S2.160. Investigation of photodynamic properties of octacationic complexes of magnesium and zinc phthalocyanine on a model lipid membrane

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The method of photodynamic therapy is effective for combating cancer and as a way of deactivating pathogenic microorganisms resistant to antibiotics [1]. Thesearch for new effective photosensitizers (FS),requires the methods to study their adsorption and photodynamic properties in vitro in a system similar in structure to the cell membrane. In the present work, such a system was a bilayer lipid membrane, which is a good model of a cell membrane. The adsorption and photodynamic activity ofocta-cationic complexes of phthalocyanine (Pc) with magnesium ion (8bMgPc) or zinc (8bZnPc), which were synthesized at IPCE RAS, on BLM wasstudied by measuring the potential jump at the BLM-solution boundary. The molecules of these compounds have 8 positively charged groups on the periphery and are distinguished by a metal ion in the center of the macro ring.The changes in the boundary potential ΔΦb were measured by 3 methods: by the intramembrane field method (IFC) developed in the laboratory [2], by changes in the conductivity of BLM induced by nonactin, as well as by the electrophoretic mobility of liposomes.
The adsorption of FS led to a change in ΔΦb, the sign of which corresponded to the binding of positively charged molecules to BLM. The values of ΔΦb for 8bMgPc and 8bZnPc were close, which indicates a slight influence of the nature of the metal ion in the center of the molecule on their adsorption properties. The values of ΔΦb measured during the adsorption of 8bMgPc by the IFC method coincided with the values of ΔΦb determined by the change in the conductanceof BLM induced by nonactin. This indicates that 8bMgPc molecules do not penetrate through the BLM. The slopes of the dependence of ΔΦb on the concentration of Pc in solution significantly exceeded the value predicted by the modelassuming that the charges of the adsorbed compounds lie on the membrane surface, and the potential jump satisfies the Gouy-Chapman theory [2]. The values of ΔΦb measured by the IFC method significantly exceeded the values of zeta-potential. These results can beexplainedassuming that the charged groups of 8bMgPc and 8bZnPc immerse into the hydrophobic region of the BLM.
An increase in the conductanceof the membrane during the binding of 8bZnPc added to the aqueous solution of the cell, which eventually decayed to the initial value,was detected. Itwas explained by the rearrangement of lipids surrounding 8bZnPc molecules in the membrane, resulting in conductive defects in the BLM. The addition of cholesterol to DPhPC (30 mole %) did not affect the appearance of conductivity, but accelerated its decay. The increase in conductanceduring the adsorption of 8bMgPcwas not observed, allowing the conclusion that the conductance is caused by the interaction between the zinc ion in the 8bZnPc molecule and phospholipids in BLM.
The photodynamic activity of phthalocyanineswas evaluatedby measuring the rate of damageof the molecules of di-4-ANEPPS used astargets of singlet oxygen under BLM illumination. The damageof di-4-ANEPPS molecules was detected as disappearance of the dipole potential jump created by them on the surface of the BLM. The target and Pc molecules were on oppositesurfacesof the membrane to exclude their direct interaction, and the damage of di-4-ANEPPS moleculeswas due to their oxidation by singlet oxygen penetrating through the BLM. The rate R of damage of di-4-ANEPPS proportional to the stationary concentration of singlet oxygen in the membrane was determined from the kinetics of change in ΔΦb under BLM illumination and its recovery in the dark. The dependences of R on the concentration of 8bMgPc and 8bZnPc in solution were close to each other. The parameter R increases linearly with theconcentration ofall Pcin the range of 10^(-8)-10^(-6) M, and reaches a plateau at concentrations above 10^(-5) M. This decline from the linear dependence at high concentrations of Pccan be explained by the quenching of singlet oxygen by phthalocyanine molecules in the membrane [3].
Finally, one can conclude that the nature of the metal ion in the center of the molecule of the studied phthalocyanines has little effect on their adsorption and photodynamic properties, but affects the structural rearrangements of the lipid bilayer caused by the incorporation of Pc molecules into the membrane. Charged groups of the studied Pc immerse into the hydrophobic region of the BLM, and the adsorption of 8bMgPc molecules occurs without their penetration through the membrane. The rate R of damage of molecules of di-4-ANEPPS characterizing the photodynamic efficiency of phthalocyanines, increases with their concentration in solution, but ceases to grow at high concentrations due to the quenching of singlet oxygen by phthalocyanine molecules in the membrane.
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S2.161. Investigation of the membrane activity of protein E of SARS-CoV-2 coronavirus

