97. Accumulation and cellular distribution of doxorubicin – transferrin conjugate in peripheral blood lymphocytes

M. Szwed, M. Jędrzejczyk, Z. Jóźwiak

University of Łódź

Doxorubicin (DOX) is one of the most potent anti tumor drugs with a broad spectrum of use. Unfortunately, numerous side effects such as severe cardiotoxicity and bone marrow suppression limit its use. To reduce this obstacle and improve DOX pharmacokinetics, we conjugated DOX to the transferrin, a popular human plasma protein. We observed the overexpression of transferrin receptors in cancer cells because they have huge demand for iron ions. Due to lack of transferrin receptors on the surface of peripheral blood lymphocytes we compared the interaction of doxorubicin – transferrin conjugate and free drug cells with normal cells.

The *in vitro* growth-inhibition test, XTT assay, indicated that DOX was significantly more cytotoxic to normal cells then DOX-TRF. The estimation of intracellular DOX-TRF level in human lymphocytes has confirmed a greater accumulation of DOX than of the doxorubicin-transferrin conjugate. Moreover, the microscopic observation of lymphocytes during drug treatment indicated a different localization of DOX and DOX-TRF conjugate inside the cells. We conclude that peripheral blood lymphocytes are less damaged by doxorubicin-transferrin conjugate than by DOX alone.

98. Induction of apoptosis by doxorubicin – transferrin conjugate in human erythroleukemia cells

M. Szwed, M. Jędrzejczyk, Z. Jóźwiak

University of Łódź

Leukemia, the most common malignancy associated with the hematopoietic system, is moderately sensitive to several cytotoxic agents. It has been demonstrated that development of multidrug resistance to the anthracycline drugs, doxorubicin and daunorubicin limits the success of chemotherapy in this disease. In our study, to decrease the resistance of cells to anthracycline, we used a doxorubicin – transferrin conjugate.

The aim of this work was to estimate the effect of doxorubicin – transferrin conjugate on the induction of apoptosis in K562 human leukemia cells. The sensitivity of cells to DOX-TRF and DOX alone was measured by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT assay). Apoptosis was detected by using DNA ladder assay and by measuring the activation of caspase-3. Moreover, morphological changes of nuclei connected with apoptosis were analyzed by acridine orange/ethidium bromide double staining. The results demonstrate that DOX-TRF is more cytotoxic to K562 cells than free doxorubicin. Morphological and biochemical cell changes were dependent on both the drug concentration and the time of incubation.

99. May the near-infrared radiation (NIR) destabilize the DNA molecule?

K. Szymborska-Małek, M. Komorowska

Wrocław University of Technology

The structure of bound water determines the conformation of biological macromolecules and controls their metabolic activity. In the further layer such molecules are surrounded by bulk water. The structure of water which surrounds the molecule can be changed by magnetic field, pressure, temperature, presence of ions or organic solvents [1, 2]. Another efficient structural modification factor is the near infrared radiation (NIR) [3, 4].

DNA molecule has been investigated in order to clarify processes that take place under the influence of NIR. Such choice is motivated by the fact that the DNA hydration layer has a first-rate influence on this molecule stability. UV-VIS spectroscopy results obtained for aqueous DNA solutions show that melting process of the macromolecules has three stages. The stage sensitive to NIR is the dissociation of bases of the molecule. Studies have shown that all used doses of radiation destabilize the system [5]. Opposite results can be obtained after introduction of modifiers to the solution of DNA.

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100. Model equations of the membrane transport of non-electrolyte solutions with concentration polarization

A. Ślęzak[1], S. Grzegorczyn[2]

[1]Częstochowa University of Technology;
[2]Silesia Medical University, Zabrze

Polymer membranes both natural and artificial are very sensitive on changes of physical and chemical outside

environment. One of the most important spontaneous processes appearing in that environment is concentration polarization, which leads to time and space evolution of concentration fields. These processes cause local non-homogeneities, which control the membrane transport and lead to creation on both sides of the membrane concentration boundary layers (CBLs). CBLs fulfill a role of additional kinetic barriers in the membrane transport. One of the consequences of this process is the change of solute concentration on the border membrane/solution. This means that by reduction of concentration gradient across the membrane, both osmotic and diffusive membrane fluxes undergo a decrease. The equation describing passive volume osmotic flux (J_{vm}) in membrane system was derived, by means of the methods of the Kedem-Katchalsky's membrane thermodynamics. The equation can be written as

$$J_{vm}{}^3 + \gamma_1 J_{vm}{}^2 + \gamma_2 J_{vm} + \gamma_3 = 0$$

where γ_1, γ_2 and γ_3 are the functions of transport parameters of the membrane (L_p, σ, ω) and solutions (D), constant thermodynamic forces ($\Delta\pi$, ΔP), thicknesses of concentration boundary layers (δ), etc. It has to be marked that parameters: L_p, σ, ω, D and δ can be determined in series of independent experiments. Above equation, in contradiction to classical Kedem-Katchalsky equations, is the equation of third row and can be used to describe nonlinear effects in membrane transport of convective character.

101. Simvastatin as inhibitor of human adenocarcinoma cancer cell growth and lipid phase perturbing agent

K. Środa, A. Palko-Łabuz, A. Uryga, K. Michalak

Wrocław Medical University

Statins or 3-hydroxy-3 methylglutaryl CoA reductase inhibitors are the most potent drugs available for treating hypercholesterolemia because of their efficacy in reducing LDL (Low Density Lipoprotein) blood level and their excellent tolerability and safety.

However, exploring the effects of statins on cancer development and progress at the molecular level, scientists have found that statin may also play a potential role in cancer prevention and treatment because of their influence on essential cellular processes such as cell proliferation and differentiation. For example, it was demonstrated both *in vitro* and *in vivo* studies that statins inhibit tumor growth and induce apoptosis in a variety of tumor cells, including melanoma [1], glioma [2], neuroblastoma [3], and leukemia cell lines.

The aim of our study was to check the biological activity of simvastatin as inhibitor of cancer cell growth and its ability to change the physical state of lipid membranes. We investigated the cytotoxic properties of simvastatin in doxorubicin-sensitive (LoVo) and doxorubicin-resistant resistant (LoVo/Dx) human adenocarcinoma cell lines using the SRB assay. The effect of simvastatin on biophysical properties of lipid membranes formed of DPPC have been studied by fluorescence spectroscopy technique.

