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Role of electrostatics in the binding of charged metallophthalocyanines to neutral and charged phospholipid membranes

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Abstract

Binding of the cationic tetra(tributylammoniomethyl)-substituted hydroxoaluminum phthalocyanine ($AlPcN_4$) to bilayer lipid membranes was studied by fluorescence correlation spectroscopy (FCS) and intramembrane field compensation (IFC) methods. With neutral phosphatidylcholine membranes, $AlPcN_4$ appeared to bind more effectively than the negatively charged tetrasulfonated aluminum phthalocyanine ($AlPcS_4$), which was attributed to the enhancement of the coordination interaction of aluminum with the phosphate moiety of phosphatidylcholine by the electric field created by positively charged groups of $AlPcN_4$. The inhibitory effect of fluoride ions on the membrane binding of both $AlPcN_4$ and $AlPcS_4$ supported the essential role of aluminum–phosphate coordination in the interaction of these phthalocyanines with phospholipids. The presence of negative or positive charges on the surface of lipid membranes modulated the binding of $AlPcN_4$ and $AlPcS_4$ in accord with the character (attraction or repulsion) of the electrostatic interaction, thus showing the significant contribution of the latter to the phthalocyanine adsorption on lipid bilayers. The data on the photodynamic activity of $AlPcN_4$ and $AlPcS_4$ as measured by sensitized photoinactivation of gramicidin channels in bilayer lipid membranes correlated well with the binding data obtained by FCS and IFC techniques. The reduced photodynamic activity of $AlPcN_4$ with neutral membranes violating this correlation was attributed to the concentration quenching of singlet excited states as proved by the data on the $AlPcN_4$ fluorescence quenching.

Keywords: Sensitized photoinactivation; Gramicidin A; Sulfonated phthalocyanine; Bilayer lipid membrane

1. Introduction

Phthalocyanines represent a continuously growing class of dyes based on tetrapyrrole nucleus and thus are closely related to porphyrins [1]. They are widely used in different fields of chemistry as colourants, photoconducting agents in photocopying machines, electrocatalysts, etc. [2]. Those of them having a diamagnetic central metal atom have proved to be potent photosensitizers producing singlet oxygen with a high quantum yield [2] and thus have been a focus of research for use in photodynamic therapy [2–4]. Recently there has appeared a new field of their application — photodynamic inactivation of bacteria, which is of particular interest as an alternative approach to treating infectious diseases [5,6]. The benefit of this method consists in its independence to antibiotic resistance of pathogenic bacteria.

Positively charged photosensitizers, in particular cationic phthalocyanines have proved to be most efficient in photodynamic inactivation of both gram-negative and gram-positive bacteria [5,7–9]. The reason is believed to lie in the electrostatic interaction of cationic dyes with negatively charged sites at the outer surface of the bacterial cell wall, which facilitates the binding of photosensitizer molecules to bacterial cells. So far the binding of cationic phthalocyanines has not been characterized. Here we present the results of studying the adsorption and photodynamic activity of

Abbreviations: BLM, bilayer lipid membrane; gA, gramicidin A; DPhPC, diphytanoylphosphatidylcholine; DPhPG, diphytanoylphosphatidylglycerol; DGEPC, dipalmitoyl-glycero-ethylphosphocholine; $AlPcS_4$, aluminum tetrasulfophthalocyanine; $AlPcN_4$, hydroxoaluminum (III) tetra(tributylammoniomethyl)phthalocyanine chloride; FCS, fluorescence correlation spectroscopy; IFC, intramembrane field compensation method; $\Delta\phi_b$, difference of boundary potentials; CTAB, cetyltrimethylammonium bromide

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a positively charged aluminum phthalocyanine on model lipid membranes in comparison with the data on the properties of tetrasulfonated aluminum phthalocyanine ($AlPcS_4$) bearing the negative charge.

