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Posters

AN EFFECT OF CAROTENOIDS ON ION TRANSPORT ACROSS MODEL LIPID MEMBRANES

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Carotenoids are ubiquitous pigments present both in the plant and animal kingdoms, playing important physiological roles. Among diverse biological functions of carotenoids protection against oxidative damage and light harvesting in the photosynthetic apparatus are the most frequently reported. The photoprotection of carotenoids is realized via quenching of the triplet states of photosensitizers, quenching of singlet oxygen and scavenging free radicals. These mechanisms are essential for maintaining integrity of both the functional membrane proteins and the lipid phase. Protection of lipid membrane by carotenoid pigments is also realized via decreasing fluidity of the membrane.The polyene chain of carotenoid pigments incorporated into lipid membranes is localized in the hydrophobic core of the bilayer. Polar carotenoids have to adopt localization in the lipid membranes, such that the hydrophilic groups remain in contact with the polar head-groups of the lipid bilayer (in the opposite polar zones).

In the present work we analyze the effect of zeaxanthin, β-carotene and violaxanthin on transmembrane proton transfer. A pH sensitive fluorescence label, piranine trisulfonate, entrapped inside small unilamellar liposomes formed with egg yolk phosphatidylcholine, was applied to investigate effect of carotenoids on proton transport across lipid membranes. Time dependencies of fluorescence-monitored pH changes inside lipid vesicles, upon sudden acidification of the liposome suspension, were analyzed. It appeared that addition of xanthophylls to the liposomes, suppressed rapid pH changes. The effect was not observed in the case of β-carotene addition. The effect of the xanthophylls on transmembrane proton transport con be interpreted in terms of modification of the lipid phase fluidity. An alternative explanation is based upon binding of protons to the transmembrane molecular structures formed by the xanthophylls.

CALCIUM REGULATED POTASSIUM CHANNEL IS PRESENT IN ENDOTHELIAL MITOCHONDRIA

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In the present study, we describe the existence of a large-conductance Ca^{2+}-activated potassium (BK_{Ca}) channel in the mitochondria of the human endothelial cell line EA.hy926. A single-channel current was recorded from endothelial mitoplasts using the patch clamp technique. A potassium-selective current was detected with a mean conductance equal to 270 pS in a symmetrical 150/150 mM KCl isotonic solution. The channel activity, which was determined as the open probability, increased with the addition of calcium ions and the potassium channel opener NS1619. Conversely, the activity of the channel was irreversibly blocked by paxilline and iberiotoxin, BK_{Ca} channel inhibitors. The open probability was found to be voltage-dependent. The substances known to modulate BK_{Ca} channel activity influenced the bioenergetics of mitochondria isolated from human endothelial cells. In isolated mitochondria, 100 μM Ca^{2+}, 10 μM NS1619 and 0.5 μM NS11021 depolarized the mitochondrial membrane potential and stimulated non-phosphorylating respiration. These effects were blocked by iberiotoxin and paxilline in a potassium-dependent manner. Under phosphorylating conditions, NS1619-induced, iberiotoxin-sensitive uncoupling diverted energy from ATP synthesis during the phosphorylating respiration of the endothelial mitochondria. Immunological analysis with antibodies raised against proteins of the plasma membrane BK_{Ca} channel identified a pore-forming α-subunit and an auxiliary β2 subunit of the channel in the endothelial mitochondrial inner membrane. In conclusion, we show for the first time that the inner mitochondrial membrane of human endothelial cells EA.hy926 contains a large-conductance Ca^{2+}-activated potassium channel with properties similar to those of the surface membrane BK_{Ca} channel.

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ORGANIZATION OF THE THYLAKOID MEMBRANE MODEL AND LHCII PHOSPHORYLATION

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In higher plants capturing and conversation of light energy, to the form that can be used to perform the metabolic processes of living organisms during photosynthesis, takes place in the thylakoid membranes of the chloroplasts. This inner system of membranes is highly dynamic and able to adapt to variable environments condition.

