## MOLECULAR DYNAMIC OF DONUT-LIKE FORM OF HUMAN CYSTATIN C IN SOLUTION

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Cystatin C (HCC) is small (MW=13343 Da, 120 amino acid residues), nonglycosylated protein with amyloidogenic properties [1-3]. The structure is stabilized by four cysteine residues forming two disulfide bridges. Due to the presence of HCC in most of human body fluids and tissues, cystatin C is excellent marker for various diseases e.g. rheumatic disorders and osteoporosis or as flag of kidney transplant rejection and function. This protein is also considered to be a guard of central nervous system. For the proper performance of its functions, cystatin C should occur in the form of monomers. Due to low structural stability of wild type monomers, cystatin C exhibits a tendency to aggregation. This tendency to form oligomers as well as the Leu68Gln pathological mutation causes the formation of HCC fibrils in cerebral blood vessels (hemorrhagic amyloidosis).

The aim of this work was a structural characterization of HCC dodecamers and a attempt to create model of this system through molecular dynamic. The molecular dynamic simulations were performed using AMBER program package and several structural models of HCC dodecamers were created.

Obtained structural models of HCC dodecamers were also compared with microscopic data. Obtained micro-images revealed that HCC dodecamers have donut-like morphology.

ACKNOWLEDGMENTS

The present study was supported by HARMONIA3 grant (Project No. DEC-2012/06/M/ST4/00036) from the National Science Centre, Poland.

REFERENCES

[1] Janowski R., Kozak M., Jankowska E., Grzonka Z., Grubb A., Abrahamson M., Jaskolski M. (2001). *Nature Structural Biology* **8**, 316-320.
[2] Orlikowska M., Jankowska E., Kołodziejczyk R., Jaskolski M., Szymańska A. (2011). *Journal of Structural Biology* **173**, 406–413.
[3] Grubb A. (2000). *Adv Clin Chem*. **35**, 63–99.

## SMALL ANGLE X-RAY SCATTERING (SAXS) STUDIES OF HUMAN CYSTATIN C IN SOLUTION

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Human cystatin C (HCC) is an inhibitor of papain-like and legumain-like cysteine proteases. This protein is present in many body fluids like urine, cerebrospinal fluid and in tissues such as cerebral cortex. HCC was observed as coprecipitate of pathological amyloid fibrils in the brains of patients with Alzheimer's disease. For correct functioning HCC should occur in the monomeric form. In the crystal, native HCC forms dimers via the domain swapping mechanism [1,2].

The study presented was aimed at developing low-resolution structure of monomeric, dimeric and trimeric forms (stabilized by disulfide bonds) of human cystatin C in solution and comparison with the HCC crystal structures.

The small angle X-ray scattering (SAXS) data were obtained using BioSAXS system and synchrotron radiation (beam line BLi911-4 [3], MAXII storage ring of the MAX-Lab Lund, Sweden; l=0.091 nm). Low-resolution structures of the HCC oligomers (covalently stabilized monomers, dimers and trimers) in solution were restored using program DAMMIN [4]. This study clearly indicated that the preferred conformation of monomeric form of HCC in solution, is almost identical with the crystal structure. HCC dimer structure is compatible with structure with elongated conformation as in tetragonal crystal form, and low-resolution structure of HCC trimer shows the structure with 3-fold axis of symmetry.

ACKNOWLEDGMENTS

This study was supported by HARMONIA3 grant (Project No. DEC-2012/06/M/ST4/00036) from the National Science Centre, Poland.

REFERENCES

[1] Janowski R., Kozak M., Jankowska E., Grzonka Z., Grubb A.,. Abrahamson M, Jaskolski M. (2001). *Nature Structural Biology* **8,** 316-320.
[2] Grubb A. (2000). *Adv Clin Chem*. **35**, 63–99.
[3] Mammen C.B., Ursby T., Cerenius Y., Thunnissen M., Als-Nielsen J., Larsen S., Liljas A. (2002). *Acta Physica Polonica* A **101**, 595.
[4] Svergun D. I. (1999). *Biophys J*. **76** (6), 2879-2886.