2. Materials and methods

2.1. Synthesis of $AlPcN_4$

Hydroxoaluminum (III) tetra(tributylammoniomethyl)phthalocyanine chloride (Fig. 1) was synthesized according to the reaction:

$$HOAlPc(4\text{–}CH_2Cl)_4 + N(C_4H_9)_3 \rightarrow HOAlPc(4\text{–}CH_2\text{–}N^+(C_4H_9)_3Cl^-)_4$$

Hydroxoaluminum (III) 2,9,16,23-tetrakis(chloromethyl)phthalocyanine (150 mg, 0.20 mmol) was dissolved in 6 ml DMF, 24.2 ml 5% alcohol tributylamine solution was added. The reaction mass was heated to boiling and kept during seven hours. After cooling the reaction mass was poured into 50 ml of water and filtered. A filtrate was vapoured under vacuum. The solid was washed with dimethyl ether and dried to yield 190 mg (63%) of hydroxoaluminum (III) tetra(tributylammoniomethyl)phthalocyanine chloride ($AlPcN_4$). Anal. Calc.,%: C 68.53; H 9.27; N 10.99; Cl 8.71. $C_{84}N_{12}H_{129}Cl_4AlO$. Found,%: C 67.63; H 8.72; N 11.27; Cl 9.51.

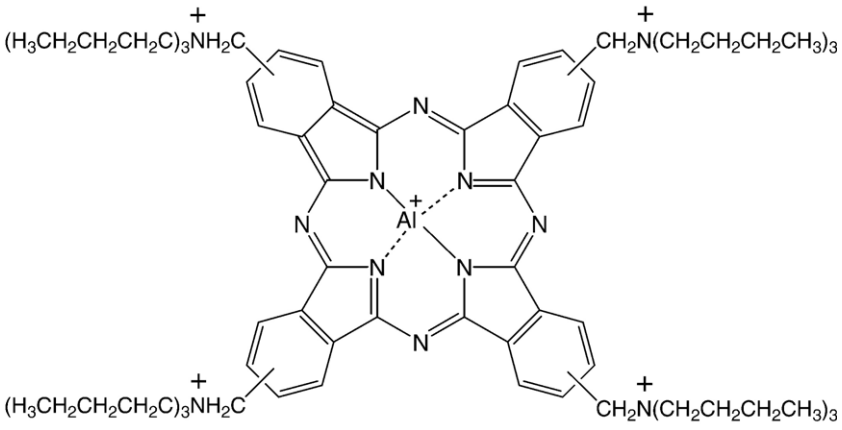
2.2. Binding of phthalocyanines to phopholipid vesicles measured by fluorescence correlation spectroscopy (FCS)

FCS was shown to be a useful technique in the measurements of binding of fluorescent molecules to lipid vesicles [10]. We carried out FCS measurements on a home-made FCS setup including an Olympus IMT-2 inverted microscope with a 40×, NA 1.2 water immersion objective (Carl Zeiss, Jena, Germany). A 633-nm He–Ne laser was used for excitation. The fluorescence that passed through a dichroic beam splitter and a long-pass filter (640DRLP and 670DF40, Omega Optical, Brattleboro, VT) was imaged onto a 100 μm core fiber coupled to an avalanche photodiode (PerkinElmer Optoelectronics, Fremont, CA). The signal from an output was correlated by a correlator card (Correlator.com, Bridgewater, NJ). The data acquisition time at each location within the mixer was 30 s, and three to five curves were averaged.

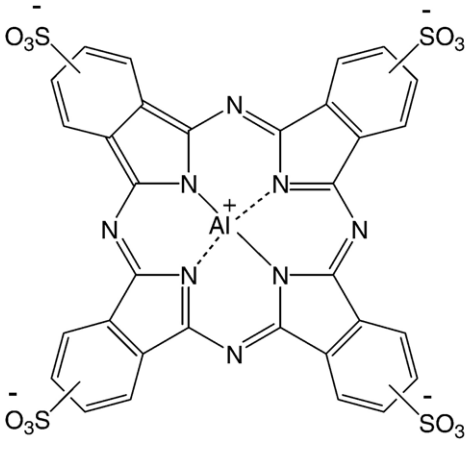
Experimental curves were fit by the correlation function for three-dimensional diffusion:

$$G(\tau) = 1 + \frac{1}{N}\left(\frac{1}{1+\frac{\tau}{\tau_D}}\right)\left(\frac{1}{\sqrt{1+\frac{r_0^2\tau}{z_0^2\tau_D}}}\right) \quad (1)$$

with τ_D being the characteristic correlation time during which a molecule resides in the observation volume of radius r_0 and length z_0, given by $\tau_D = r_0^2/4D$, where D is