The lipid multi-bilayer formed with plant lipids: monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) and modified by the largest photosynthetic antenna complex LHCII have been studied. In the research, there have been used two types of LHCII antenna complex: non-phosphorylated (isolated from dark-adapted spinach leaves) and phosphorylated (isolated from spinach leaves pre- illuminated by strong light).

X-ray diffraction, spectroscopic and microscopic measurements revealed, that membranes which contained the non-phosphorylated complexes were formed trans-layer, supramolecular structures, which are stabilized by hydrogen bonds. These structures provide a scaffold for lipid bilayers and allow the formation of the grana structures. In the lipid bilayer which contain phosphorylated LHCII have been observed aggregated lamellar structures in the plane of the membranes. The obtained results show that the process of antenna protein phosphorylation and its side reorganization in the thylakoid membranes constitute a regulating mechanism of grana formation from lipid-protein bilayers. Moreover, LHCII phosphorylation facilitates creation of aggregate structures, capable of quenching of excess excitation via non-radiative excitation energy dissipation.

The results have been used as a basis for creating a model of the thylakoid membranes of a chloroplast under normal light and under light stress co

THE INFLUENCE OF *Scutellaria baicalensis* MAIN FLAVONOIDS ON THE PROAPOPTOTIC ACTIVITY OF ANTICANCER DRUGS IN CANCER AND NORMAL HUMAN CELLS

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The beneficial effects of flavonoids in cancer therapy are often linked with their lack of toxicity against normal cells, the possibility of oral administration, low cost and general acceptance.

Accumulating evidence demonstrates that *Scutellaria baicalensis*, multi-purposed herb used in traditional Chinese medicine, possesses potent anticancer activity. In particular, its clinically important active compounds baicalin (BLIN), baicalein (BLEIN) and wogonin (W) have been reported to be primarily responsible for the cytotoxicity of this herb toward a range of cancer cell lines.

In this study the effects of above mentioned flavonoids on the proapoptotic activity of doxorubicin (DOX) and taxanes: docetaxel (DTX) and paclitaxel (PTX) in estrogen-responsive MCF-7 breast cancer cell line has been investigated. Human endothelial cell line HUVEC-ST as a model for normal cells has been also included in the experiments.

Externalization of membrane phosphatidylserine as an early marker of apoptosis was estimated by flow cytometry. Cells were preincubated for 24 hours with IC_{10} and IC_{50} concentrations of flavonoids and then incubated for 2 hours with DOX, DTX or PTX. After treatment the cells were cultured in fresh medium for 0, 12, 24 and 48 h.

Our studies showed that flavonoids did not hamper the proapoptotic activity of DOX and taxanes. Moreover, baicalin enhanced cytotoxic effect of chemotherapeutics. In contrast, combinations of anticancer drugs with BLIN displayed the lowest toxicity towards normal cells. These results showed an attractive activity of baicalin in combination with DOX and taxanes – decreased toxicity of anticancer drugs toward normal cells and enhancement of their proapoptotic activity in cancer cells.

POLYUNSATURATED FATTY ACIDS AND THEIR METABOLIC PRODUCTS IN THE DIAGNOSTICS OF HUMAN BREAST CANCER TISSUE

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Techniques commonly used in breast cancer diagnostics are: mammography, ultrasound, and finally confirming diagnosis the histopathological examination of tissue taken during a biopsy. We will present results obtained by: Raman spectroscopy, Raman imaging, and IR spectroscopy, on the identification and spectroscopic analysis of benign and malignant human breast cancer changes. Particular attention will be paid to the spectral ranges characteristic for lipids, carotenoids, proteins and water. In the analysis of the vibrational spectra characteristic for lipids range, will be presented a comparative analysis of the spectra of the human noncancerous and cancerous breast tissues with spectra of fatty acids: oleic acid, representatives of n-6 fatty acids: linoleic acid, g - linolenic acid, arachidonic acid and representatives of n-3 fatty acids, such as a-linolenic acid, eicosapentaenoic and decosahexaenoic acids and products of lipid peroxidation, [1-4].