## CHANGES IN SECONDARY STRUCTURE OF WHEAT GLUTEN AFTER USING Ag NANOPARTICLES

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Crops storage processed in inadequate conditions causes heavy losses in crops quality. Post-harvest losses are mainly due to bacteria and fungi infections. Silver in form of nanoparticles is well-known from its antimicrobial properties. For this reason, silver nanoparticles (AgNPs) were used as a protective layer on the grain surface against bacterial and fungal infections (antimicrobial agent). Silver nanoparticles stabilized by trisodium citrate were used. Trisodium citrate is commonly applied as a food additive in the food industry but is not regarded as antimicrobial agent. Fourier transform infrared (FT-IR) spectroscopy was used to examine conformational changes in secondary structure of wheat gluten washed out from grain treated by aqueous solution of silver nanoparticles (AgNPs) stabilized by trisodium citrate. Analysis of the amide I band revealed significant changes in secondary structure after using both kinds of AgNPs. It was observed a slight increase in β-sheet content (from 36.2% to 39.2%) at the expense of α-helix and beta-turns content. The percentage distribution of beta-turns decreases from 13.1% for control sample to 11.7% for AgNPs-treated sample. To find factors causing these changes, the wheat grain were treated by aqueous solution of trisodium citrate and water. Obtained results indicated that the changes in gluten structure were connected mainly with the trisodium citrate action due to presence of small amount of free molecules of the stabilizer in AgNPs solution. Additionally, the conformational changes in gluten pointed out that gluten flexibility increased (decrease in α-helix/beta-sheet ratio from 1.40 for control sample to 1.26 for AgNPs-treated samples) as well as solubility of gluten decreased.

## SPECTROSCOPIC CHARACTERISTIC OF ESTER-TYPE DERIVATIVES OF α-TOCOPHEROL IN HOMOGENOUS ENVIRONMENTS

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Alpha-tocopherol (α-Toc) is one of the most potent natural antioxidant known in nature, which can protect the membranes from damages induced by lipid peroxidation. However, alpha-tocopherol is very unstable under the influences of light and oxygen. Therefore, less susceptible to oxidation than α-Toc many ester-type derivatives of this vitamin were developed. The synthetic esterified forms of α-Toc are frequently added to many foodstuffs, pharmaceuticals and cosmetics.

In this study, spectroscopic properties (absorption and fluorescence) of α-Toc and two ester-type derivatives of α-tocopherol: a novel ester, di-α-tocopheryl maleate (TM), and commercially available α-Toc derivative: α-Tocopheryl succinate (TS), in some organic solvents were measured.

In organic solvents with different physical properties, the absorption maxima for TS and TM were located at similar positions (285-286nm), with extinction coefficients ranging from $2350M^{-1}cm^{-1}$ for TS in methanol to $5150M^{-1}cm^{-1}$ for TM in hexane. The investigated ester-type derivatives exhibit a blue shift of 7-10nm compared to α-Toc. The fluorescence maximum of TS and TM in investigated solvents is found at the wavelengths range 304 to 308nm, which is blue shifted at about 18nm compared to α-Toc. The positions of maxima of investigated derivatives are held within a wide fluorophores concentration range. Increasing the concentration of these esters results in the linear fluorescence increase only in initial range. At higher esters concentrations (about 80μM) the increase becomes non-linear with further fluorescence quenching. Such behavior may indicate the formation of esters dimers or aggregates.

The measured absorption and emission spectra of α-Toc esters show that esterification of α-Toc modifies its spectroscopic and physico-chemical properties compared to parent tocopherol compound. Observed electronic energy increase of the esters is very probably due to electron rearrangement in the chromanol ring due to attached moiety.

## MULTI-METHOD APPROACH TO STRUCTURE AND FUNCTION OF THE RNA 5' cap-BINDING PROTEINS RESPONSIBLE FOR REGULATION OF EUKARYOTIC GENE EXPRESSION: eIF4E AND PARN

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A biophysical bases of molecular mechanisms underlying the recognition of the mRNA 5' terminal structure called "cap" by proteins is crucial both for understanding of the complex process of regulation of eukaryotic gene expression at the levels of translation and mRNA surveillance, as well as for putative drug design.

Recognition of the 5' cap by the eukaryotic initiation factor 4E (eIF4E) is the rate limiting step of protein biosynthesis, while poly(A)-specific ribonuclease (PARN) is a 5' cap-dependent enzyme that plays a key role in 3' deadenylation, is involved in nonsense-mediated mRNA decay, and also in regulation of cytoplasmic polyadenylation.

The goal of the studies was to find structural requirements for the affinity of the cap-binding proteins to the cap [1], thermodynamic driving forces [2,3] and kinetic characteristics of the intermolecular recognition, as well as to gain an insight into the structure and structural dynamics [4], that are biologically relevant.