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102. Pirolin influences antioxidant enzyme activities in heart tissue of rats bearing experimental breast tumors and treated with anticancer drugs doxorubicin and docetaxel

S. Tabaczar[1], M. Talar[1], J. Czepas[1], J. Piasecka-Zelga[2], A. Koceva-Chyła[1], K. Gwoździński[1]

[1]University of Łódź;
[2]Nofer Institute of Occupational Medicine, Łódź

Combination of doxorubicin (DOX) with docetaxel (DTX) can cause different side-effects, e.g. cardiotoxicity, which is mainly a result of oxidative stress, generated by these drugs. Nitroxides as low molecular weight antioxidants might potentially decrease oxidative stress in the heart tissue.

The aim of this study was to assess the effect of nitroxide Pirolin (PL) and combination of anticancer drugs doxorubicin (DOX) and docetaxel (DTX) on activities of antioxidant enzymes in the heart of rats bearing experimental breast tumors. Animals were divided into 4 groups (I–IV) and injected with: 0.9% NaCl (group I, control), PL (10 mg/kg b.w., group II), DOX (2.5 mg/kg b.w.) + DTX (3.75 mg/kg b.w., group III), DOX and DTX at the same doses as in group III + PL (10 mg/kg b.w., group IV).

Activities of four antioxidant enzymes: catalase (CAT), total superoxide dismutase (SOD), MnSOD and Cu,ZnSOD were assayed in the homogenates of hearts of control and treated rats.

We have found that Pirolin alone caused a significant increase in the activities of all enzymes. DOX and DTX, used in combination, did not change significantly enzyme activities. PL injected in conjunction with drugs caused a significant increase in the activities of MnSOD and catalase in the group IV as compared with the group III, which suggests that this nitroxide can act as a modulator of oxidative stress induced by anthracyclines and taxanes in heart myocytes.

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103. Structural changes in the bone tissue of pregnant female rats treated with caffeine

E. Tarnowska-Sokołow, G. Olchowik, M. Tomaszewski, M. Tomaszewska

Medical University of Lublin

Osteoporosis is the most common metabolic illness of bones. It consists in the loss of bone mass with impaired microstructure, leading below a critical mass to pain and fractures. The metabolism of bone tissue is controlled by many factors, both internal: genetic, physiological, and external, such as diet, medication, stimulants and perspiration. The physiological status like pregnancy also exerts significant influence on the bone tissue. Presumably, caffeine, which is now the most commonly used stimulant in population, has also an adverse effect on the bone has. Many experimental and clinical studies conducted by the authors [1] demonstrated that caffeine has adverse effects on the bone tissue. Also young women (18–22 years), which consume caffeine with insufficient calcium intake have reduced bone density [2].

The purpose of this work was to assess changes in the bone tissue in animals exposed to caffeine during pregnancy. The experiment was carried out on pregnant female Wistar rats. Before administration caffeine was dissolved in water at 1:10 ratio. The so obtained suspension of a temperature of 25°C was administered intragastrically in a volume of 2 ml/kg b.w. to the females from the experimental group once daily, from the 8th till the 21st day of pregnancy. Assessment of bone structural changes were based on microscopic images, measurements of bone density and strength tests. Thickness and bone microarchitecture were evaluated in microscopic images.

Results of the study show that administration of caffeine at a dose of 120 mg/kg/day adversely affected the bone tissue of pregnant females and resulted in changes of bone density and structure, biomechanical parameters determined at maximal load and elasticity limit.

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104. Studies on the influence of selected plant-derived compounds on the activity of voltage-gated potassium channels Kv1.3

A. Teisseyre, J. Bronowicka, K. Michalak

Wrocław Medical University

Voltage-gated potassium channels of Kv1.3 type are expressed in many cell types. These channels play an important role in: setting the resting membrane potential, shaping of action potentials, cell proliferation, apoptosis and volume regulation. Activity of Kv1.3 channels is necessary for proliferation of many cell types. Inhibition of Kv1.3 channels can also inhibit cell proliferation at an early phase of this process.

Inhibition of Kv1.3 channels may be helpful in treatment of T-lymphocyte-mediated autoimmune diseases, insulin resistance, obesity and breast and colon cancer. Therefore, a big effort is done to discover new potent and selective inhibitors of the channels.

Among the recently discovered inhibitors of Kv1.3 channels there are some natural plant-derived compounds from the groups of flavonoids and substituted stilbenes and also some synthetic derivatives of these substances. Studies performed recently provided evidence that plant-derived compounds: the isoflavone genistein and a substituted stilbene resveratrol applied at micromolar concentrations are both effective inhibitors of Kv1.3 channels in human T lymphocytes. Also a synthetic tetramethoxy derivative of piceatannol and two synthetic methoxy derivatives of naringenin: 4',7-dimethylether and 7-methylether, inhibited the channel activity at such concentrations. On the other hand, plant derived compounds: daidzein, silybin, naringenin, aromadendrin, piceatannol and a synthetic tetraacethoxy derivative of piceatannol did not inhibit the activity of Kv1.3 channels at micromolar concentrations.

Further research work on the influence of other selected plant-derived compounds, such as the alkaloid berberine, on the activity of Kv1.3 channels is continued now.

105. NMR study of the mode of interaction of neurotoxic divalent cation (Mn^{2+}) with polysialic acid

A. Timoszyk, K. Wilkosz

University of Zielona Góra

Polysialic acid (polySia) is a α(2-8)-linked homopolymer of N-acetyloneuraminic acid (Neu5Ac). It is present in the N-glycans of neural cell adhesion molecules (NCAM) of vertebrate cells as well as in capsular polysaccharides of some pathogenic bacteria. In nervous tissue, polySia is thought to modulate NCAM-

mediated cell adhesion events, such as axonal guidance in nerve developing and regeneration, as well as establishing of a new neural connection during learning and memory formation. NCAM-polySia is present on growth cones of propagating axons and its expression is strictly temporally regulated, peaking in humans at the time of birth.

Manganese is essential for the brain. Insufficient brain Mn^{2+} can lead to central nervous system dysfunction. Excessive brain Mn^{2+} is neurotoxic, producing a Parkinsonian syndrome (manganism). However, it has also been reported that manganese concentrations increase in the brain with ageing, but not in patients with Alzheimer's disease. Inhibition of choline transport in red blood cells and synaptosomes, as well as the inhibition of release of neurotransmitters and glycolysis in nerve cells by manganese, suggests an elective effect of these metals on neuronal structures. Additionally, Mn^{2+} was recently shown to specifically promote the interaction of L1 cell adhesion molecules with β1 integrins, a similar interaction controlled by extracellular Ca^{2+}.

