/Dx) cell line.Our results indicated that these derivatives are able to reverse the resistance of cancer cells against doxorubicin and may be regarded as promising agents improving doxorubicin efficacy in drug-resistant cancer cells.Doxorubicin can be used as fluorescence marker of drug accumulation inside the cells and its modification for eg. By MDR transporters' inhibition.The fluorescence signal derived from the drug accumulated inside LoVo/Dx cells increased in the presence of the phenotiazines.The effect of studied compounds on the expression of P-gp, MRP1 and BCRP has been also checked and determined by RT-PCR and immunohistochemical methods. These experiments revealed that one of the studied compounds increased the expression of BCRP in LoVo/Dx cells.Applying the QSAR methods allowed us to describe electronic, structural and topological parameters and hydrophobicity of tested compounds and to correlate these properties with ability of phenothiazines to influence on MDR phenotype.

POTENTIAL USE OF HALLOYSITE IN PHYTOREMEDIATION OF SOILS CONTAMINATED WITH HEAVY METALS

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The present study aimed to investigate the effect of the addition of halloysite to soils contaminated with heavy metals on the growth rate of common flax (*Linum usitatissimum* L.) and cock's foot (*Dactylis glomerata* L.*)* in pot culture and on the bioaccumulation of elements in these plants.

The peat-muck soil with the structure O-Mt-n-Mt-Ot-D formed on light loamy sand from the former Białogon Pond bowl contaminated with heavy metals (Pb 910,4 mg/kg d.w., Cu 121,3 mg/kg d.w., Zn 1140,5 mg/kg d.w.) was used for experiments. The influence of halloysite concentration on growth of test plants and the content of heavy metals (Pb, Cu, Zn) in biomass of plants, growing on contaminated soils enriched in different doses of halloysite (from 10% to 50%) were recorded. The content of heavy metals in soils and biomass of test plants was determined using X-ray fluorescence (XRF) method.

The highest growth rate and biomass growth were observed in plants cultivated on soil supplemented with 25 % halloysite while the slowest values were recorded for 50% halloisyte. The greatest heavy metal bioaccumulation factors were seen for lead at 50% halloysite (WB_{Pb}= 0.35), for copper at 25% halloysite (WB_{Cu}=2.2) and for zinc also at 25% halloysite (WB_{Zn}= 0.65). The addition of halloysite to the contaminated soil induced changes in many of its physico-chemical properties, e.g. decreased the contents of Pb to 80%, Cu to 30% and Zn to 20%. A positive correlation was found between biomass and Cu content in cock's foot and between heavy metal contents in plant biomass and soil pH or calcium carbonate content.

THE INFLUENCE OF SELECTED PRENYLATED CHALCONES AND FLAVONOIDS ON THE ACTIVITY OF Kv1.3 CHANNELS IN HUMAN JURKAT T CELLS

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It is known that small-molecule organic inhibitors of voltage-gated potassium channels Kv1.3 channels may potentially find a clinical application to support a chemotherapy of some cancer disorders characterized by an overexpression of Kv1.3 channels, such as breast, colon and lymph node cancer, melanoma or chronic lymphocytic leukemia. Promissing candidates may be some plant polyphenolic compounds that combine a high efficiency with a good bioavailability and a low cytotoxicity. Studies performed previously in our laboratory showed that a plant-derived prenylated flavonoid - 8-prenylnaringenin, in contrast to its precursor, naringenin, was an effective inhibitor of Kv1.3 channels both in normal human T lymphocytes and in human cancer T lymphocyte cell line - Jurkat [1]. Studies on the influence of prenylated chalcones and flavonoids on the activity of Kv1.3 channels in cancer cells were then extended on other compounds from both groups: xanthohumol, isoxanthohumol and isobavachalcone. The influence of these compounds on the activity of Kv1.3 channels in human Jurkat T lymphocytes was studied applying the whole-cell patch-clamp technique. Obtained results provide evidence that all selected compounds are inhibitors of Kv1.3 channels in Jurkat T lymphocytes. The inhibitory effect occurred, in case of all compounds, in a concentration-dependent manner. The value of a half-blocking concentration (EC_{50}) was about 3 μM for xanthohumol, 5 μM for isobavachalcone and 7.8 μM for isoxanthohumol, respectively. The inhibitory effect was reversible for all the compounds tested. These results may confirm our earlier hypothesis that the presence of a prenyl group in the molecule is a factor that facilitates the inhibition of Kv1.3 channels by flavonoids and chalcones [1].