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COVID-19 infection, caused by the SARS-CoV-2 coronavirus, has led to the largest pandemic since the Spanish flu in 1918. In view of this, development vaccines and antiviral drugs that can stop the spread of this infection has become an acute issue. Currently, in the search for antiviral drugs against COVID-19, much attention is paid to the study of the structure of the receptor-binding domain of the surface protein S. However, the emergence of new SARS-CoV-2 coronavirus strains indicates its high variability, which reduces the effectiveness of vaccines and antiviral drugs. At the same time, the envelope protein E of this virus is membrane-active and shows a rather high conservatism. Despite the critical importance of this protein in the coronavirus life cycle, the physicochemical mechanisms of its interaction with cell membranes still remain unclear.
The aim of this work was to investigate the membrane activity of protein E of the SARS-CoV-2 coronavirus on models of giant unilamellar vesicles and lipid nanotubes. As a result, it was found that the protein forms pores in the lipid bilayer, i.e. performs the main function of viroporin. In addition, protein E is able to deform lipid membranes and form double-membrane vesicles depending on the concentration.
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S2.162. Isolated rats heart lvp dynamics hyperpolarization activated currents blockade in acute myocardial infarction model

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Hyperpolarization activated current present on the membrane of atypical cardiomyocytes, is the main initiator of diastolic depolarization of action potentials in the sinoatrial and atrioventricular nodes, regulating the heart rate. Some cardiac dysfunctions, sinus node dysfunction, atrial fibrillation, ventricular tachycardia, and atrioventricular block, have been associated with altered function of HCN channels present on the membrane of working cardiomyocytes. Increased ventricular HCN current is seen in hypertrophy, ischemic cardiomyopathy, and heart failure.

The study of the effect of If blockade in an isolated heart with an experimental model of acute myocardial infarction is an important and perspective direction.

The aim of this study is to investigate the effect of If blockade on the force of contraction of the Langendorff-isolated rat heart with an experimental model of acute myocardial infarction.

The model of acute myocardial infarction was reproduced by placing a ligature on the left descending coronary artery. Twenty-four hours after coronary artery ligation, the acute stage of myocardial infarction developed, confirmed on the electrocardiogram by the presence of a pathological ST wave. The control group included healthy animals. LVP (left-ventricular pressure) dynamics were studied in experiments on isolated rat hearts by Langendorff method. Hyperpolarization-activated currents were blocked with ZD7288 at concentrations of 10-9 M and 10-5 M (Sigma).

When the If blocker (10-9 mol) was added to the perfused solution in the control group of healthy animals we observed a 13% ($p<0.001$) increase in pressure developed by left ventricular myocardium, in the group with experimental model of acute myocardial infarction LVP increased by 12% ($p<0.05$). When ZD7288 (10-5 M) was added to perfusion solution in healthy animals the pressure developed by the left ventricle decreased by 13% ($p<0.001$), and in the group with acute myocardial infarction model the studied index decreased by 29% ($p<0.05$).

Thus, blockade of hyperpolarization-activated currents has a multidirectional effect on the pressure developed by the left ventricle of the isolated heart of healthy rats and with the experimental model of myocardial infarction. Possibly, 24 hours after ligation of the left coronary artery, the density and number of HCN channels change, affecting the inotropic function of the isolated rat heart with an experimental model of myocardial infarction.