Polysialic acid contains hydroxycarboxylate moiety, which can chelate metal ions. In this way, in principle, it can initiate membrane degradation processes. Our hypothesis is that toxic effects of Mn^{2+} are partly due to degenerative processes induced in the cellular membranes, and it is possible that the mobility of polySia bound to membrane proteins is affected by the presence of Mn^{2+}. To verify the hypothesis, in this paper, the Mn^{2+}/polySia interaction was studied by NMR.

106. Slowly activating vacuolar channels (SV) in the presence of trimethyltin chloride

Z. Trela[1], W. Karcz[2], S. Przestalski[1]

[1]Wrocław University Environmental and Life Sciences;
[2]University of Silesia, Katowice

Using the patch clamp technique, we studied the influence of trimethyltin chloride ($(CH_3)_3SnCl$, Met_3SnCl) on the electrical characteristics of SV channels in the tonoplast of *Beta vulgaris*. Registration of current was performed both for macroscopic ionic currents (in *whole-vacuole* configuration), and microscopic currents of single SV channels of isolated patches of tonoplast (*inside-out vacuole*). Trimethytin chloride added to the incubation medium caused a decrease of macroscopic outward current by 48% as compared to the control. The effects were irreversible, after re-exchange for control solution there was no indication of an increase of the macroscopic outward current to the control value.

Taking into account single channel recordings we have showed that conductance of a single channel (calculated as slope of current-voltage characteristic) to be about 75 pS, which is in good agreement with literature data for *Beta vulgaris*, at 100 mM symmetrical K^+ concentration. Histograms of microscopic SV currents shows that openings of the channel are suppressed in the presence of trimethyltin. Using FitMaster software we calculated the opening probability of SV channels. In the control, the opening probability significantly increased with the holding potential in the range 40–100 mV. Below 40 mV the opening was practically not observed in the current traces. After supplementation of the bathing medium with trimethyltin, the opening probability drastically decreased (by about one order of magnitude in comparison to the control). Conductivity of single SV channel showed a slight decrease (from 75.5 pS in control to 71 pS in the presence of 100 μM organotin).

We assume that the tested compounds reduce the conductivity of SV channels, affecting them indirectly. It cannot be excluded that the effect of Met_3SnCl in this case is analogous to that of K^+ channel blockers.

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107. Calorimetric evaluation of peritoneal tissue in surgical patients

H. Trębacz[1], K. Torres[1,2], A. Chrościcki[2]

[1]Medical University of Lublin;
[2]District Specialistic Hospital, Lublin

The structure of peritoneal tissue may be influenced by different diseases or during surgical procedures. The aim of this study was to evaluate the quality of collagen of peritoneal tissue obtained from patients operated because of gastrointestinal tract cancer, groin hernias and cholecytolithiasis (laparoscopic cholecystectomy).

Stability of collagen in peritoneal tissue was investigated using differential scanning calorimetry at temperatures from 40°C to 85°C. In all samples a nonreversible endothermic process with a maximum between 66°C and 70°C and enthalpy from 6 J/g to 31 J/g was found. Considering that the main structural protein in the tissue under study is collagen, we attribute the thermal activity of the sample to the denaturation of collagen molecules resulting from breaking of hydrogen bonds that stabilize the collagen triple helix. The broad range of enthalpies obtained for different samples of peritoneum presumably results from different contents of collagen in the samples.

In samples of peritoneal tissue from patients with groin hernias the peak temperature of the endotherm was, on the average, significantly lower than in the other two groups. We attribute that difference to the lowered stability of collagen molecules in peritoneum of these patients. This might be one of the factors that facilitate the occurrence of hernia. It has been proven previously, that in the group of patients affected by groin hernia the changes of peritoneal tissue collagen are essential for the occurrence of this disease.

In the most of samples the denaturation endotherm was followed by an equilibrium state adequate to the new configuration of the protein molecules. All peritoneum samples that did not return to a thermal equilibrium at higher temperatures were from the group of patients with tumors.

108. Effect of collagen glycation on the stability of organic phase in bone tissue

H. Trębacz, K. Wójtowicz, W. Dyś, T. Gieroba, E. Wlizło

Medical University of Lublin

Nonenymatic glycation of proteins is a spontaneous process and its products accumulate in tissues of living organisms with ageing resulting in increasing stiffness of collagen fibers in the connective tissue and depressed solubility of collagen and its lower susceptibility to enzymatic digestion.

In diabetes an increased concentration of glucose results in a considerable increase of intensity of nonenzymatic glycation. Accumulation of destructive advanced glycation endproducts is one of the causes of diabetes-related disorders like renal failure, retinopathy, cardiovascular diseases or diabetic dermadromes that are relevant to changes in connective tissue collagen.

The aim of this work was to find if glycation influences the stability of the structure of collagen matrix of bone tissue. Samples from bovine cortical bone were incubated in ribose solution for three weeks. One halve of glycated samples and one halve of controls were then decalcified in formic acid. Stability of collagen in intact and decalcified samples was investigated using differential scanning calorimetry at temperatures from 50°C to 220°C.

In each of samples at least two endothermal processes of different cooperativity were stated at temperatures above 100°C. We attribute the first of these processes to denaturation of collagen molecules and the other to decay of intra- and intermolecular cross-links in collagen fibrils. In decalcified bone matrix, glycation resulted in an increase of the denaturation temperature and a lowering of the enthalpy of the process. In mineralized bone samples, glycation influenced only the second, high-temperature endotherm.

In conclusion, glycation endproducts influence stability of the structure of bone collagen, both in organic matrix itself and in the mineralized bone tissue.

109. The effect of NIR pulse sequence and energy on erythrocyte susceptibility to oxidative stress

T. Walski, K. Grzeszczuk, M. Komorowska

Wrocław University of Technology;
Regional Specialist Hospital in Wrocław

Rapid growth of medical technology contribute to raise in invasiveness of healing procedures. Biomaterials are agonists of the complement system and leukocyte activation during inflammation. Blood-biomaterial interaction results in a massive production and releases of Reactive Oxygen Species (ROS). Oxidative stress is one of the major factors which leads to erythrocytes destruction during extracorporeal circulation. The antioxidant effects of near-infrared radiation (NIR, 700–2000 nm) on blood *in vitro* is known. This work presents influence of NIR pulse sequence and energy on red blood cell susceptibility to oxidative stress.