We have established a precise method of the protein-ligand binding constants determination [5] and found that eIF4E exploits conformational changes to provide tight binding of the cap and the synergy of interactions with eIF4G/4E-BP1 [1-4], while PARN is the only one among 3' exoribonucleases which interacts with the 5' mRNA terminal structure [6] to provide the processivity of deadenylation. PARN is thus the minimal protein context to bind two mRNA termini concurrently [7]. eIF4E and PARN share similar structural cap-binding motif (Trp-$m^7$G) but they have different thermodynamic and kinetic binding properties that correlate with the biological functions of these proteins. We have also visualized single PARN molecules by Atomic Force Microscopy in liquid [8] that provided mesoscopic structural description complementary to the protein fragments known from cryslallography.

ACKNOWLEDGEMENTS

This work was partially performed in the laboratories founded by NanoFun POIG.02.02.00-00-025/09

REFERENCES

[1] Niedzwiecka A., Marcotrigiano J., Stepinski J., Jankowska-Anyszka M., Wyslouch-Cieszynska A., Dadlez M., Gingras A.C., Mak P., Darzynkiewicz E., Sonenberg N., Burley K., Stolarski R. (2002). *J Mol Biol*. **319,** 615-635.

[2] Niedzwiecka A., Stepinski J., Darzynkiewicz E., Stolarski R. (2002). *Biochemistry* **41**, 12140–12148.

[3] Niedzwiecka A., Darzynkiewicz E., Stolarski R. (2004). *Biochemistry* **43**, 13305–13317.

[4] Rutkowska-Wlodarczyk I., Stępiński J., Dadlez M., Darzynkiewicz E., Stolarski R., Niedźwiecka A. (2008). *Biochemistry* **47**, 2710-2720.

[5] Niedzwiecka A., Stepiński J., Antosiewicz J., Darzynkiewicz E., Stolarski R. (2007). *Methods Enzymol* **430**, 209–245.

[6] Nilsson P., Henriksson N., Niedzwiecka A., Balatsos N. A. A., Kokkoris K., Eriksson J., Virtanen A. (2007). *J Biol Chem.* **282**, 32902–32911.

[7] Wu M., Nilsson P., Henriksson N., Niedzwiecka A., Kiat Lim M., Cheng Z., Kokkoris K., Virtanen A., Song H. (2009). *Structure* **17,** 276–286.

[8] Niedzwiecka A., Lekka M., Nilsson P. et al. (2011). *Biophys Chem*. **158,** 141−149.

## EVALUATION OF THE PHYSICAL PROPERTIES OF THE ANIMAL BONES AFFECTED BY LEAD

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Lead exposure is an important public health problem. There are many sources of lead, including: ceramic glazes, electronic waste, cosmetics, toys, water pipes, solder in canned food and lead from soils. However, lead contaminated dust and lead-based paints are the main sources of lead poisoning. Clinical studies have shown that lead is devastating to the human body. This chemical element enters the human body from the environment by inhalation and through the digestive system and one is accumulated in the kidneys, liver, brain, lungs and muscles. More than 90% of the Pb in adult human body and 70% in child body is stored in the bones. Lead is released very slowly, from calcified tissue. According to Rabinowitz et al. the elimination half-life of Pb in cortical bone is approximately 10 to 30 years. The aim of researches were evaluation of the changes in the bone tissue in rats intoxicated with lead acetate. To determine the possibility of bones quality reduction by Pb two studies were conducted: biomechanical strength assay and FTIR spectroscopy measurement. Femur strength was measured in three-point bending test, whereas infrared spectroscopy was used to measure molecular structural changes, specifically, to study ratio of area of two types of vibrational transitions, determining to mineral to matrix ratio. The results of the biomechanical study show that femurs of rats treated by Pb-acetate appear to be weaker than bones of the control group and may produced condition for development of higher risk of fractures. FTIR spectra of the processed rat femoral head samples show significantly differences in the mineral to matrix ratio between the control and lead-treated bones.

The lower bone mineral content and the weaker mechanical properties of bones from Pb-treated rats are associated with the pathologic state dependent of the exposure of lead.

## CHANGES IN PHYSICAL PROPERTIES OF THE BISPHOSPHONATE-ENRICHED BONE CEMENT

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Bisphosphonates (BPs) are a class of drugs that has very efficient antiresorptive properties. The use of bisphosphonate include the prevention and treatment of ostheoporosis and similar diseases. Benefits that accrue from use of bisphosphonates are better mineral density, bone microarchitecture, strength and quality of bones.

PMMA (methyl polymethacrylate) bone cement is widely used material to anchor artificial joints. The bone cement fills the free space between the prothesis and the bone, that means that this material should be highly biocompatible and biotolerant.