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CHANGES IN THE VIABILITY AND ANTIOXIDATIVE SYSTEM OF ERYTHROCYTES AND HUMAN BLOOD MONONUCLEAR CELLS CAUSED BY BROMFENVINPHOS CONTAMINANT

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Bromfenvinphos is organophosphorus insecticide, which is considered to be effective in controlling of ectoparasite in farm animals, dogs and cat's fleas as well as other domestic insects. Since 2003, bromfenvinphos (as Apifos preparation) has been successfully used against the *Varroa destructor*, mite that causes bees varroosis in the species *Apis mellifera* and *Apis Cerana*. Unfortunately, this preparation was withdrawn due to lack of its specific MRL (*maximum residue limit*) values. In order to indroduce Apifos in the market again, it is necessary to conduct additional toxicity tests of bromfenvinphos and its impurities.

We investigated the effect of bromfenvinphos contaminant: 1-bromo-2-(2,4-dichlorophenyl)-2-etho-xy ethene on erythrocytes and human blood mononuclear cells (*in vitro*). The cells were exposed to different concentrations of these compound (0.1; 0.5; 5; 10; 50; 250 and 500 μM) for 1 and 4 h.

The following parameters were determined in the erythrocytes: the level of methemoglobin and reduced glutathione, reactive oxygen species formation, size and shape of the cells and the activity of acetylcholinesterase.

In human peripheral blood mononuclear cells the following parameters were analyzed: viability, size and granulation of the cells and lipid peroxidation level.

The bromfenvinphos contaminant induced a slight increase in hemolysis level (about 2%), oxidation of hemoglobin (about 20%) and oxidation of R123 (about 200%) and a slight change in shape and size of the erythrocytes. No effect was observed in acetylcholinesterase activity and the concentration of reduced glutathione. This compound showed high toxicity to human peripheral blood mononuclear cells as evidenced by the decrease in viability (about 60%) and considerable changes in size (decreased about 55%) and granularity (increased about 35%) of the cells studied.

THE NANOSTRUCTURE OF PECTINS DURING THEIR PHYSIOLOGICAL DEGRADATION

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Pectins are cell wall polysaccharides which undergo dynamic changes during growth, pre- and postharvest ripening. Pectin network is considered as a matrix surrounding cross-linked glucans. It also links neighboring cells through middle lamella, plays a role of plastificator and is a main water binding factor in the cell wall. In pectins dynamic enzymatic transformations occur such as de-esterification and depolymerization, what has a significant influence on the texture of fruits and vegetables.

The aim of the work was characterization of structural changes in pectins during natural postharvest ripening. Pectins were isolated from carrot during three months of storage as three fractions: water soluble pectins (WSP), chelator soluble pectins (CSP) and

diluted alkali soluble pectin (DASP). Atomic force microscopy and infrared spectroscopy were the main tools applied to observation of pectins structure.

Direct visualization of the pectin fractions clearly depicted structural differences. The WSP molecules characterized granular structure with very rare chain-like molecules. The CSP fraction contained branched molecules chains of several hundred nanometers long and diameter of few nanometers. Fibers extracted in DASP fraction were the longest and had a capability to create regular network-like structures. During storage a reduction of dimensions of WSP was observed as a result of enzymatic degradation. In CSP fraction both decrease of fibers length and number of junction zones between fibers was found. DASP fraction clearly lost regular cross-linking between main and side chains during postharvest ripening. Enzymatic degradation proceeded sequentially, starting from demethylation following de-polymerization and separation of side chains.

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AMPHOTERICIN B - AN OLD DRUG, NEW IDEAS

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Very high efficiency, but also the high toxicity of amphotericin B lead to the search for modifications of the drug to obtain more favorable pharmacological properties. These modifications can be made at the appropriate antibiotic formulation or modification of its chemical structure. Another possibility of the modification is the complexation of the antibiotic with transition metal ions such as ions of copper (II). Analysis of the electron absorption spectra as well as the circular dichroism indicate that the amphotericin B in aqueous solutions at pH values ranging from 10.5 to 11.0 complexes with Cu^{2+} ions with 2:1 stoichiometry. These complexes are stable at physiological pH values. In the Raman spectra of the Cu $(AmB)_2$ complex it was observed the ν_1 band shift towards lower frequencies, which was associated with the change of the polarizability of the AmB chromophore, induced by Cu^{2+} ions. The change of the chromophore polarizability may increase the hydrophobicity of the complex, which can influence its biological activity. In studies on standard strains of *Candida albicans* increase in fungistatic and fungicidal properties of the Cu $(AmB)_2$ complex was observed as compared to Fungizone - the formulation currently used in therapy. The increased antifungal activity of the complex did not result from the sum of the toxic effect of AmB and copper ions, but it is a unique feature of this complex. Among factors influencing the increased biological activity of the complex may be a different spatial structure of the complex, compared to AmB. Another factor can be transport of Cu^{2+} ions, together with complexes, directly to the cell membrane of the fungal cell.