Whole, heparinized (10 IU/ml), bovine blood (hematocrit 20%, volume 10 ml) was incubated in a thermomix container to obtain a temperature of 37°C. Then blood cells (RBC) were treated with *tert*-butyl hydroperoxide (t-BOOH) at a final concentration of 9 mM. Samples (except a control probe) were modified by a Near Infrared Radiation pulse sequence. Incubated blood was gently shaken in the thermomix for next 6 hours and hemolysis was measured.

Single sequence of impulses did not result in any significant change of t-BOOH induced RBC hemolysis. A protective effect was observed after 12 pulses. Hemolysis ratio of the irradiated sample was lower by about 32%. Energy accumulation into one pulse did not improve the obtained results.

Suitable energy of Near Infrared Radiation in san appropriate pulse sequence restricts destructive effects of oxidative stress in blood.

110. Influence of cationic phosphorus-containing dendrimers on amyloid peptide fragment Aβ 1–28 studied by FT-IR

T. Wasiak[1]; M. Granell[2]; J. Cladera[2]; J. P. Majoral[3]; B. Klajnert[1]; M. Bryszewska[1]

[1]University of Łódź, Poland;
[2]Autonomous University of Barcelona, Spain;
[3]Laboratoire de Chimie de Coordination du CNRS, France

The formation of neuronal inclusions is a hallmark pathology of neurodegenerative diseases. The inclusions are principally composed of proteins such as β-amyloid. One of the essential problems left unresolved is the physical nature of the bonding that stabilizes the highly insoluble inclusions rich in β-sheet structure. In this work we used synthetic β-amyloid peptide fragment Aβ 1–28 (JPT Peptide Technology GmbH, Ger-

many), cationic phosphorus-containing dendrimers generation 3 (pD-G3) and 4 (pD-G4) synthesized by the Laboratoire de Chimie de Coordination du CNRS. The stock solution of dendrimers was 2 mmol/L in 10 mmol/L HEPES buffer in D_2O, pD 7.2. The aggregation process was monitored using infrared spectroscopy with Fourier transformation (FT-IR). Stock solution of Aβ 1–28 peptide (250 μmol/L) in 10 mmol/L HEPES buffer, pH 7.4 was subjected to 24h-liofilization and again dissolved in 10 mmol/L HEPES buffer in D_2O, pD 7.2. We used three concentration of dendrimers: 0.01 μmol/L, 1 μmol/L and 100 μmol/L. Heparin in 10 mmol/L HEPES buffer in D_2O, pD 7.2 was added to a final concentration 0.041 mg/ml as trigger of aggregation process and pD was adjusted to 5.3 with DCl. Fourier transform infrared spectra were recorded with Varian 7000e FT-IR spectrometer in 37°C with HgCdTe detector, equipped with a liquid nitrogen, at the normal resolution of 2 cm^{-1}. Forty microliters of a sample were placed between two CaF_2 windows separated by teflon spacer. 1000 scans for each sample were made.

MTT test was used to check cytotoxicity of cationic phosphorus-containing dendrimers on two cell lines. Cell were seeding 1.6×10^4 per well in 96-well in 100 μl of grow media. Twenty-four hours after seeding, cell were washed with PBS, fresh grow medium was added, and dendrimers were put in to final concentrations 10 to 200 μmol/L. After 24h incubation, medium with dendrimers was removed, cell were washed twice with PBS and fresh grow medium with MTT solution (at a final concentration of 0.5 mg/ml) were added to each well. After four hours incubation, the supernatant containing the unreacted dye was replaced with dimethyl sulfoxide (DMSO) (Sigma) (100 μl/well). Plates were shaken and absorbance was measured at 540 nm and reference at 720 nm by fluorescence multiplate reader (VICTOR, Perkin Elmer).

An increase of the band intensity at 1616 cm^{-1} indicates the formation of β-sheet structures whereas the broad band located at 1645.5 represents random or helical structure. Only one concentration (1 μmol/L) for pD-G3 and pD-G4 slowed the aggregation process down. Therefore, 0.01 μmol/L and 100 μmol/L concentrations of dendrimers did not affect amyloid fibril formation.

Dendrimer pD-G4 was more cytotoxic (IC_{50} pD-G4 = 15.36 μmol/L) than pD-G3 (IC_{50} pD-G3 = 207.04 μmol/L). We observed a generation-dependent effect. Higher generations of dendrimers possess more cationic groups on the surface. Cationic groups react with cell membrane causing cell lysis. PC12 cell line is non-differentiated. For high concentrations of dendrimers we could see adaptation of cells to the presence of dendrimers in grow medium.

111. Influence of antibiotic drug amphotericin B on lipids in liposome investigated with using fluorescence anisotropy

P. Waśko, W. I. Gruszecki

Maria Curie-Skłodowska University in Lublin

Amphotericin B (AmB) is an antifungal antibiotic commonly used in the treatment of deep-seated mycosis, so it is important to discover the mechanisms of its interaction with fungal and human cells. From other investigations, it is known that AmB binds to lipid membranes.

Auto-fluorescence of AmB incorporated into DPPC liposomes may be used to investigate dynamics and structural properties of lipid membranes and, simultaneously, molecular organization of the drug with using fluorescence anisotropy. The conclusion from our investigations is that AmB incorporated to the system at concentrations of 0.2 mol% and 1mol% (with respect to lipid) is bound in the monomeric and dimeric form to membranes in the region of polar lipid heads.

112. Differential interaction of 8-prenylnaringenin with zwitterionic phosphatidylcholine and charged phosphatidylglycerol bilayers

O. Wesołowska, J. Bronowicka-Gąsiorowska, K. Michalak

Wrocław Medical University

8-Prenylnaringenin is a prenylated flavonoid isolated from the common hop. 8-Prenylnaringenin was identified as the most potent plant phytoestrogen, and also an effective inhibitor of aromatase, a key enzyme in estrogen biosynthesis. Apart from the estrogenic activity, it may also modulate the processes of inflammation, angiogenesis, cancer cells proliferation, and cellular detoxification. Differential scanning calorimetry together with fluorescence spectroscopy were employed to study the interaction of 8-prenylnaringenin with zwitterionic (DMPC) and negatively charged (DMPG) lipid bilayers. Microcalorimetric experiments demonstrated that 8-prenylnaringenin caused a reduction of the main phospholipid phase transition temperature and co-operativity in both model systems. Additionally, in the case of DMPG, two separated calorimetric peaks observed at higher 8-prenylnaringenin concentrations pointing to a flavonoid-induced phase separation. Several fluorescent probes that locate at different depths inside lipid bilayer have been used. The studies on Laurdan and Prodan generalized polarization as a function of temperature demonstrated similar influence of 8-prenylnaringenin on thermotropic properties of lipid bilayer as that recorded by means of microcalorimetry. 8-Prenylnaringenin interacted more

strongly with fluorescent probes of more superficial membrane localization (e.g. Prodan, NPN, TMA-DPH) than with labels buried deeper within membrane (like Laurdan and DPH). The effect exerted by the flavonoid on the fluorescence intensity of Prodan and Laurdan was greater in PG than in PC bilayers. This observation suggests that the charge on the membrane surface is likely to influence 8-prenylnaringenin interaction with model membrane.