$AlPcN_4$



$AlPcS_4$

Fig. 1. Structure of $AlPcN_4$ and $AlPcS_4$.

the diffusion coefficient, N is the mean number of molecules in the confocal volume.

Liposomes were prepared by evaporation under a stream of nitrogen with a 2% solution of egg yolk phosphatidylcholine, diphytanoylphosphatidylglycerol (DPhPG) or brain phosphatidylserine in chloroform (all from Avanti Polar Lipids) followed by hydration with a buffer solution containing 100 mM KCl, 10 mM Tris, 10 mM MES, pH 7.0. The mixture was vortexed, passed through a cycle of freezing and thawing, and extruded through 0.1-μm pore size Nucleopore polycarbonate membranes using an Avanti Mini-Extruder.

2.3. Measurements of the boundary potential difference of planar bilayers by the intramembrane field compensation (IFC) method

Bilayer lipid membranes were formed by the brush technique [11] from the solution of diphytanoylphosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) or monooleoyl-glycerol (Sigma) in decane (15 mg/ml) on the 0.8–1.2 mm hole in the teflon septum. Electrical measurements were performed using silver-chloride electrodes with agar bridges. The bridges were made from standard plastic pipette tips (for a 200-μl sampler), the bottom part of which was closed by agar, and the remaining volume was filled by the 0.1 M KCl solution. Total electrical resistance of the electrodes with the bridges did not exceed 50 kΩ. Thinning of the membrane was monitored as an increase in its capacitance by measuring the electric current initiated by alternating voltage of the triangle waveform applied to the membrane. As it was shown in [12,13], the dependence of the planar bilayer capacity on the transmembrane voltage can be applied for the estimation of the difference in the surface potentials of two membrane–water interfaces. This technique was improved considerably by [14,15] and was applied successfully for studying the binding of charged peptides [16] and other polymers [15] to planar membranes. Our setup was described in [17]. Briefly, 350 Hz sine voltage (G3-112 functional generator, Russia) after filtration from the 700 Hz sine component was applied to an Ag/AgCl reference electrode connected to one side of the membrane by an agar–agar bridge. The other electrode (from the other side of the membrane) was connected to a Keithley 428 current amplifier with feedback resistance of 1 MΩ. The second harmonic 700 Hz signal was detected with a model 5209 lock-in amplifier (Signal Recovery, Oak Ridge, TN). The minimum in the 700 Hz signal upon application of different DC offset voltages was searched manually every minute in order to measure the kinetics of the changes in the difference of boundary potentials. The sensitivity of the setup was enough to measure the boundary potential difference with the accuracy of less than 1 mV.