The goal of the presented study was to assess whether the enriching bone cement with bisphosphonate has changed its physical properties. Investigation of pure bone cement and bisphosphonate-enriched bone cement sample's biomechanical parameters included compressive test and three point flexural test. During the three point flexural test we recorded the load and stress to the point of maximal load which caused fracture and deflection and strain of the sample to this point. From obtained characteristics the transverse Young's modulus, energy which was absorbed by the sample before the fracture occurred and stiffness of the samples were determined. Longitudinal Young's modulus and stiffness was determined from compressive test. Besides the biomechanical parameters the density and the Fourier-transform infrared spectra of the samples were recorded.

The studies have shown that enrichment of bisphosphonates cause yielding of the bone cement material which results in increase of elastic region. It also did not change the density of the samples. Any significant differences between FTIR spectra of pure bone cement and bisphosphonates-enriched bone cement were recorded, which means that BP-enrichment did not cause any visible changes in the chemical composition of bone cement.

## STUDY ON FINITE ELEMENT ANALYSIS OF PLANT TISSUE MICROMECHANICS

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The goal of this research was to create a computational model that incorporates micro-scale geometrical features of plant tissue, and which will provide qualitative and quantitative predictions of mechanical properties. The proposed technique of simulation of micromechanical cellular systems was demonstrated on case study of onion (*Allium cepa* L.) upper epidermis. Onion epidermis was chosen due to its simple single-layer structure, the lack of intercellular spaces and ease of sample preparation. The geometry of the FEM model was created on basis of images obtained using a confocal scanning laser microscope CSLM (OLYMPUS FluoView300, Olympus Corporation, Tokyo, Japan). The geometrical features of onion tissue were reconstructed in FEM environment by means of vectorization procedure. Then, uniaxial tensile test were carried out to determine the mechanical parameters of tissue samples. Mechanical testing was carried out up to 50% of strain with a deformation speed of 1.5 mm/min. During mechanical test the tensile force and elongation of the sample were recorded. Both values were converted into stress and strain respectively.

The uniaxial tensile tests were simulated in FEM environment using created virtual models of onion epidermis. The qualitative validation was based on the comparison of the force-strain curves from laboratory tensile test and the simulations. On the basis of calculated mechanical parameters we were able to provide a qualitative and quantitative validation of FEM models. The values of cell wall mechanical properties from the experiments were compared with those from FEM models that gave the best fit of the force deformation curves.

The developed model showed good qualitative and quantitative agreement with experimental results. The curves obtained through simulation preserved all the key characteristics of the real object. The FEM model was able to predict mechanical properties of cell wall with average estimation error of 15%.

## INTERACTION BETWEEN POLYPHENOL COMPOUNDS OF BILBERRY FRUIT EXTRACTS AND MODEL LIPID MEMBRANES

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Phenolic compounds contained in plant extracts are nowadays extensively studied, because they exhibit many beneficial effects on living organisms, mostly due to their antioxidant properties. The subject of the study was extracts from the fruit of low, high and black (wild) bilberry of genus *Vaccinium.* The UPLC–ESI/MS and HPLC-DAD analysis showed that these fruits are rich in many nutrients, including polyphenols. The most predominant phenolic group was anthocyanin derivatives that constituted ca. 25 % in high

blueberries, 28 % in low blueberries, and 34 % in wild blueberries of fruit extract.

The aim of the study was to determine changes incurred by polyphenol compounds from blueberry fruit in model lipid membranes. In particular, the effect of the extracts on the packing order in the hydrophilic lipid phase and fluidity of the hydrophobic phase was studied. Model membranes were formed of DPPC, egg phosphatidylcholine, and lipids extracted from erythrocyte membranes. The interaction of the extracts with the lipids was examined with differential scanning calorimetry (DSC), infrared spectroscopy (ATR IR), and fluorometry, using the Laurdan, Prodan, and DPH probes. All the experimental results indicate that the biggest changes occurred in the hydrophilic part of the lipid bilayer. The polyphenol compounds had practically no influence on fluidity in the hydrophobic region of the membranes. No changes in temperature of the main phase transition of DPPC were observed and only a small change in pretransition temperature for high concentration of the compounds. The results obtained with the ATR IR method did not reveal any changes in the alkyl chain region of the bilayer; however a small shift of bands was observed for the phosphate and choline groups, the broadest shift being for wild bilberry.