113. Free radicals in beta-lactam antibiotics sterilized by gamma irradiation

S. Wilczyński[1], B. Pilawa[1], R. Koprowski[2], Z. Wróbel[2], M. Ptaszkiewicz[3], J. Swakoń[3], P. Olko[3]

[1]Medical University of Silesia, Katowice;
[2]University of Silesia, Sosnowiec;
[3]Institute of Nuclear Physics, Kraków

In modern medicine a number of methods of sterilization of pharmaceutical substances have been developed. According to Polish Pharmacopoeia, sterilization is the process leading to elimination of microbes in both vegetative and spore forms [1]. Free radicals in radiation sterilized beta-lactam antibiotics: piperacillin, ampicillin and crystal penicillin were studied by electron paramagnetic resonance (EPR) spectroscopy. β-Lactam antibiotics are a class of antibiotics that include a β-lactam nucleus in its molecular structure [2].

Sterilization of the analyzed antibiotics was performed by ^{60}Co gamma irradiation in THERATRON 780E. A dose of 25 kGy was applied.

First-derivative spectra were measured in an X-band (9.3 GHz) EPR spectrometer with modulation of magnetic field 100 kHz. Microwave power in the range of 2.2–70 mW was used. Free radical concentration was determined. Ultramarine and a ruby crystal were used as the references. Spin-spin and spin-lattice relaxation in the tested beta-lactam antibiotic were analyzed. Changes in the system of paramagnetic centers were observed during storage of the drugs. The fastest changes of concentrations were observed in the initial period after irradiation. Changes of integral intensity of EPR spectra of irradiated antibiotics with increasing time of storage were fitted by a two-exponential function and identification of function parameters was performed by the Gauss-Newton and Levenberg-Marquardt methods. The characteristic g-value of 2.0048–2.0062 suggests that unpaired electrons are localized on oxygen atoms in the case of piperacillin and ampicillin and the g-value of 2.0083 for crystal penicillin points that unpaired electrons are localized on sulphur atoms.

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114. The effect of aclarubicin (ACL) on human erythrocytes

P. Witczak, A. Matusiak, A. Marczak

University of Łódź

Aclarubicin (ACL) is a II generation anthracycline antibiotic administered in the treatment of acute leukemias, lymphomas and other tumors. This drug induces less side effects than the I generation anthracyclines, but nevertheless it still is a health hazard to patients. Erythrocytes are among the first target of the ACL cytotoxic activity after intravenous administration. In this paper the influence of ACL on human red blood cells was investigated. We determined reactive oxygen species (ROS) production and changes in some parameters of red blood cells e.g. catalase activity, methemoglobin content and the level of reduced form of glutathione (GSH). In our study we observed a significant growth in ROS level after treatment with ACL. The depletion of catalase activity and increase of metHb content were demonstrated and attributed to ACL-induced ROS production. Changes in GSH concentration were not significant statistically. The presented results confirm ROS generation as a primary mechanism of ACL cytotoxic activity.

115. Inhibition of oxidation of membrane lipids by hawthorn extracts

A. Włoch, J. Oszmiański, H. Kleszczyńska

Wrocław University of Environmental and Life Sciences

Hawthorn *(Crataegus laevigata)* is a plant that grows throughout Europe, Asia and North Africa. It was known in ancient Greece, where its fruit was used for treating podagra; and since the 17th century the plant was also used for treating circulatory ailments, internal bleeding and pleuritis. Hawthorn extracts find application in medical treatments of heart diseases. Hawthorn possesses a broad spectrum of healing properties, and recently it has caught the attention of researchers as a very potent antioxidant, owing to the content of polyphenolic compounds.

The aim of the work was to determine the antioxidant activity of extracts from leaves and bark of hawthorn with respect to membrane lipids. The research was performed on isolated erythrocyte membranes and lipids obtained from erythrocyte membranes. Lipid oxidation was induced with UV-C radiation and AAPH radical, by using two methods, fluorimetric with DPH-PA probe and spectrophotometric. The fluorimetric method was applied for the determination of free radi-

cal concentration in the solution based upon fluorescence intensity, while the spectrophotometric method was employed for determination of concentration of MDA, a the compound that evolves in the lipid oxidation process.

The research has shown a high antioxidative activity of both the hawthorn extracts, the extract from hawthorn bark showing a slightly higher activity.

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116. Effect of incorporated dendrimers on thermotropic parameters of DMPC/DPPG liposomes

D. Wróbel[1], H. Mourelatou[2], M. Ionov[1], J. P. Majoral[3], B. Klajnert[1], C. Demetzos[2], M. Bryszewska[1]

[1]University of Łódź, Poland;
[2]School of Pharmacy, Greece;
[3]Laboratoire de Chimie de Coordination du CNRS, France

The aim of this work was to study changes in thermotropic parameters caused by dendrimers incorporated in lipid bilayers. Cationic phosphorus-containing dendrimers, generation 3 or 4, were incorporated at different concentrations of 0.1%, 0.3%, 0.5%, 1% to liposomes made of DMPC (dimyristoylphosphatidylcholine) with 3% of DPPG (dipalmitoylphosphoglycerol). Experiments were performed in 10mM buffer Hepes without NaCl with 150 mM concentration of NaCl, therefore the influence of NaCl on the dendrimer effect on lipid phase transitions was checked. Differential scanning calorimetry (DSC) was used as a method. Three parameters: enthalpy of transition, temperature at which heat capacity at constant pressure is maximal and the width of the transition peak were monitored.