COMPARISON OF TRANSPORT POLYMER MEMBRANE MODIFIED WITH ONE- AND TWO-SIDED METHOD OF ION IMPLANTATION

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The topic of research is to compare the transport properties of polymer membranes with a thick-ness of 12μm made of polycarbonate (PC) and poly (ethylene terephthalate) (PET), single and double-sided modified by ion implantation. Implantation ion in polymer samples using ion beam implanter UNIMAS 79. In these studies, we use N^{+1} ion beams with energies 180 keV and a dose of $1 \times 2 * 10^{14}$ (modified single-sided) and $2 \times 1 * 10^{14}$ ions/cm^2 (double-sided modifica-tion). The idea of this task is to obtain the dou-ble-sided modified membrane whose surface is modified by ions of the same dose per unit area of the membrane, as in the case of one-sided membrane.

The aim of research is to explain the conduc-tivity changes of diffusion of polymer membra-nes resulting from implantation. In this study, we have shown previously that a change in membrane surface topography (increase the active surface area) as well as the change of surface wettability improves conductivity coefficient of diffusion. Current research is aimed to explain how the coefficient changes depending on the method of implantation. In order to explain this the polymeric films were subjected to infrared spectroscopy studies (FTIR), atomic force microscopy (AFM) and the contact angle (CA). However, transport prope-rties, i.e. conductivity coefficient of diffusion have been studied using laser interferometry.

THE IMPACT OF NEAR-INFRARED RADIATION (NIR) ON THERMO-TOLERANCE IN HUMAN ERYTHROCYTES

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Human erythrocytes exposed to temperature 44°C become resistant to second thermal shock at a temperature 48.5°C. The phenomenon of thermotolerance in human erythrocytes reaches a maximum after 3 hours incubation at 37°C between the shock and disappears the after 7, 8 hours.

The aim of the study was cause thermotolerance in human erythrocytes by NIR (wavelength of 700-200 nm). Human erythrocytes (2% hematocrit) were subjected to radiation of 6.9 mW/cm^2 in 5-20 minutes

Obtained results indicate that 10 and 15 minute exposure to NIR causes resistance to temperature 48.5°C (2nd shock). The kinetics of appearance and disappearance of the thermotolerance caused by NIR is different than caused by thermal shock. Increase in ATPase activity is accompanied with thermotolerance caused by thermal shock and by near-infrared radiation.

EFFECT OF PIROLIN AND DOXORUBICIN ON MITOCHONDRIAL MEMBRANE POTENTIAL IN MCF-7 AND MDA-MB-231 BREAST CANCER CELLS

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Breast cancer chemotherapy employing doxorubicin (DOX) is associated with numerous side effects, especially severe cardiotoxicity. It is believed that oxidative stress generated by DOX is the main reason of its high cardiotoxicity due to naturally impaired antioxidant system of heart myocytes. In this context low molecular, nonimmunogenic and cell permeable compounds with antioxidant and chelating properties, such as nitroxides, seem to be good candidates for serving as cytoprotectors. A desirable property of such compound is to not hamper anticancer activity of chemotherapeutic.

In this study the effect of pyrroline nitroxyl derivative Pirolin (PL) on the collapse of mitochondrial transmembrane potential (DY_m) of DOX-treated breast cancer cells was investigated. Impaired mitochondrial potential is one of the fastest response of cells to proapoptotic stimuli. Changes in DY_m were assessed using the fluorescent probe JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolocarbocyanine iodide). Fluorescence of JC-1 monomers and dimmers was measured over 0-180 min period after the treatment in order to estimate the kinetics of DY_m changes caused by the investigated compounds. Cells preincubated with 5 μM of carbonyl cyanide m-chlorophenylhydrazone (CCCP), an uncoupler of oxidative phosphorylation, served as a positive control.