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MULTI-ION SENSOR SYSTEM FOR REAL-TIME ION TRANSPORT MONITORING

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Cystic fibrosis (CF) is the most common human genetic disorder caused by disturbed ionic transport in cells [1,2]. The direct cause of anomalous ion transport in CF is the mutation of CFTR gene encoding chloride-conducting channel. This leads to increased thickness of mucus layer in bronchial epithelium what is advantageous for development of chronic lung infections resulting in patient death. There is a lot of contradictory hypotheses of ion transport mechanism in CF what is the result of complex interactions between numerous transporting proteins found in bronchial epithelium [3]. Usually ion transport studies

are carried out in Ussing chamber system. However, this method is limited because of lack of selectivity toward specific ion. The current signal obtained from Ussing chamber measurements results from total ion fluxes in the cell layer and it is not possible to distinguish particular ion contribution in the observed current change. Hence, conclusions drawn from Ussing chamber experiments are incomplete. Reliable ion transport mechanism may be obtained only in the system where particular ion fluxes are monitored simultaneously. Potentiometric methods based on ion-selective electrodes (ISE) meet this expectations and have become indispensable tools for the determination of ionic constituents of human body. Beside selectivity toward specific ion, simple construction and possibility of miniaturization were the main reasons for ISE to be paid much attention and gain popularity in biological and medical applications.

In this work novel ISE-based system for the simultaneous determination of K^+, Cl^-, Na^+ ions and pH in cell monolayer was described. The designed system allows for real-time monitoring of ion transport within cell layer. The constructed miniaturized electrodes were integrated with reference electrode in one system allowing direct observation of changes in the concentration of ions in surface layer of the cells. The ISE-based system for conducting continuous flow measurements was successfully applied for in vitro studying of ion fluxes in human bronchial cells monolayer.

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PROTECTIVE ROLE OF THE ELECTRIC FIELD AGAINST ACCESS TO BIOLOGICAL MEMBRANE POTENTIALLY TOXIC CATIONS

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The aim of this study is to search of an effective method of protection of biological membrane against potentially toxic cation, including organometal cations. As demonstrated by our earlier studies some amphiphilic cations from the group of quaternary ammonium salt used in properly small concentrations, in which practically do not disturb cell membrane, significantly inhibit erythrocyte hemolysis induced by organic cations of lead. This effect can be explained by changing the polarity of the membrane (increase in positive surface charge after incorporation of amphiphilic cations) and, consequently, difficult access to the membrane of organometallic ions. In this case, an electrostatic field can serve a protective function against toxic effects of organometallic cations. In this study we investigated the effect of the electric polarization of the monomolecular lecithin membrane on access to it potentially toxic cations present under the monolayer (in the water subphase). As a protective factor, positively polarizing the monolayer, dihexadecylammonium bromide ($C_{34}H_{72}N^+Br^-$) was used. Surface pressure changes were measured after addition of the test compound to the subphase (due to the relatively small changes in surface pressure caused by organometallic compounds of lead, here we used a cationic surfactant, hexadecylpyridynium chloride, $C_{21}H_{38}ClN$). It was found that the change in surface pressure monomolecular lecithin membranes, under the influence of the test compound present in the subphase, decreases with membrane electrical polarization. This demonstrates the effective inhibition (under the influence of the electric field generated by the protective cations, $C_{34}H_{72}N^+$) of access to the membrane of cations present in the subphase.

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EFFECT OF GLYCATION ON THE THERMODYNAMICS OF DENATURATION OF COLLAGEN TYPE I

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Glycation of collagen - the primary structural component of connective tissue , and accumulation of advanced glycation products (AGEs) is one of the reasons for dysfunctions affecting diabetics and elderly people. The experiment tested the hypothesis that glycation can affect the thermodynamic stability of collagen fibers.

Chemically pure fibers of the main organic component of bone tissue and tendons , collagen type I , were used. Collagen was incubated in aqueous solutions ribose or glucose in different concentrations for a period of 4-14 days. The formation of AGEs was evaluated spectrofluorimetrically at 420-440nm (370 nm excitation) and at 390-400 nm (335 nm excitation). The latter signal is specific for pentosidine –a marker for AGEs in the aging processes. A high level of pentosidine fluorescence, dependent on the incubation period and sugar concentrations, was stated. The level of fluorescence related to other AGEs was lower, however correlated with pentosidine.

Thermodynamic parameters of denaturation and melting of collagen fibers were measured using differential scanning calorimetry (DSC). The parameters depend on the configuration of molecules, their hydration and cross-linking within and between fibers. Both hydrated and dry fibers were investigated.