The obtained results allow for a conclusion that sodium chloride does not influence interactions between dendrimers and the lipid bilayer. Interactions with lipid head groups were reflected by a pre-transition abolition, while changes in the main transition confirmed existence of dendrimers interaction with lipids chains. Moreover, dendrimers of higher generation interact stronger with membranes and their concentration as well as generation are equally important.

117. Extrinsic luminophores in the examination of neurodegenerative diseases

A. Wypijewska[1], B. Sikora[2], J. Gałązka-Friedman[3], E. R. Bauminger[4], Z. K. Wszolek[5], K. J. Schweitzer[5], D. W. Dickson[5], A. Jaklewicz[7], A. Baranowska-Korczyc[2], D. Elbaum[2], A. Friedman[7]

[1]University of Warsaw, Poland;
[2]Polish Academy of Sciences, Warsaw, Poland;
[3]Warsaw University of Technology, Poland;
[4]Hebrew University in Jerusalem, Israel;
[5]Mayo Clinic, Jacksonville, FL, USA;
[7]Medical University of Warsaw, Poland

A common feature of neurodegenerative diseases is the occurrence of oxidative stress, which may be responsible for the disorder or death of nerve cells. It is debated whether oxidative stress is a cause or a consequence of neurodegeneration. Oxidative stress is the result of overproduction of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), nitric oxide (NO), peroxyl radicals (ROO$^{\bullet}$) or the highly reactive hydroxyl radical ($^{\bullet}$OH). High oxygen consumption, relatively low levels of antioxidants, low regenerative capacity and lipid composition make the brain tissue vulnerable to oxidative damage. Despite the controversy many researchers believe that oxidative stress contributes to neurodegeneration occurring in Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis [1]. Although the main chemical source of ROS production is the reaction between molecular oxygen and the copper and iron cations (e.g. Fenton reaction) [2], other ROS could actively contribute to the overall oxidative stress, as well.

We determined the levels of labile iron dependent ROS activity by visible light luminescence, in *substantia nigra* of Parkinsonian and control samples. We determined levels of several ROS: hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}$OH) and superoxide radical ($O_2^{\bullet-}$). We used the organic dye 2'7'-dichlorodihydrofluorescein (H_2DCF) to determine the total metal dependent ROS activity. Measurements were conducted in the presence and absence of metal chelators. The *substantia nigra* of parkinsonian patients contained more labile iron compared to the control samples, thus generated higher ROS activity [3].

Hydroxyl radical activity was determined by the terephthalic acid reactivity. Although terephthalic acid has no native fluorescence, it fluoresces when hydroxylated by $^{\bullet}$OH. It formed only a single isomer [4]. The superoxide radical activity was investigated fluorimetrically by the dihydroethidium oxidation [5]. Oxidation of scopoletin in the presence of horseradish peroxidase (HRP) by hydrogen peroxide caused a loss of scopoletin fluorescence [2].

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118. A biophysical view of mRNA cap binding by *C. elegans* DcpS

A. Wypijewska, J. Żuberek, E. Bojarska, A. M. Rydzik, J. Jemielity, E. Darzynkiewicz

University of Warsaw

Two major processes of mRNA degradation occurring in living cells involve activities of decapping enzymes. In the 3'-5' pathway, the enzyme responsible for cap degradation is a specific mRNA cap pyrophosphatase, DcpS (Decapping Scavenger). It catalyzes the cleavage of the triphosphate bridge of capped short oligonucleotides, as well as synthetic mRNA cap analogs, producing 7-methylguanosinemonophosphate. The cellular function of this enzyme is not fully understood. One of the obvious roles of the enzyme is removal of the residual 5' mRNA end, after 3'-5' mRNA degradation, which prevents translation inhibition. Recently other roles of DcpS have been suggested (e.g. in splicing [1]). Moreover, it has been recognized as a novel therapeutic target for spinal muscular atrophy (SMA) [2].

The biological roles of DcpS make it an interesting object for biophysical and biochemical investigations. Here, we present studies of DcpS interactions with non-hydrolysable bisphosphonate mRNA cap analogs using fluorescence time-synchronized titration method, which allows the determination of the association constant, K_{AS}. The measurements were carried out by adding 1 µl aliquots of the cap analog with increasing concentration to protein solution of *C. elegans* DcpS. The important aspects of this method that enable us to compute the binding affinity of chosen ligands to the DcpS protein are corrections for sample dilution and for the inner filter effect, introduced by empirical calibration curve. Prior to the calculation of the K_{AS}, this method provides also a quantitative estimate of the free energy of binding ($\Delta G°$). For the first time, the data of *C. elegans* DcpS affinity will be presented. The values of association constants of various modified dinucleotides are invaluable data for designing of DcpS inhibitors as well as for better understanding the decapping process at the molecular level.

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119. Types of paramagnetic centers in melanin complexes with netilmicin

M. Zdybel[1], B. Pilawa[1], E. Buszman[1], D. Wrześniok[1], R. Krzyminiewski[2], Z. Kruczyński[2]

[1]Medical University of Silesia, Katowice;
[2]Adam Mickiewicz University, Poznań

Paramagnetic centers of melanins and their complexes with drugs and metal ions play an important role in free radical processes in organisms and may be responsible for toxic effects [1]. The aim of this work was to determine the types and properties of paramagnetic centers in DOPA-melanin and its complexes with netilmicin, Zn(II) and Cu(II) ions.

Electron paramagnetic resonance (EPR) spectroscopy with microwave frequency of 9.3 GHz (an X-band) was used as the experimental technique. The first derivative EPR lines were recorded. EPR studies of melanin samples at 100–300 K were performed.

The studies of correlations between EPR parameters (integral intensities I) and temperature point out complex character of paramagnetic centers in melanin complexes. It was pointed out that *o*-semiquinone free radicals with spin S = 1/2 which fulfill the Curie law and biradicals with spin S = 1 which do not fulfill the Curie law [2] exist in all samples studied. Free radical properties of melanin were characterized earlier [3, 4]. For the first time, the existence of biradicals in DOPA-melanin complexes with netilmicin and Zn(II) and Cu(II) was shown.

Effect of netilmicin and metal ions on concentrations of paramagnetic centers with spins of 1/2 and 1 was determined. The complex character of the system of paramagnetic centers in melanin samples, especially in melanin complexes with kanamycin and copper(II) was detected spectroscopically before [4]. Comparative analysis of our results for DOPA-melanin complexes with netilmicin and the results for DOPA-melanin-kanamycin complexes [4] was done.