2.4. Sensitized photoinactivation of gramicidin-mediated current in planar bilayers

Bilayer lipid membranes (BLMs) were formed on a 0.55-mm diameter hole in a Teflon partition separating two compartments of a cell containing aqueous solutions of 100 mM KCl, 10 mM MES, 10 mM Tris and 0.05 mM EDTA at pH 7.0. The membrane-forming solutions contained 20 mg diphytanoyl-glycero-phosphocholine (DPhPC, Avanti Polar Lipids) in 1 ml *n*-decane, or a mixture of 14 mg DPhPC and 6 mg diphytanoyl-glycero-phosphoglycerol (DPhPG, Avanti Polar Lipids) in 1 ml *n*-decane (negatively charged lipid), or a mixture of 14 mg DPhPC and 6 mg Dipalmitoyl-Glycero-3-Ethylphosphocholine (DGEPC, Avanti Polar Lipids) in 1 ml *n*-decane (positively charged lipid). Gramicidin A (gA) was added from stock solutions in ethanol to the bathing solutions at both sides of the BLM and routinely incubated for 15 min with constant stirring. Experiments were carried out at room temperature (24–26 °C). Aluminum tetrasulfophthalocyanine ($AlPcS_4$) was from Porphyrin Products, Logan, UT). The dyes were added to the bathing solution at the *trans*-side (the *cis*-side is the front side with respect to the flash lamp). The electric current (I) was recorded under voltage-clamp conditions. The electrical current (I) was measured with a Keithley 428 amplifier, digitized by a LabPC 1200 (National Instruments, Austin, TX) and analyzed using a personal computer with the help of WinWCP Strathclyde Electrophysiology Software designed by J. Dempster (University of Strathclyde, UK). Ag–AgCl electrodes were placed directly into the cell and a voltage of 30 mV was applied to BLM. BLMs were exposed to 20-s continuous illumination with a halogen lamp ("Novaflex", World Precision Instruments, USA) providing an incident power density of 30 mW/cm^2 or single flashes produced by a xenon lamp with flash energy of about 400 mJ/cm^2 and flash duration <2 ms. A glass filter cutting off light with wavelengths <500 nm was placed in front of the lamp. To avoid electrical artifacts, the electrodes were covered with black plastic tubes. Illumination of BLM by visible light in the presence of a photosensitizer is known to suppress the gramicidin-mediated transmembrane current (I) [18–21]. The photoinactivation of gramicidin A in BLM results from the damage to its tryptophan residues caused by reactive oxygen species that are generated upon interaction of excited photosensitizer molecules with oxygen [22–24]. It has been shown that the light-induced decrease in the gramicidin-mediated current is due to the reduction of the number of open channels, while the single channel conductance remains unaltered [18]. Therefore, the relative decrease in the current, $\alpha=(I_0-I)/I_0$, induced by illumination is equal to the damaged portion of gramicidin channels. This parameter enables to compare the efficacy of different photosensitizers.

2.5. Optical measurements

Absorption spectra were measured with a Hitachi 557 spectrophotometer (Japan). Fluorescence emission spectra of phthalocyanines excited at 365 nm were recorded with a Panorama Fluorat 02 (Lumex, Russia) fluorescence spectrophotometer with excitation and emission slits adjusted to 5 nm.

3. Results

According to our previous paper [25], the addition of tetrasulfonated metallophthalocyanines to the bathing solution of a planar bilayer lipid membrane results in the appearance of a difference of boundary potentials ($\Delta\phi_b$) which enables to study the adsorption of these negatively charged dyes on the membrane by the intramembrane field compensation (IFC) method. Fig. 2 compares the data on the binding of $AlPcS_4$ (curve 1) and $AlPcN_4$ (curve 2) on DPhPC (electrically neutral) membranes obtained by this technique. It is seen that $AlPcN_4$ (curve 2) binds to DPhPC membranes more effectively than $AlPcS_4$ (curve 1) as judged by the concentration dependences of the magnitude of $\Delta\phi_b$. As expected, the sign of $\Delta\phi_b$ was positive in the case of $AlPcN_4$ and negative in the case of $AlPcS_4$.

To further elucidate the mechanism of phthalocyanine binding to lipid membranes, we monitored the fluorescence of ensembles of dye molecules by the fluorescence correlation spectroscopy (FCS). First we applied this method to lipid bilayer membranes formed of egg yolk phosphatidylcholine whose surface is