Glycation in ribose, even for small concentrations of sugar and a short period of incubation, resulted in a higher temperature of denaturation, both in fully hydrated, and dry samples.

Incubation in glucose, resulted in an increase of denaturation temperature only after two weeks of glycation, and only in the dry samples , where the molecules of collagen, without being surrounded by water, are more strongly influenced by cross-links between fibers. However, after the first week of glycation changes in enthalpy of denaturation and cooperativity of the thermal processes were observed.

The results obtained confirm that the measurements of thermodynamic parameters of collagen can be used to assess the changes in connective tissue in diabetes and advanced age.

THERMODYNAMIC CHARACTERISTICS OF BONE COLLAGEN DENATURATION

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The aim of the study was to examine thermodynamic characteristics of collagen in bone tissue and demineralized bone matrix and an assessment of the impact of glycation induced bonds on thermodynamic stability of bone collagen. Parameters of denaturation and decomposition of collagen were estimated on the basis of differential scanning calorimetry (DSC) performed for temperatures from 40 °C up to 220^{o}C. Unmodified cortical bone samples , samples of fully demineralized bone matrix and bone samples modified by glycation in vitro were tested. Changes of heat capacity, enthalpy, entropy and Gibb's energy during denaturation of bone collagen were calculated from the thermograms.

It was shown that, compared to other proteins, collagen in bone tissue is thermally very stable, both in natural, and in the demineralized bone matrix. Is was stated that denaturation of collagen occurs gradually in a few separate steps which are followed by meting of collagen fibers into smaller structural units. That gradual denaturation results from existence of different populations of collagen with different level of cross-links inside and between fibers. It was alsofound that the complex process of bone collagen denaturation is influenced by additional bonds induced by glycation, which can contribute to the nonphysiological changes in bone tissue and an increased fracture risk among diabetics.

PLASMONIC FLUORESCENCE ENHANCEMENT IN PERIDININ-CHLOROPHYLL-PROTEIN-SILVER NANOWIRE HYBRID NANOSTRUCTURE

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Collective elecface plasmon resonance, can affect absorption and emission of light by placed nearby fluorophores.

In our experiment we investigate the influence of plasmon excitation in silver nanowires on fluorescence of the photosynthetic complex peridinin-chlorophyll-protein (PCP) using a wide-field fluorescence microscope equipped with EMCCD detector.

First we took white-light transmission images to localize positions of silver nanowires on the surface. Then we recorded map of the PCP fluorescence off this area. We observed that in the vicinity of the silver nanowires emission of the PCP complexes is strongly enhanced. The enhancement is higher at the ends of the silver nanowires. The enhancement can be observed for both 405 nm and 480 nm excitation wavelength, and is present for samples with different concentrations and arrangement of silver nanowires versus the PCP complexes. In particular, for structures were the nanowires are mixed with the PCP complexes prior the deposition on the surface the enhancement factor values are generally higher than for a sample where the PCP complexes are deposited on previously prepared silver nanowire layer. Analysis of kinetics of fluorescence yields a surprising result that the presence of the silver nanowires has no influence upon the photostability of the PCP fluorescence.

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QUININE INFLUENCE ON THE DYNAMIC PROPERTIES OF LIPOSOME MEMBRANES MODIFIED BY N-METHYLATED PEPTIDOMIMETICS – EPR STUDY

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The susceptibility to proteolytic degradation hindering the use of peptides as drugs. Thus, extensive studies on modifications of naturally occuring peptides are carried out to overcome this difficulty. The most common modifications are incorporation of non-coded amino acids into peptide backbone and replacement of a hydrogen atom with a methyl group on the nitrogen atom [1]. The main aim of research concentrates on the improvement of the therapeutic index of biologically active substances too. One of the most promising intelligent drug carriers tend to be liposomes [2].

In the current studies we used quinine in the presensce of three peptidemimetics [two peptides with Phe residue: Ac-Phe-NHMe (1) and Ac-Phe-NMe_2 (2) and one with ΔPhe amino acid Ac-DPhe-NMe_2 (3)] to explore structural and dynamic changes for model EYL (**E**gg **Y**olk **L**ecithin) bilayer. The effect of such dopants on the plasticity and fluidity of bilayer was studied by electron spin resonance (ESR) technique enhanced by typical spin probe – TEMPO.