Pirolin used alone and in combination with DOX caused both depolarization and hyperpolarization of mitochondrial membrane, nevertheless cells treated with PL recovered faster their DY_m than cells incubated with DOX. Preincubation with PL did not improve DY_m alterations caused by DOX. Changes in DY_m persisted longer in estrogen-negative MDA-MB 231 cells which suggested their higher sensitivity to the investigated compounds.

APPLICATION OF QUANTUM DOTS TO DETECT ANTIOXIDANT PROPERTIES OF PHENOLS

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Absorption, emission and fluorescence lifetimes of CdTe quantum dots in aqueous and deuterated solutions as well as in the presence of some polyphenols with antioxidant properties have been measured. It has been shown that fluorescence quenching of cadmium telluride quantum dots occurs in the presence of substances with antioxidant properties. We observed the linear relation between fluorescence quenching of CdTe quantum dots and content of polyphenols. The obtained results were used to set up a simple and fast method for determination of total antioxidant activity and total content of naturally occurring polyphenols. The possible mechanisms responsible for the observed interaction between CdTe quantum dots and catechin have been discussed.

Applied fluorescence quenching method of CdTe quantum dots to estimate the total antioxidant activity, due to its better specificity, may be alternative to more elaborate Folin-Ciolteau method.

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MOLECULAR TRANSPORT OF AMINO ACIDS IN GELS PROBED BY INTERFEROMETRIC TECHNIQUE

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We study molecular transport of amino acids in a diffusion system consisting of two cells separated by a horizontally located polymer membrane. We have filled the upper cuvette of the diffusion cell with agarose hydrogel solvent while in the lower one there has been an aqueous gel solution of transported substance. Then, the substance diffuses from the lower cuvette to upper one. Since the concentration gradient is in vertical direction only, the diffusion is expected to be one-dimensional. We follow the diffusion process of amino acids. For each measurement we prepared different concentration solution of agarose in water (gek samples) and the same gel dripped by the amino acid. The diffusion can be characterized by a time evolution of the so-called near -membrane layer (NML) or concentration boundary layer (CBL) where the concentration of diffusing substance drops *k* times [1]. When the thickness of NML- %u0111, grows in time as $t^{ß}$ with ß *= 0.5* we deal with normal or gaussian diffusion. If ß *> 0.5* there is superdiffusion and when ß *< 0.5* we have a subdiffusive behaviour [1,2,3]. To observe the time evolution of CBL we employed the laser interferometric technique [1,2,3]. The analysis of interferograms allows reconstructing the time dependent concentration profiles of the substance transported in gels.

Our results show that the thickness concentration boundary layers are smaller than in normal diffusion, and transport is consequently said to be subdiffusive.

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THE EFFECT OF DIAMOND NANOPARTICLES ON THE LEVEL OF GLUTATHIONE, AND ENZYMES INVOLVED IN ITS METABOLISM IN LUNG CANCER CELLS

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With the development of nanotechnology, the new methods of synthesis and surface modification of diamond nanoparticles are seen. Diamond nanopowders are diamond particles with sizes below 100 nm, although in practice, this limit is about 10 nm. Nanodiamonds with the smallest sizes could penetrate cell membranes, which would affect the functioning of the cells, causing changes in redox homeostasis. Cells have antioxidant system which protects them against changes in redox homeostasis and involves the sweeping of free radicals, which includes glutathione and enzymes involved in the metabolism.

In this study we used lung cancer cell line (A549), which was treated by nanodiamonds at concentration 0-100 μg/ml for 24, 48 and 72 hours. We observed changes in the level of glutathione, as well as the activity of glutathione peroxidase, glutathione reductase and glutathione transferase. The changes in the enzyme activities were founded which was associated with glutathione in a tested cell line, and these changes were dependent on the concentration of this diamond and time incubation.