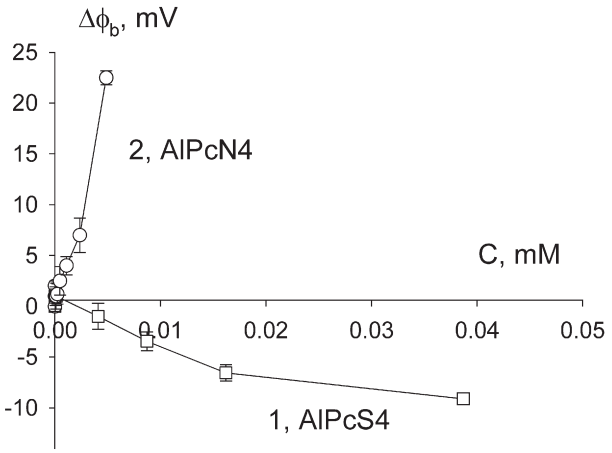


Fig. 2. Concentration dependence of the binding of $AlPcS_4$ (curve 1) and $AlPcN_4$ (curve 2) as measured by the boundary potential difference ($\Delta\phi_b$) after the addition of $AlPcS_4$ and $AlPcN_4$ at one side of the membrane. The solution was 10 mM KCl, 2 mM Tris, 2 mM MES, pH 7.0. The membrane was formed of DPhPC.

uncharged. As it is seen from Fig. 3, the addition of egg phosphatidylcholine liposomes at a concentration of 6.7 μg/ml to the aqueous solution of $AlPcN_4$ led to the appearance of bursts in the fluorescence time trace (A, curve 2) and a shift in the corresponding autocorrelation curve (B) showing an increase in the correlation time from 100 μs to about 10 ms. By contrast, in the case of $AlPcS_4$, bursts in the fluorescence time trace (C, curve 3) and a shift in the autocorrelation curve (D) appeared only at a lipid concentration of 0.67 mg/ml. These results support the conclusion derived from the $\Delta\phi_b$ measurements that $AlPcN_4$ binds to electrically neutral lipid bilayers more effectively than $AlPcS_4$. It should be noted that with $AlPcN_4$ the increase in the egg phosphatidylcholine concentration from 6.7 μg/ml to 0.67 mg/ml led to reduction of the intensity of fluorescence bursts (Fig. 3A, curve 3) which can be attributed to an increase in the amount of lipid vesicles and the corresponding decrease in the number of dye molecules per single vesicle. The similar effect was detected in the study of peptide binding to liposomes [10].

The addition of potassium fluoride to the dye solution reversed the effect of the egg phosphatidylcholine addition on the autocorrelation curve (Fig. 4) both with $AlPcN_4$ (A) and $AlPcS_4$ (B), showing desorption of the dyes from the membrane surface. The inhibitory action of fluoride on the binding of $AlPcS_4$ to DPhPC membranes was earlier detected by other methods [25–27].

To gain further insight in the contribution of electrostatic interactions to the phthalocyanine adsorption on lipid bilayers, we used membranes formed of charged lipids in FCS experiments. With liposomes formed of brain phosphatidylserine, $AlPcN_4$ exhibited practically complete binding at low lipid concentration (6.7 μg/ml) similarly to the case of egg phosphatidylcholine liposomes, whereas $AlPcS_4$ did not bind to these