ACKNOWLEDGEMENTS

This work was sponsored by the Ministry of Science and Education, scientific project no. N N312 422340

## INFLUENCE OF PLASMONIC EXCITATION ON THE ENERGY TRANSFER IN PERIDININ-CHLOROPHYLL-PROTEIN COUPLED TO SILVER NANOWIRES

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The motivation of this work was to investigate the influence of plasmon excitations in silver nanowires upon the dynamics of the energy transfer in photosynthetic complex peridinin-chlorophyll protein that was reconstituted with both, Chl *a* and Chl *b* (Chl *a*/*b*-N-PCP). In order to control the strength of this interaction, we separated the silver nanowires from the PCP complexes with silica spacers with thickness of 5 nm and 40 nm.

The PCP complex from *Amphidinium carterae* is unique in a sense, that it features bidirectional energy transfer, that is from Chl *a* to Chl *b* and from Chl *b* to Chl *a*.

The samples were prepared by spin-coating a solution of Chl *a*/*b*-N-PCP on substrates with both 5-nm-thick and 40-nm-thick silica spacers covering the silver nanowires. The fluorescence properties of such hybrid nanostructures were examined using wide-field fluorescence microscopy for both Chl *a* and Chl *b* emissions out of the same locations across the sample. z In this was we obtain spatially resolved maps of fluorescence intensity ratio of both Chl emissions.

In the case of the sample with the thinner silica spacer we observe strong interaction between the pigments comprising the PCP complex and plasmon excitations in the silver nanowires. The intensity ratio between Chl *a* and Chl *b* emissions is equal to 2,7, while it amounts to 4 away from the nanowires. In contrast, in the case of the thicker silica spacer, the intensity ratio is identical across the whole sample, regardless of whether it is monitored on or off a nanowire. This result indicates that the plasmon excitation in silver nanowires significantly influences the energy transfer between chlorophylls in the PCP photosynthetic complex.

ACKNOWLEDGEMENTS

Financial support provided by the Welcome/2008/2 project awarded by the Foundation of Polish Science.

## INCORPORATION OF LHCII INTO CHLOROPLAST LIPID MONOLAYERS

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LHCII is a light-harvesting pigment-protein complex of photosystem II , responsible for the absorption of light energy and regulation of its transfer in the photosynthetic apparatus. The efficiency and mecha-nism of these processes depend on the specific of the molecular complex in the thylakoid membrane . The monolayer formed from chloroplast lipids ( MGDG and DGDG) with built-in LHCII complex reflects well the organization of LHCII in the thylakoid membrane.

Langmuir-Blodgett technique enables the formation of collated lipid monolayers with controlled thickness and accurate composition.

LHCII complexes were incorporated into the lipid monolayer using two methods:

1) a chloroplast lipid mixture was applied to the subphase surface, and was then compressed to the required pressure surface. LHCII complex suspension was injected under this stable monolayer lipid . The increase of the molecular surface with kept constant pressure was inter-preted as incorporation of protein-pigment structures into the lipid membrane.

2) monolayers were prepared out of a protein-lipid mixture and then applied to the subphase surface. The

mixture was compressed to the surface pressure as mentioned earlier.

Afterward, the-prepared samples were trans-ferred to a solid substrate while monitoring the size of the transfer. The resulting structures were visualized with Atomic Force Microscopy (AFM) and fluorescence-lifetime imaging spectroscopy - (FLIM)**.** A lipid membrane containing LHCII complexes built-in with subphase showed the presence of monomeric, trimeric and aggregated forms of LHCII. A monolayer received from applying a prepared protein-lipid mixture on the subphase surface showed the presence of aggregated configurations in form of multilayer units.

ACKNOWLEDGEMENTS

The author is a scholarship holder of the project carried out in the Team of the Foundation for Polish Science, co-funded by the European Union under the European Regional Develop-ment Fund.

## THE INFULUENCE OF HUMIC AND FULVIC ACIDS ON THE CONCENTRATION OF FREE RADICALS IN AQUEOUS ENVIROMENT: ESR TECHNIQUE

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The aqueous solutions containing humic substances (fulvic and humic acids extracted from peat) and free radicals (using probes 2,2,6,6 - tetramethylpiperidine - 1 - OXYLANE - TEMPO, by the ESR method was studied. The concentration of the probe in relation to the water molecules was 0.1 ppm , the concentration of humic substances: humic or fulvic acids was in a weight ratio of water to 0.1 %. In order to precise stir, each of the samples before measurement were shaken 60 seconds and then placed in the measuring chamber ESR spectrometer. ESR spectrometer operating parameters were: sweep time t = 128 s, sweep range Δ H = 7mT, amplitude modulation dH = 0.08mT. The total duration of the measurement was 60 hours. Based on the spectra of the probe TEMPO, the changing of the concentration of the free radicals in the solution was determined (according to the time). Our research shows that the probe is strongly " poisoned " by humic substances , both the larger (humic acids) and less (fulvic acids) molecular weight.