UTILIZATION OF MAGNETOTHERAPEUTIC DEVICE IN THE STIMULATION OF THE SEED GERMINATION AND DEVELOPMENT OF ONION (*Allium cepa* L.)

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The alternating magnetic field is widely used in rehabilitation and therapy from many years. Magnetotherapy and magnetostimulation are used as supporting therapy as well as individual therapies.

Utilization of magnetic field in stimulation of seeds and plants development is a topic of long standing experiments carried out in many research centres. Application of magnetic field leads to better germination capacity and plant development. It affects also some taste values of selected, economically significant plants.

In the carried out researches the VIOFOR JPS device used in medicine and exploited in Poland was utilised for magnetostimulation of onion seeds.

Obtained results show the improvement in germination capacity and taste values of some varieties of onion such as *Allium cepa* L. cultivated in Poland.

INFLUENCE OF PHOTOSYSTEM II ANTENNA COMPOSITION ON THE TRAPPING RATE OF ELECTRONIC EXCITATION

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Photosystem II (PSII) is a large membrane protein complex composed of a core, consisting of two reaction centers and core antenna, and peripheral antenna. LHCII complexes (consisting of apoproteins Lhcb1, 2, and 3) are the major part of the peripheral antenna and are connected to the core via minor peripheral antenna, called CP29, CP26, and CP24 (the apoproteins of these antenna are called Lhcb4, 5, and 6, respectively). The antenna absorb the light, convert its energy into electronic excitation of chlorophyll , and transfer it to reaction centers, where it is utilized to initiate the electron transfer. Thus, reaction centers act as quenchers of electronic excitation. Consequently, fluorescence decay measurements in PSII allows determination of excitation lifetime in antenna. In the presented work, influence of Photosystem II peripheral antenna composition on the trapping rate of electronic excitation by reaction centers was studied. Modifications of the antenna composition were introduced by mutations. The polypeptide composition in each mutant was determined biochemically. PSII-enriched membrane fragments were then studied by fluorescence time-resolved technique and correlations between polypeptide composition and fluorescence decay lifetimes were observed in order to determine the role of particular polypeptides in excitation energy transfer from the peripheral antenna to reaction centers.

STRUCTURAL CAUSES OF SEMI-LAMELLAR AGGREGATION OF PROTEOLIPOSOMES

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Chloroplast membranes which conduct the „light" photosynthetic phase are the most common type of membrane bilayer in the world. The specific mechanism of protein-lipid interactions within these membranes is essential for photosynthetic, energetic efficiency of the process. Photosynthetic efficiency is directly bound to the degree of membranes' stacking. Membrane aggregation and fluidity are varying due to environmental conditions for example. That's why it is very important to create simpler, useful model of such a membrane for more elementary study.

The attempt was made to create such a model. We used proteoliposomes build with plant galactolipids, with incorporated LHCII trimeric antennae. This is our basic model, with some modifications like the level of saturation acyl lipid chains or the level of aggregation each of the components (lipid and protein alike), it could be very useful for explanation of mechanisms which occurs in natural, thylakoid membranes.

Our preliminary model of aggregating proteoliposomes was based on spectroscopic analyses (Fourier Transformed Infrared Spectroscopy, fluorescence in 77K), as well as on microscopic studies (confocal laser scanning microscopy, atomic force microscopy). For the very first time such a model was used. We shown that proteoliposomes' structure is very similar to the native ones, so we think that this model can be used with success as thylakoids' research simplifier.

EXCITATION ENERGY TRANSFER AND PRIMARY STEPS OF ELECTRON TRANSFER IN PHOTOSYSTEM I FROM GREEN ALGA *Chlamydomonas reinhardtii*

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Photosystem I (PSI) is a large pigment-protein complex embedded in the thylakoid membrane, which uses light energy to drive the transmembrane electron transport. It consists of the core and the peripheral light harvesting complexes (LHCI). PSI core has its own antenna system containing ~90 chlorophyll *a* molecules bound to the protein matrix. The central part of the PSI core, called the reaction center (RC), is provided with two quasi-symmetrical branches of electron carriers (A and B). The purpose of the antenna chlorophylls is to absorb the light and transfer the excitation energy to the RC, where in the charge separation process the electron transport is initiated.