Based on the analysis of the EPR signal of EYL liposomes we observed a significant change of bilayer fluidity:

- Quinine in connection with studied peptides modify spectroscopic parameter F of TEMPO spin probe.
- Petide 1 in the presence of quinine liquefies liposome membrane in proportion to the concentration of quinine in the range of 0 to 7%. However, above the 7% we observed a rapid growth of membrane fluidity, which suggest a phase transition in the membrane bilayer.
- Peptide 2 with quinine cause liquidate the model bilayer in the range of 0 to 8%. Over 8% we found the similar trend as in the case of peptide 1.
- In the case of peptide 3 we didn't observe quinine effects on fluidity of liposome membrane.

These findings are important and they show a synergistic effect of both quinine and the peptides 1 and 2 as potential means of controlling liquidity of lipid bilayer. The results may have application significance in design of drugs.

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MOLECULAR ORGANIZATION OF POLYENE ANTIFUNGAL ANTIBIOTIC DRUG Amphotericin B IN STEROL CONTAINING MODEL LIPID MEMBRANE

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Amphotericin B (AmB) is a polyene antifungal antibiotic, widely used for the treatment of systemic mycoses. The risk of fungal infections is particularly high in patients with immune system weakened by long term treatment with immunosuppressive drugs or *Acquired Immuno-Deficiency Syndrome* (AIDS). According to the studies performed since 1970s, the pharmacological effect of the drug but also the toxic side effects are determined by the molecular organisation of the antibiotic in lipid membrane. As follows from the hitherto proposed and investigated models, AmB selectively forms specific structures that aggregate with ergoterol, which brings about the pharmacological effect of the drug. However, similar mechanisms of interaction with cholesterol seem to responsible for the side effects of the drug. Comprehensive understanding of molecular mechanisms responsible for organisation of the drug in model systems of biological importance is of great significance for possible design and development of a drug showing much reduced toxicity.

Amphotericin B was characterised in model lipid membranes either with or without sterols (ergosterol or cholesterol) by UV-vis linear dichroism spectra and FTIR. Results obtained by UV – Vis linear dichroism spectra revealed that AmB molecules were incorporated into the ergosterol-containing lipid membrane at a lower angle than into a cholesterol-containing membrane or a membrane without sterols. This finding has considerably increased the probability of formation of a trans-membrane channel in the form of AmB tetramer. FTIR linear dichroism analysis for the drug built into the membrane permitted to check the influence of the antibiotic and sterols on the lipid membrane at the specific sites, in the hydrophobic layer and in the region of polar heads. The outcome of the study has brought a contribution to understanding the origins of the toxic side effects of the drug.

APPLICATION OF MEMBRANE SYSTEM FOR INVESTIGATING SORPTION PROPERTIES OF HALLOYSITE

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To study the sorption properties of minerals the single-membrane system as a component of the interferometric set-up was used.The interferomeric method allows a comprehensive examination of the substance diffusion process. This method also permits the visualization of concentration diffusion layers creating process.

The absorption capacity of halloysite was investigated with reference to glucose, which is often found in industrial waste water and the glucose excess can disturb the environmental eco-balance. The sorption capacity of halloysite was thus determined indirectly, based on the comparison of concentration profiles as well as time characteristics of glucose quantities released from the control solution and from the solution incubated with a halloysite adsorbent. Glucose diffusion analysis from these solutions was carried out in a two-chamber system with the horizontally situated membrane. Concentration profiles for various times were obtained for the control glucose solution of initial concentration 0.05 M, as well as for solution exposed to halloysite. The time characteristics of glucose quantities released from the control solution and from the solution incubated with a halloysite adsorbent were also obtained. On the basis of concentration profiles the evolution of concentration field was defined, and adsorption efficiency (34%) as well as the amount of glucose adsorbed at equilibrium state (6.12 mg/g) were determined.

The obtained results confirm the halloysite good sorption properties with respect to the investigated substance and the usability of the method for this kind of investigations. The present study indicates the possibility of optimizing the measurement system so that it is possible to visualize and study the kinetics of the adsorbed substance release directly from the mineral.