This provides that both fractions of humic substances are characteristic of free radical sweepers. In the initial phase of the experiment, the more activity were humic acids, which showed intensively impact on the signal recording by the spectrometer.

However, after a longer period - 40 hours of the experiment, stronger effect of the fulvic acids was observed. The observed antioxidant properties of humic substances, especially in the context of the production of paramedical preparations derived from peat and used in herbal medicine. Humic substances could be a large natural reservoir of free radicals and could be important in controlling the decomposition processes of organic matter in the soil.

## DISORDERS OF ERYTHROCYTE'S ANTIOXIDANT SYSTEM IN PEOPLE WITH CORONARY ARTERY DISEASE TREATED WITH STATINS

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Oxidative stress is one of the most important factors in the development of cardiovascular disease. Increased level of free radicals is also observed in coronary artery disease (CAD). The reactive oxygen species cause pro- and antioxidant imbalance. That imbalance can cause disorders of natural antioxidant system.

The aim of our study was to evaluate the effects of oxidative stress associated with CAD on the selected parameters in erythrocytes and the effect of statin therapy to improve of disorders parameters. The study included 34 patients with CAD after myocardial infraction within 6 months at the age of 62.8 ± 6.1 years. Qualified patients were divided into two groups. The first group has taken atorvastatin in dose 40 mg/day and the second group has taken rosuvastatin in dose 40 mg/day. Control was healthy individuals in appropriate age. In the erythrocyte were determined antioxidant enzymes activity (catalase, glutathione peroxidase and superoxide dismutase), lipid peroxidation and concentration of thiol groups.

The results show disorders of antioxidant system in patients with CAD whose are manifested in the reduction of antioxidant enzyme activity (11% catalase and 22% superoxide dismutase) compared to the control group and 16% higher level of lipid peroxidation. Monthly treatments statins resulted in statistically significant increase in catalase activity: 13% atorvastatin and 14% rosuvastatin and superoxide dismutase: 19% atorvastatin and 16% rosuvastatin. Both treatments have also influence on reduction of lipid peroxidation level: 18% atorvastatin and 20% rosuvastatin.

In conclusion, statin treatment has a positive effect on the balance of pro-and antioxidant properties of erythrocytes in patients with CAD.

## PLASMA LIPID PEROXIDATION IN PEOPLE WITH CORONARY HEART DISEASE (CAD) AND STATIN TREATMENT

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Coronary artery disease (CAD) is associated not only with increased cholesterol levels but also oxidative stress. In the treatment of lipid disorders as well as in primary and secondary prevention of CAD the most important role plays statins.

In our study, we tried to determined changes in plasma antioxidant capacity in patients with CAD before and after treatment with statins. The study involved 30 patients with coronary artery disease after myocardial infarction within 6 months, at the age of 63.5 ± 5.9 years Qualified patients did not have high blood pressure, no burning and alcohol abusers. Patients were divided into two groups in which one group have taken, for a period of one month , atorvastatin in dose 40 mg/day and second rosuvastatin in dose 40 mg/day. Red blood cells from healthy individuals were a control group. In the plasma were determined: lipid peroxidation and total antioxidant capacity depends on the fast and the slow antioxidants.

The results show that patients with CAD have any statistically significant changes in plasma total antioxidant capacity but show 25% increase in plasma lipid peroxidation level. Monthly treatment atorvastatin resulted in reduction of lipid peroxidation level by 20%, while treatment rosuvastatin by 23% compared to the results before treatment. Both the treatments did not cause statistically significant changes in total antioxidant capacity of plasma.

These data suggest that CAD is accompanied by high lipid peroxidation but does not change the total antioxidant capacity of plasma.

## EFFECT OF TREATMENT OF HIPOLIPEMIC DRUGS ON THE STRUCTURE OF ERYTHROCYTES IN PATIENTS WITH CORONARY ARTERY DISEASE (CAD)

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Statins, as inhibitors of 3-hydroxy-3-methyl-coenzyme A (HMG-CoA) reductase are used as hipolipemic drugs. But not always they are able to decrease of the desired lipid parameters, especially in monotherapy. Combining statins with hipolipemic drugs with different mechanism of action, which are representative by ezetimibe, allows by lower dose of statins achieving a similar reduction in cholesterol.