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S2.163. Kinetics and mechanisms of oxidative hemolysis of erythrocytes under the action of azo- and peroxide initiators

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When the cell antioxidant system is disrupted, the amount of active oxygen metabolites increases. It causes various oxidative damage, leading the cell to oxidative stress [1]. The most dangerous manifestation of oxidative stress is membrane lipid peroxidation (LPO). In such state, the cell ceases to perform important physiological functions. It leads to the development of a number of pathological conditions of the body, including cardiovascular diabetes mellitus, and different forms of neurodegenerative diseases. Therefore, at present one of the basic areas of chemical biology and medicinal chemistry is the search and investigation of substances with antioxidant properties and the subsequent development of pharmacological preparations based on them. This determines the relevance and practical significance of the development of biological models for effective testing of these compounds for antioxidant activity.

The effectiveness of the developed model is primarily determined by the quality of LPO initiation in a biological and by the presence of a reliable quantitative criterion, which will effectively differentiate the pro- and antioxidant components in the action of chemical compounds on a biological object. In our work erythrocytes was used as biosubstrate. An important step in the development of such a model is the selection and study of possible chemical initiators of lipid peroxidation in the cell membrane, belonging to different classes of chemical compounds. This work is devoted to the study of peroxide hemolysis of erythrocytes under the action of two initiators of lipid peroxidation, 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH), belonging to the class of diazo compounds, and tert-butyl hydroperoxide (t-BuOOH), from the class of organic peroxides.

AAPH in an aqueous medium undergoes monomolecular thermal decomposition, with the formation of two alkyl radicals and the release of molecular nitrogen. The process proceeds with sufficient efficiency already at 35-40 ○C. In an oxygen environment, alkyl radicals react rapidly with oxygen to form peroxyl radicals, which attack the double bonds of membrane lipids from the outside, initiating lipid peroxidation in the membrane.

Tert-butyl hydroperoxide (t-BuOOH), unlike AAPH, is not able to spontaneously decompose into radicals at physiological temperatures. Easily penetrating through the plasma membrane of the cell due to the highly lipophilic tert-butyl fragment, t-BuOOH triggers a cascade of complex, not fully understood, reactions involving hemoglobin, during which radical products are formed that initiate the LPO process in the membrane.

We found that under the action of the studied initiators, the content of TBARS in the erythrocyte membrane increases and erythrocyte hemolysis is observed, which is consistent with the available literature data. At the same time, the kinetic patterns of hemolysis under the action of these compounds differed significantly.

In a wide range of concentrations, the kinetics of hemolysis of the 0.2% suspension of mouse erythrocytes under the action of AAPH and t-BuOOH was studied. The experimental values characterizing the change in the degree of hemolysis over time were approximated in the Origin program by the sigmoidal Boltzmann function. Both compounds caused a concentration-dependent hemolytic effect.

The hemolytic activity of AAPH and t-BuOOH was characterized by the value of the hemolysis induction period, which was determined graphically by the time to reach 10% hemolysis. In the case of AAPH, the hemolysis induction period depended linearly on the initial concentration of the initiator, while a similar dependence for t-BuOOH was significantly nonlinear and was well approximated by a biexponential function, where k1 and K2 are 2•10-2 and 65•10-2, respectively. Such a character of the dependence may indicate the presence in the system of two different factors that affect the osmotic balance of the cell in opposite ways, which can lead to a slowdown in the rate of hemolysis with an increase in the concentration of the initiator. This is directly confirmed by the earlier plateauing of hemolytic curves at high concentrations of t-Buoy, which leads to hemolysis arrest.

Although both studied initiators are capable of activating LPO in erythrocyte membranes, their hemolytic effect has some peculiarities. The linear change in the hemolysis induction period with increasing AAPH concentration is consistent with the concept of AAPH as a monomolecular generator of peroxide radicals in an aqueous medium. At the same time, the hemolytic response of the system to the action of t-BuOOH appears to be essentially non-linear. This indicates that the interaction of t-BuOOH with the cell may include processes that are not limited to peroxidation of the lipid substrate. These circumstances should be taken into account in the possible practical use of these compounds as inducers of oxidative hemolysis of erythrocytes.

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