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MICELLIZATION STUDY OF ENVIRONMENTAL FRIENDLY DICEPHALIC AMINE DIBROMIDE IN COMPARISON WITH GLUCONAMIDE-TYPE CATIONIC SURFACTANTS

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In recent years, the basic and applied research interest in cationic surfactants has increased, partly because of urge for cationic amphiphiles to be useful in medical applications. The most popular trend here is the pursuit to obtain environmental friendly compounds with desired physicochemical properties. We have recently studied the aggregation processes of various cationic surfactants. The worth of our special interest are sugar-based compounds (w-(alkyldimethyl-ammonium) alkylaldonamide bromides) showing unique properties such as mild production conditions, lower toxicity and higher biodegradability. As a continuation of our research we were examined the physicochemical behaviour in the water solutions of N,N- bis[3,3-(trimethylammonio) propyl]alkylamide dibromides $C_n(TAPABr)_2$ with different chain lengths (n= 12, 14, 16). The critical micelle concentration (cmc) were otained using the Isothermal Titration Calorimetry (ITC) as the main investigation technique. The thermodynamic parameters – the enthalpies, the entropies of micellization as well as the contributions of headgroups to the Gibbs free energies $\Delta G°(hy)$ were calculated. The aggregation processes of $C_n(TAPABr)_2$ were also studied by means of conductance method to calculate the degree of micelle ionization b. The obtained results were compared with those previously reported and literature data for compounds with monomeric head structure as well as sugar-based surfactants with analogical chain lengths. Sugar-based surfactants and dicephalic amine bromide studied have been considered to be promising candidates as gene- and drug-delivery vehicles for biomedical applications, as was find in other study. In the view of these practical applications the comparison of chemical-physical data among different groups of cationic surfactants can lead to better insight into the structure-property relationships.

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mRNA CAP ANALOGS – WHAT IS IN THEM FOR A BIOPHYSICIST?

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In terms of structure, mRNA cap analogs are similar to a cellular cap, consisting of 7-methylguanosine attached to the first transcribed nucleoside via a triphosphate bridge (m^7GpppN), that flanks the 5' end of eukaryotic mRNAs. However, their modifications impose various physicochemical properties. Thus, cap analogs may serve as tools specialized for the structural and functional examination of cap-binding properties. One example of such proteins is decapping scavenger (DcpS) enzyme catalyzing the hydrolysis of a cap in the 3'→5' mRNA decay, avoiding inhibition of other cap-binding proteins. Notably, DcpS is specific to very short cap-containing oligonucleotides, with the highest activity towards dinucleotides.

We applied a set of 50 intrinsically fluorescent mono- and dinucleotide cap analogs to characterize *C.elegans* DcpS. Using cap analogs modified within nucleosides, which are hydrolyzed by DcpS, the Michaelis constants and maximal velocities were determined. Selecting unhydrolysable cap analogs modified in the phosphate chain the association constants and Gibbs free energies of binding were calculated. The representative cap analogs were further used for computational docking studies, revealing enzyme residues involved in the cap-binding. This approach enabled us to find that *C. elegans* DcpS catalysis relies on the recognition of a positively charged 7-methylguanosine and on interactions with the ribose 2'O and 3'O hydroxyls of 7-methylguanosine. Diphosphate chain of a cap is sufficient for an efficient binding to DcpS, whereas triphosphate or longer one is required for hydrolysis. The second nucleoside is not absolutely necessary for hydrolysis, but plays a role in the stabilization of a cap in the cap-binding pocket. Our studies extend the knowledge about DcpS enzyme and serve as an example of potential applications of cap analogs in the biophysical studies of proteins playing a role in the cap-dependent mRNA metabolism.

APPLICATION OF MEMBRANE SYSTEM FOR INVESTIGATING SORPTION PROPERTIES OF HALLOYSITE

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Living organisms have developed many manners of ion transport, in which peptides are involved (channels, pumps, transporters). Change of osmotic pressure, caused by active ion transport across the cell membrane, causes the passive water transport through water channels existing in lipid bilayer called aquaporin's. Transport of one chemical molecule through the membrane is accompanied by transport of about 450 molecules of water. Transport across the lung epithelium is especially interesting since the defect in anion channel CFTR is responsible for the most common fatal human genetic disorder – cystic fibrosis. Through epithelial cells in lungs, ions like Na^+, K^+, Cl^-, $HCO3^-$ are transported, and exist the system of pH stabilization. Measurements of this parameters as fast as it is possible can seem to be the key to understand the mechanism of cystic fibrosis. That is why we have developed our integrated electrode system.

MATERIALS AND METHODS

Electrodes

Silver wires were mounted in poly(methyl metacrylate) capillary with ion selective membrane and filled by suitable inner solution.

Integrated electrode system for biological measurements

Build of two different poly(methyl metacrylate)-based modules containing five places for ion selective electrodes each and solution inlet/outlet. Modules are placed vis-à-vis at a distance of approximately 100 μm. The insert with monolayer of epithelial cells grown on porous support is placed between them.

Cell line

Immortal cell line of Human Bronchial Epithelium - 16HBE14-σ.

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