Our study was aimed to investigate the effect of combination therapy to improve the structural parameters of erythrocytes, that disorder observed in CAD. The material was erythrocytes isolated from the blood of patients with CAD after myocardial infarction within 6 months. The study group included 20 patients, at age 64.2 ± 5.9 years. Patients qualified for the study were divided into two groups: the first has taken 40 mg/day atorvastatin and the second 10 mg/day atorvastatin + 10 mg/day ezetimibe. Control was healthy individuals in appropriate age. The structure parameters determined erythrocytes: lipid peroxidation, concentration of thiol groups in membrane proteins, total cholesterol, and erythrocyte membrane fluidity.

Our results show disorders in the structure of erythrocytes in people with CAD. In these patients, we observe an increased level of lipid peroxidation (18%), total cholesterol (19%) and a decrease in erythrocyte membrane fluidity (in the subsurface layers of 14%, in the deeper layers of 7%). Monthly treatment with atorvastatin resulted in reduction of lipid peroxidation (12%), while the monthly atorwasatyną + eztymibem therapy resulted in reduction of lipid peroxidation (19%) and increase membrane fluidity in subsurface layers (9%).

The results suggest that combination therapy of statin with hipolipemic drugs with different mechanism of action, allows achieving similar results as the use of statin in monotherapy in larger doses.

## INTERACTIONS OF XANTHOPHYLL PIGMENTS WITH PROTEINS

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Carotenoid pigments are important constituents of human eyes and they are responsible for vision. Deficiency of macular xanthophyll's, lutein and zeaxanthin, has been correlated with macular retinopathy referred to as AMD (Age Related Macular Degeneration). It seems very likely that xanthophyll difference in eyes is associated while impeared transport across the blood-macula barrier. in the current work we study interactions of carotenoid pigments (lutein, zeaxanthin and β-carotene) with proteins which can play role of carotenoid transporters in the blood: BSA and GST. The results of electronic absorptions and FTIR studies show strong interactions of carotenoids with protein studied.

ACKNOWLEDGEMENTS

The author is a scholar of the project carried out within the TEAM project from the Foundation for Polish Science, co-funded by the European Union under the European Regional Development Fund.

## SCOTS PINE NEEDLE SURFACE WETTABILITY PARAMETERS AS INDICATORS OF AIR POLLUTION IMPACTS

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Wettability represents a fundamental property of any solid material, it reveals information on the chemical structure and surface architecture of the surface-modified biological substrata strongly correlated to the environmental pollution stress. An investigation of water contact angles (CA), contact angle hysteresis (CAH) was carried out for 1-year to 4-year old needles (Pinus sylvestris) collected in urban (Gdansk) and rural (Karsin) locations using an original measuring technique based on the geometry of the drop on a vertical filament. Concentrations of air pollutants ($SO_2$, $NO_x$, $O_3$, F and SPM-suspended particular matter), currently considered to be most important in causing direct damage to vegetation, were simultaneously monitored. A set of the surface wettability parameters: the apparent surface free energy, adhesive film pressure, work of adhesion, and spreading were determined from CAH data using the approach developed by Chibowski (2003) to quantify the surface energetics of the needle substrata. Since CAH depends on the outermost wax layer surface roughness and spatial physicochemical heterogeneity of a solid surface, CA data were corrected according to the Wenzel and Cassie equations, respectively using surface architecture profiles. It was found that the roughness parameter r is significantly negatively correlated (R=-0.74) with the needle age (collected at Karsin). The needle surface becomes smoother with an increase in sample ages in the village area whereas such a relation does not appear (R=-0.24), for samples collected in industrialized regions (Gdansk). The wettability parameters were closely correlated to pollutant concentrations as evidenced from Spearman's rank correlation procedure ($R = 0.63\text{-}0.91$; $p < 0.05$). The aim of the study was to validate the established CA methodology to create a new non-invasive, low cost technique suitable for monitoring of structural changes at interfaces of biological systems.

## EFFECT OF RESVERATROL ON NEUROBLASTOMA (Neuro-2a) AND HIPPOCAMPAL CELLS (mHippoE-18) UNDER OXIDATIVE STRESS CONDITIONS

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Resveratrol (RSV) is a polyphenol, produced naturally by some plants in response to several harmful factors such as attack by pathogens, UV radiation, or increased oxidative stress. Resveratrol has been shown to exert anticancer, neuroprotective and cardiovascular effects.

The main aim of this research was to investigate the effect of resveratrol on neuroblastoma (Neuro-2a) and hippocampal cells (mHippoE-18) under conditions of oxidative stress.

Cells were treated with hydrogen peroxide and incubated for 24 hours with or without the presence of resveratrol. Different concentrations of resveratrol (2.5 μM to 40 μM) were added to cell culture 3, 6 or 12 hours prior to $H_2O_2$ treatment. The cytotoxicity of the studied compounds was checked with MTT assay. To estimate percent of apoptotic cells, the Annexin V Detection Kit was used.