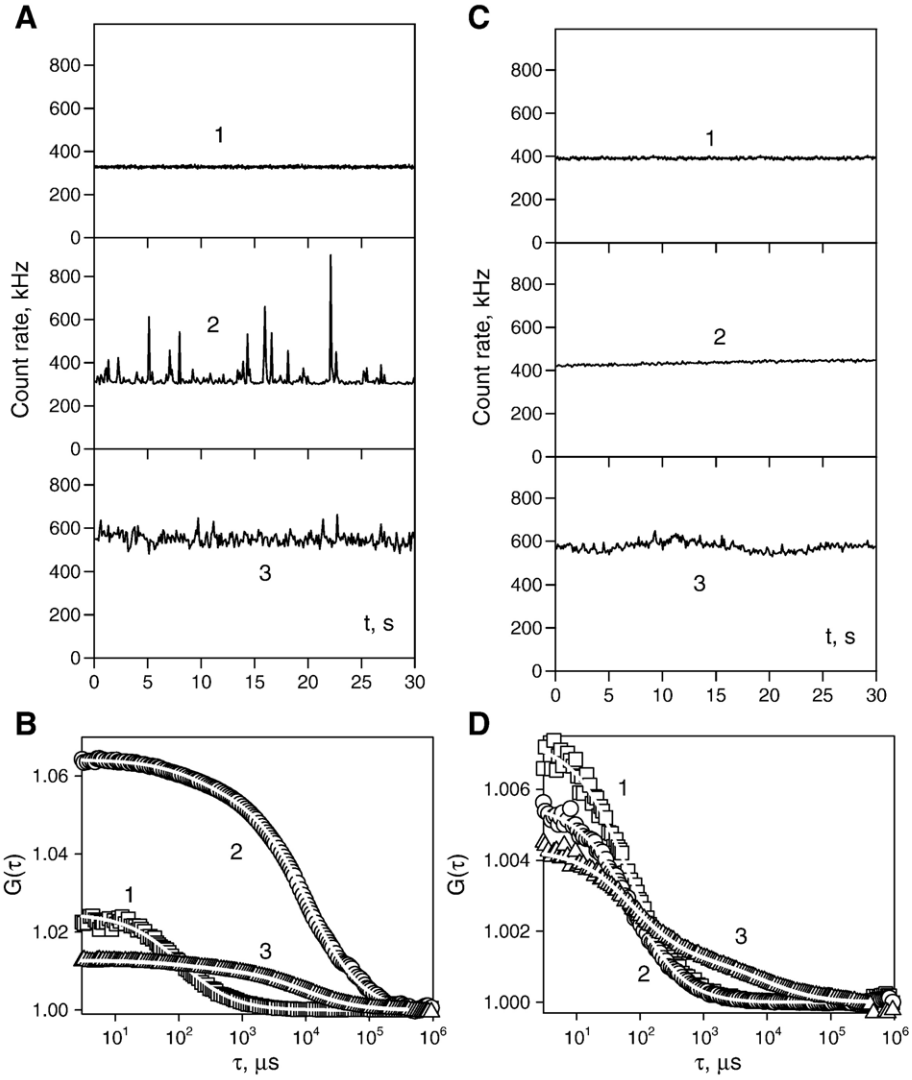


Fig. 3. Binding of $AlPcN_4$ (left side, panels A, B) and $AlPcS_4$ (right side, panels C, D) to phosphatidylcholine liposomes measured by FCS. Count rate traces (panels A, C) and autocorrelation functions (panels B, D). Curves 1: control measurements without liposomes with 300 nM of $AlPcN_4$ (left side) and of $AlPcS_4$ (right side); curves 2: in the presence of 6.7 μg/ml liposomes; curves 3: in the presence of 0.67 mg/ml liposomes. White curves are best fits by the sum of the components described by Eq. (1) with the following values of τ_D: panel B, curve 1, 0.103 ms; curve 2, 0.20 ms (14%) and 11 ms (86%); curve 3, 0.126 ms (18%) and 9.2 ms (82%); panel D, curve 1, 0.066 ms; curve 2, 0.072 ms; curve 3, 0.076 ms (72%) and 9.6 ms (28%).