The aim of our research is to obtain a consistent description of the electronic excitation migration and charge separation in Photosystem I, using two complementary ultrafast spectroscopic techniques: femtosecond transient absorption and time-resolved fluorescence measurements by streak camera. Our measurements are carried out for preparations based on green alga *Chlamydomonas reinhardtii*, both for isolated PSI core and PSI-LHCI complexes, as well as whole living cells.

So far we have managed to clarify some controversial issues relating to the functioning of Photosystem I, such as: (1) reversibility of the primary charge separation leading to reproduce the excited state, (2) the involvement of each of the electron carriers branches in the charge separation process, (3) the time of excitation energy transfer from LHCI to the PSI core, (4) the presence of so-called *red chlorophylls* in the PSI antenna systems, (5) the emission spectrum of the excited RC.

EXCITATION ENERGY TRANSFER BETWEEN FMN MOLECULES MONITORED BY FLUORESCENCE INTENSITY DECAY

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Flavins play an important role in biological processes. The existence of FMN dimers in biological and photoreceptor systems has been shown by many authors. Presence of dimers may be important in photoreception phenomena. Therefore it is very interesting to examine the nonradiative energy transfer processes in the ensemble of FMN monomers and dimers.

The task of this paper was to abtain additional information on the mechanism of monomer – dimer energy transfer for FMN in water using time-resolved techniques. The FMN concentrations (from $1.05 \cdot 10^{-5}$ M to $2.84 \cdot 10^{-1}$ M) were prepared in water (pH 7.0). Mean fluorescence lifetimes were determined from fluorescence intensity decays using the Fluotime 200 spectrofluorometer (Picoquant) ($lambda_{ex}$=473nm).

It was found that at low concentrations, in the absence of energy migration and trapping, the FMN fluorescence intensity decay is single exponential. From C= $1.98 \cdot 10^{-2}$M the decay is accelerated with initially slight deviations from single exponential character because of multistep energy transfer between FMN monomers and trapping. It was found that fluorescence intensity decays are strongly accelerated in the presence of dimers due to excitation energy trapping. The mean fluorescence lifetime of FMN at low concentration t= 4.67 ns, whereas at the highest concentration it attains 0.43 ns. Mean localization time of excitation energy and the number of its jumps between FMN molecules were calculated versus concentration by Monte–Carlo method.

The localization time $\tau_{loc}=\tau_0 /(n+1)$, where n is the mean number of excitation energy jumps. The mean fluorescence lifetime was significantly longer than the mean localization time at monomers because excitation energy walks randomly within the set of monomers and is transferred finally to dimers. The effect of material diffusion enhances the efficiency of energy migration.

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THE PHOTOACOUSTIC SIGNAL OF SCOTS PINE NEEDLES FROM DIFFERENT ENVIRONMENTS

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At present, photoacoustic spectroscopy is one of the measuring techniques providing detailed information on the photosynthesis system and structure of plants. Unfortunately, the applicability range of most of parameters is limited to the homogeneous samples. However, as evidenced in our previous studies (Szurkowski, 2001), such a condition is not fulfilled in the case of Scots pine (*Pinus silvestris* L.) needles.

The aim of the work was to demonstrate the differences in the photoacoustic signal of Scots pine needles from different environments. The needles used in the studies were collected in the spring of 2011. The sampling stations were situated in the Tri-city (Gdańsk) area and in a place about 100 km from the agglomeration (Sominy).

Since the measurements were performed at the modulation frequency of 20 Hz, the photoacoustic signal originated from mesophyll of parenchyma cells containing chloroplast, for Scots pine needles. Both the signatures of the photoacoustic signal amplitude, in the presence of the strong light background (i.e., equivalent of adsorption spectrum in photoacoustic spectroscopy), and of the signal phase, measured under the condition of the sample illumination with the measuring light beam alone, exhibited an apparent variability with the needle age and place of the sample collection.The variability of the photoacoustic spectra had already been studied earlier, for Scots pine needles, although the phase spectra were obtained and interpreted here for the first time. These phase spectra allow one to determine the change in oxygen evolution yield with the light wavelength of the irradiation. With the needle aging, the ratio of phase for the Soret to red bands decreases. For the samples collected in Sominy the ratio was significantly higher than determined for the samples originating from Gdansk center.