The concentration of the hydrogen peroxide that caused about 50% reduction in cell viability was 20 μM for Neuro-2a and 30 μM for mHippoE-18 cells. Comparing Neuro-2a viability, it decreases in cells treated with both – resveratrol and hydrogen peroxide (especially with higher concentrations of RSV) than in cells treated by hydrogen peroxide only. Higher doses of resveratrol combined with hydrogen peroxide reduces cells viability substantially.

Our data show that resveratrol does not demonstrate any statistically significant effects on cell viability of hippocampal cells. We also observed that resveratrol in low concentrations (7.5 μM preincubated for 6 hours) reveal antioxidant effect.

In conclusion, resveratrol in combination with hydrogen peroxide increases apoptosis and cellular cytotoxicity of tumor cells (Neuro-2a). It does not influence on normal, hippocampal cells (mHippoE-18).

## THE BEHAVIOR OF THE POLAR HEAD GROUP OF NEW 2-(ALKYLDIMETHYL-AMMONIO)ETHYLGLUCONAMIDE BROMIDES IN WATER SOLUTION

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Sugar-based surfactants are known for their improved surface and performance properties, and reduced environmental impact due to easy biodegradation. They may be also considered as main molecular factors in the gene delivery. The newly synthesized 2-(alkyldimethylammonio) ethylgluconamide bromides($C_n$GAB) with different chain lenghs ($n$ = 10, 12, 14, 16) were proved to be able to compact plasmid DNA with great efficiency. This happens due to a number of intermolecular interactions in which the polar head group plays an significant role. Thus, the exploration of the behavior of the polar head group of $C_n$GABs in water solution is very important. It is also interesting for defining structure-activity relationships.

The enthalpies of dilution of $C_n$GABs were measured by means of Isothermal Titration Calorimetry at 298 K and 313 K. The calorimetric curves were normalized to infinite dilutions, which approximately correspond to relative partial molar enthalpies of dilution. Analyzing and comparing the trends with those of other quaternary ammonium bromides with long chains, we draw the conclusion that the polar head group of $C_n$GABs in comparison with trimethylammonium group decreases the hydrophobicity of the surfactant to the same extent as shortening its alkyl chain by one methylene group. This may be due to the presence of amide group. The heat capacity of micellization for $C_n$GABs and alkyltrimethylammonium bromides was also estimated and the number of "dry" hydrogens in micelles was calculated. This suggests that in micelles of $C_n$GABs are hydrated for one methylene group deeper than alkyltrimethylammonium bromides. This may be also attributed to amide group, especially to its ability to form hydrogen bonds with water.

These findings are supported by the results of molecular modeling studies performed for the two types of surfactants in solution.

ACKNOWLEDGEMENTS

This work was supported by Polish Ministry of Science and Higher Education with the grant N N305 361739.

## TRANSPORT OF 3-BROMOPYRUVATE ACROSS THE HUMAN ERYTHROCYTE MEMBRANE

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3-Bromopyruvic acid (3-BP) is a promising anticancer compound as it is a strong inhibitor of glycolytic enzymes, especially hexokinase II and glyceraldehydes 3-phosphate dehydrogenase. Since malignant cell are much more dependent on glycolysis than normal cells due to the Warburg effect, they are more sensitive to this compound. However, potential complication of anticancer therapy with 3-BP are the side effects of this compound due, i.a., to interaction of 3-BP with normal cells, especially erythrocytes.

The aim of our study was the kinetic characterization of 3-BP transport into human erythrocytes. Erythrocytes (hematocrit of 5%) in phosphate-buffered saline (PBS) were added with various concentrations of 3-BP containing 10 μM 14C (carboxyl)- 3-BPA (15 mCi/mmol, Perkin-Elmer). After 1-min incubation at room temperature, the suspensions were centrifuged through a layer of dibutyl phthalate and washed 2 times with ice-cold PBS. Radioactivity of the red cell sediment was measured after sample treatment with 0.5 M NaOH/10% H2O2 in a dioxane-based scintillation cocktail. The Km and Vm values for 3-BP transport were 0.89 mM and 0.94 mmol/min*l cells, respectively. The transport was inhibited competitively by pyruvate and significantly inhibited by SITS and 1-cyano-4-hydroxycinnamic acid.

ACKNOWLEDGEMENTS

We are indebted to Profs. Andre Goffeau and Stanisław Ułaszewski for the generous gift of 14C-3-BP and inspiration to join the research of 3-BP.