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120. Paramagnetic centers in vertebrae of newborn rats

M. Zdybel[1], B. Pilawa[1], K. Michalik[2], Z. Drzazga[2]

[1]Medical University of Silesia, Katowice;
[2]University of Silesia, Katowice

Abnormalities in the development of fetal bone tissue in response to prenatal conditions such as maternal drug treatment can have temporary or permanent effects during postnatal life. Cyclophosphamide as an alkylating anticancer drug disturbs DNA synthesis and cell divisions. This drug is also administered to pregnant women suffering from cancer. The question about the effect of cyclophosphamide therapy during pregnancy on bones of future offspring remains still unanswered. The aim of present work was to compare concentration and properties of paramagnetic centers in vertebrae as a function of newborn age and maternal drug administration.

Electron paramagnetic resonance (EPR) examination of vertebrae of 7, 14 and 28 days old rats whose mothers were treated with cyclophosphamide during pregnancy was done. Microwave frequency of 9.3 GHz (X-band) and magnetic modulation of 100 kHz were used. The first-derivative EPR spectra were measured by the Rapid Scan Unit of Jagmar Firm (Kraków).

EPR lines were measured at free radical magnetic field. Their EPR spectra were asymmetrical broad lines with linewidths of 0.69–1.03 mT. Strong dipolar interactions may be responsible for the observed line broadening [1]. Similar linewidths of EPR spectra of bones were obtained for the control group and the samples from 7- and 28-old rats whose mothers were treated with cyclophosphamide. The relatively narrower lines characterize bones after maternal administration with cyclophosphamide than the control bones of 14-day old rats. Continuous microwave saturation of EPR lines were used to study the spin-lattice relaxation of the bone samples.

The concentration of paramagnetic centers in the bones from the 7- and 28-day old rats after maternal treatment with cyclophosphamide did not change essentially in comparison with the control. However, marked decrease of free radical content in the bones of 14-day old rats was observed after maternal treatment with cyclophosphamide. Significant differences in the bones from these 14-day old rats were also obtained by the other techniques such as optical and XRF spectroscopy.

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121. Toxicity of polypropylenimine dendrimers with various degree of sugar modification

B. Ziemba[1], A. Janaszewska[1], K. Ciepluch[1], M. Krotewicz[1], W. A. Fogel[2], D. Appelhans[3], B. Klajnert[1], M. Bryszewska[1]

[1]University of Łódź, Poland;
[2]Medical University of Łódź, Poland;
[3]Leibniz Institute of Polymer Research, Dresden, Germany

Toxicity studies of polypropylenimine (PPI) dendrimers generation 4th were performed on Wistar albino rats. The rats were treated either with unmodified PPI dendrimers or with dendrimers which surface was modified by maltotriose residues in 25% or 100%. Saline treated rats were used as the controls. A dose of 4 or 16 mg/kg body weight daily for unmodified and modified dendrimers, respectively, was given for 10 days, by an intraperitoneal injection. The treatment was followed by a 30-day recovery period. During the entire experiment, a general state, behavior, body weight, feed and water intake, urinary output, protein and carbohydrate metabolism, as well as blood hematology were monitored in all rats. Over the period of treatment significant differences in all tested parameters were observed between rats receiving unmodified PPI dendrimers and a control group. The unmodified dendrimers-treated rats showed slower body weight gain, reduced feed and water intakes and urinary output and their mobility was highly reduced. In blood of these animals lower levels of uric acid, alanine aminotransferase and amylase as well as a higher percentage of granulocytes and lower percentage of lymphocytes were detected. After a recovery period, however, no further signs of toxic effects were found. Surface modification remarkably decreased dendrimers toxicity, even if only 25% of amino groups were substituted by maltotriose residues. In rats from the two groups only a decrease in uric acid level in blood was observed while they were treated with modified dendrimers and not at a final, post recovery examination.

122. Kinetic parameters of butyrylcholinesterase in blood plasma samples of patients with metabolic syndrome

A. Ziobro[1], M. Broncel[2], M. Koter-Michalak[1]

[1]University of Łódź;
[2]Medical University in Łódź

The main components of the metabolic syndrome (MS): obesity, dyslipidemia, hypertension and impaired glucose tolerance, constitute risk factors for the development of cardiovascular disease.

Butyrylcholinesterase, BChE [EC 3.1.1.8] catalyses the hydrolysis of esters of choline, including acetyl-

choline. This enzyme, besides its proven cholinergic activity, participates also in processes of lipids metabolism.

The aim of the study was to examine of V_{max} and K_m values of pseudocholinesterase in plasma samples of patients with MS. This disorder was diagnosed according to the definition of the International Diabetes Federation (IDF, 2005). The control group (n = 10) and patients (n = 19) were in the similar age range (38–68 y). Significant differences were found between healthy controls and patients with MS in plasma lipid parameters (total cholesterol 179 ± 23 vs. 248 ± 35, $p < 0.001$; LDL 98 ± 21 vs. 154 ± 26, $p < 0.001$; triglycerides 110 ± 34 vs. 213 ± 130, $p < 0.01$; HDL 59 ± 11 vs. 57 ± 19 mg/dl, respectively).

Kinetic parameters of BChE (K_m and V_{max}) were determined by a spectrophotometric assay using butyryltiocholine iodide as a substrate.

As related to control V_{max} value was higher in the patients group while the Michealis-Menten constant was decreased (approximately 2.4 ± 0.5 vs. 2.9 ± 0.6 μmol/ml/min, $p < 0.02$ and 41.2 ± 5.3 vs. 27.7 ± 10.6 μmol/l, $p < 0.001$, respectively).

These results suggest changes in the activity of plasma butyrylcholinesterase of patients with MS.

123. Changes in erythrocytes in the youth with dyslipidemia

A. Ziobro[1], E. Rapacka[2], P. Duchnowicz[1], M. Koter-Michalak[1]

[1]University of Łódź;
[2]Medical University in Łódź

Disorders of cholesterol and lipoprotein metabolism are commonly described in school children. Several studies showed a correlation between these changes and increased risk of cardiovascular disease in adulthood.

It has been described that these disturbances are accompanied by secretion of a number of pro-inflammatory agents by the adipose tissue. It can be expected that dyslipidemia causes alterations in the antioxidant levels and increases oxidative stress.

The aim of the study was to investigate whether dyslipidemia in teenagers is associated with changes in erythrocyte membrane and antioxidant system of red blood cells.