THE VOLTAGE AND FLUX CHARACTERISTICS OF TWO-MEMBRANE SYSTEMS UNDER DIFFUSIVE AND CONVECTIVE CONDITIONS

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The study was conducted for the two-membrane system with bacterial cellulose membrane (CB), and acetate cellulose membrane (N) and ternary solutions of water, ethanol and KCl. The membranes were placed in horizontal planes and separated three compartments with solutions of equal volumes . The Ag|AgCl electrodes located in outer chambers, 5 mm from the membranes surfaces, were connected to a voltage measurement circuit coupled with a computer. The outer chambers at the initial moment were filled with KCl solutions having a concentration of 10^{-5} mol l^{-1} whereas the chamber between the membranes was filled with ternarny solution with varying concentrations of ethanol and KCl at initial moment (turning off mechanical stirring of solutions). The time characteristics of the voltage between the electrodes were measured after turning off mechanical stirring of solutions. The membrane systems with upper membrane – CB and the lower membrane - N (and vice versa) were analyzed. After turning off mechanical stirring of solutions, the concentration boundary layers (CBLs) were formed at surfaces of the lower and upper membrane. In the case when density of the solution in the chamber between the membranes was lower than density of solutions in external chambers, the convection conditions in CBLs could appear at upper membrane surfaces and did not appear at surfaces of the lower membrane. The convective stirring of the solutions was the cause of voltage pulsation in time due to the periodical changes of KCl concentrations at the surface of one of the electrodes. Analyzing of received voltage characteristics it can be stated that the moment of appearance of voltage pulsations (connected with hydrodynamic instabilities in the membrane system) depend non-linearly on the initial concentrations of ethanol and KCl in the chamber between the membranes. Moreover, the nature and temporal evolution of voltage in the membrane system depend on the initial concentrations of ethanol and KCl in the chamber between membranes. The characteristics of the initial time after which the pulsations arise as a function of concentration of ethanol have also complex and non-linear character.

CONVECTION DISORDERS OF KCl CONCENTRATIONS IN THE MEMBRANE SYSTEM: TIME CHARACTERISTICS OF VOLTAGE FOR DIFFERENT ELECTRODE DISTANCES FROM THE MEMBRANE

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The studies of the membrane system with bacterial cellulose membrane, placed in horizontal plane, with aqueous solutions of KCl were carried out. Both Ag|AgCl electrodes were placed in the chamber with an initial KCl concentration equal to 10^{-5}mol l^{-1}. One of the electrodes (the reference electrode) was located 14 cm from the surface of the membrane and the second electrode (active) was placed at different distances from the membrane in the range from 1 to 12 cm. Initial KCl concentration in the second chamber was 10^{-2} mol l^{-1}. After filling the chambers with solutions of KCl and equalization of the pressure in the chambers the voltage between the electrodes was measured. The solution with higher KCl concentration (solution with higher density) was placed above the membrane. In this case, diffusive formation of concentration boundary layers (CBLs) at the surfaces of the membrane could lead to the hydrodynamic instability and could cause convective stirring of solutions in chambers. These instabilities occur when the solution density gradient in CBLs, directed oppositely to the gravitational field vector, reaches sufficiently large value for which the calculated Rayleigh number for CBL reaches critical value (1700) . The disorders of KCl concentration at the electrode surface, observed as voltage pulsations in time, were caused by hydrodynamic instability in chambers of the membrane system. The determination of the dimension of the convection cell (by changes of position of active electrode) and analysis of time needed to the appearance of voltage pulsations was the aim of the study. According to the preliminary measurements, sift of the active electrode further away from the membrane causes increase of time after which the pulsations of voltage caused by hydrodynamic instabilities appear. The time required for the appearance of concentration disorder at the surface of the active electrode, connected with the convection stirring of solution, is proportional to the distance of electrode from surface of the membrane.