The control and patients groups were in the similar age range. The values of plasma lipids parameters were different in controls and in children with dyslipidemia (total cholesterol 159 ± 21 vs. 204 ± 20, $p < 0.001$; LDL 93 ± 18 vs. 134 ± 16, $p < 0.001$; TG 76 ± 25 vs. 170 ± 58, $p < 0.001$; HDL 54 ± 12 vs. 43 ± 12 mg/dl, $p < 0.02$, respectively).

The levels of cholesterol and lipid peroxidation products (thiobarbituric acid-reactive substances, TBARS) in erythrocyte membranes were higher in the group of children with dyslipidemia than in healthy controls (3.7 ± 0.4 vs. 2.7 ± 0.8 $mg/ml_{packet\ cells}$; $p < 0.001$ and 0.42 ± 0.14 vs. 0.33 ± 0.11 $\mu mol/g_{Hb}$; $p < 0.05$, respectively).

Lower values of total ATPase and (Na^+,K^+)-ATPase activities of erythrocyte membranes were also found in the group of children with dyslipidemia (439 ± 97 vs. 502 ± 69; $p < 0.05$ and 169 ± 59 vs. 228 ± 51 nmol $o\text{-}P/mg_{proteins}/h$; $p < 0.01$ respectively).

No statistically significance changes in the red blood cell antioxidant profile (superoxide dismutase, SOD; catalase, CAT and glutathione peroxidase, GPx) and levels of reduced glutathione (GSH) and proteins thiol groups) although GPx and CAT activities and GSH level tended to be lower in children with dyslipidemia as compared with those of control.

These results show that changes in the red blood cells may be a consequence of cholesterol loading of erythrocyte membranes and/or oxidative stress induced in the blood of children with dyslipidemia.

124. Kinetic parameters of butyrylcholinesterase in the plasma of youth with dyslipidemia

A. Ziobro[1], E. Rapacka[2], P. Duchnowicz[1], M. Koter-Michalak[1]

[1]University of Łódź;
[2]Medical University in Łódź

Butyrylcholinesterase, BChE [EC 3.1.1.8] catalyses the hydrolysis of esters of choline, including acetylcholine. This enzyme, has been suggested to participates also in processes of lipids metabolism.

In dyslipidemia, disturbances of cholesterol and lipoproteins metabolism have been observed. It is possible that changes in kinetic properties of blood plasma of BChE of dyslipidemic school children occur.

The aim of the study was to examine whether dyslipidemia in teenagers is associated with alterations in plasma pseudocholinesterase activity.

Healthy control (n = 12) and patients groups (n = 24) were in the similar range of age (15–20 and 13–22 y, respectively). There were significant differences between the control group and patients in plasma lipids parameters (total cholesterol: 159 ± 21 vs. 206 ± 20, $p < 0.001$; LDL: 93 ± 18 vs. 136 ± 15, $p < 0.001$; TG: 76 ± 25 vs. 172 ± 55, $p < 0.001$; HDL: 54 ± 12 vs. 44 ± 11 mg/dl, $p < 0.05$, respectively).

Kinetic parameters (K_m and V_{max} values) of plasma BChE were determined by a spectrophotometric assay using butyryltiocholine iodide as a substrate.

The Michaelis-Menten constant of the patients group was lower and amounted to 25.0 ± 5.7 μmol/l; $p < 0.05$ while the V_{max} was higher (2.6 ± 0.8 μmol/ml/min; $p < 0.01$) as compared to the healthy controls (31.8 ± 10.5 μmol/l and 1.7 ± 0.6 μmol/ml/min, respectively).

These results suggest that dyslipidemia involves participation of BChE in the pathophysiological process. The observed changes in plasma pseudocholinesterase activity may be a consequence of disturbances in the cholesterol and lipoprotein metabolism in youth children with dyslipidemia.

125. The specific blue-light-effect on the singlet excitations quenching in protein complex LHCII

M. Zubik[1], R. Luchowski[1,2], W. Grudziński[1], M. Gospodarek[3], J. Szurkowski[4], Z. Gryczynski[2], I. Gryczynski[2], W. I. Gruszecki[1]

[1]Maria Curie-Skłodowska University, Lublin, Poland;
[2]University of North Texas Health Science Center, Fort Worth, USA;
[3]Lublin University of Technology, Poland;
[4]University of Gdańsk, Poland

Green plants have developed different photoprotection mechanisms operating at all the organization levels. Besides reorientation of leaves and translocation of chloroplasts, high-light induced reactions in the photosynthetic apparatus at the molecular level are observed.

LHCII, the major photosynthetic pigment-protein antenna complexes of plants, plays a role in collecting excitations and passing them on to the reaction centers. Regulation of photosynthetic antenna function at the molecular level at high-light conditions leads to adjusting a number of excitations to the capacity of the photosynthetic apparatus.

Examination of blue-light-effect on the level of chlorophyll *a* fluorescence emission in antenna complex LHCII revealed operation of specific mechanisms which result in singlet excitation quenching. Chlorophyll fluorescence lifetime analysis shows that the excitation with blue light (470 nm) gives rise to shorter lifetime components as compared to the excitation with red light (635 nm), despite the equal number of absorbed light quanta or energy. Simultaneous analysis of the fluorescence and photoacoustic signals in LHCII demonstrates that the light-driven fluorescence quenching is not associated with an increase in heat emission. Instead, a reversible light-induced conformational transformation of the protein takes place, as demonstrated by the FTIR technique.

126. Conformational changes of peptides in the erythrocyte membrane induced by organometallic tin compounds

R. Żyłka, J. Kupiec, S. Przestalski

Wrocław University of Environmental and Life Sciences

The functions proteins play in cell processes are closely connected and controlled by the protein structure. A change in protein conformation means a break of a process it participates in or a change in the process. This must result in a change in functioning of the cell and consequently of the entire organism.

The poster presents the results of a study on the effects of selected organic chlorides of tin on peptide conformations in erythrocyte ghosts from pig blood. The following compounds were used: dibutyltin dichloride (DBT), tributyltin chloride (TBT), diphenyltin dichloride (DPhT) and triphenyltin chloride (TPhT). Peptide conformation changes were determined on the basis of measurements done by the ATR FTIR technique. This method made it possible to measure the percent share of the peptide secondary structures in the membranes studied. The investigation showed that all the tin organic compounds studied cause a several percent decrease in quantity of peptides in the α-helix and the turn conformations, and a about 20% increase in ghosts peptides with β-sheet conformation.

The results obtained indicate that the toxic properties of organometallic compounds can importantly depend on their effect on the structure of membrane peptides.

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