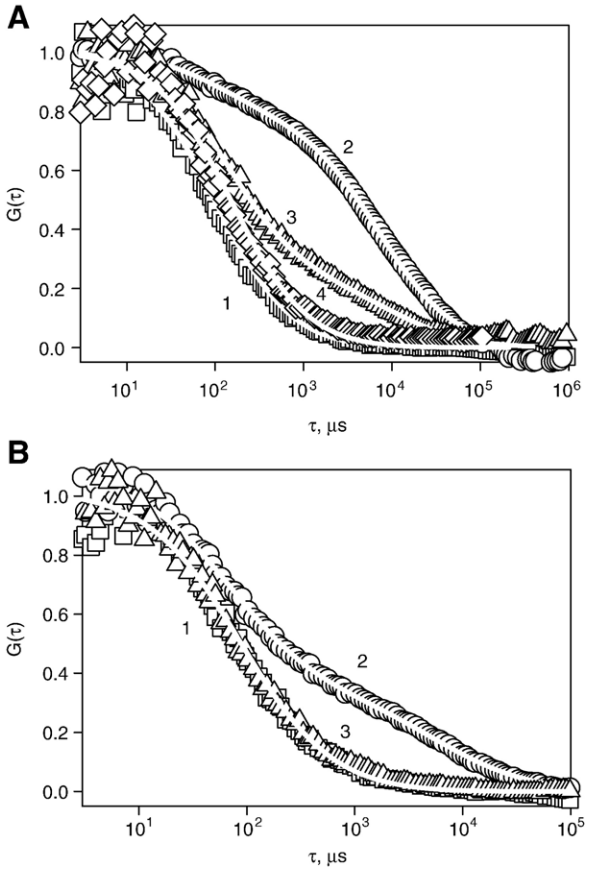


Fig. 4. Effect of KF on the binding of $AlPcN_4$ (A) and $AlPcS_4$ (B) to phosphatidylcholine liposomes measured by FCS. Curve 1 is the autocorrelation function for a control without liposomes with 300 nM $AlPcN_4$ (A) or $AlPcS_4$ (B); curve 2: in the presence of 0.67 mg/ml of liposomes; curve 3: after the addition of 1 mM KF; curve 4: after the addition of 10 mM KF. White curves are best fits by the sum of the components described by Eq. (1) with the following values of τ_D: A, curve 1, 0.10 ms; curve 2, 0.10 ms (22%) and 7.3 ms (78%); curve 3, 0.14 ms (77%) and 10.6 ms (23%); curve 4, 0.15 ms. B, curve 1, 0.09 ms; curve 2, 0.07 ms (69%) and 6.3 ms (31%); curve 3, 0.08 ms.

negatively charged liposomes both at low and high lipid concentrations (data not shown) in contrast to neutral egg phosphatidylcholine liposomes.

To render membranes positively charged, cetyltrimethylammonium bromide (CTAB) was added to the suspension of egg phosphatidylcholine liposomes. Control experiments showed that the concentration of CTAB used (0.2 mM) was far below bromide concentrations which quenched the fluorescence in our system (about 100 mM). As seen from Fig. 5, with $AlPcN_4$, the addition of 200 μM CTAB reversed the effect of the egg phosphatidylcholine addition (0.67 mg/ml) on the autocorrelation curve (A). It can be calculated that this concentration ratio of CTAB and egg phosphatidylcholine corresponds to one positive charge per 12 lipid molecules. By contrast, with negatively charged $AlPcS_4$, the addition of 200 μM CTAB under similar conditions led to disappearance of the ~100-μs component and appearance of the ~10-ms component (B), thereby evidencing the complete binding of $AlPcS_4$ to the positively charged membranes. These results demonstrated the essential role of membrane charge in the adsorption of $AlPcN_4$ and $AlPcS_4$ on the membrane.

According to our earlier study [26], where we examined the photoinactivation of gramicidin channels in planar BLM sensitized by aluminum phthalocyanines of different degrees of sulfonation, binding to a lipid membrane is a prerequisite for the photodynamic action of phthalocyanines on targets residing in the membrane. In the previous work [25] we applied the method of gramicidin photoinactivation to studying the photodynamic activity of tetrasulfonated metallophthalocyanines with different central metal atoms in relation to their adsorption on neutral lipid membranes as measured by the IFC technique. Fig. 6 presents the data on the gramicidin photoinactivation sensitized by $AlPcN_4$ and $AlPcS_4$ in membranes differing in the electric charge, i.e. neutral (curve 1, DPhPC), negatively charged (curve 2, DPhPC/DPhPG) and positively charged (curve 3, DPhPC/DGEPC). It is seen that in the case of DPhPC, at 10^{-7} M $AlPcN_4$ (panel B) displayed higher photodynamic potency than $AlPcS_4$ (panel A),

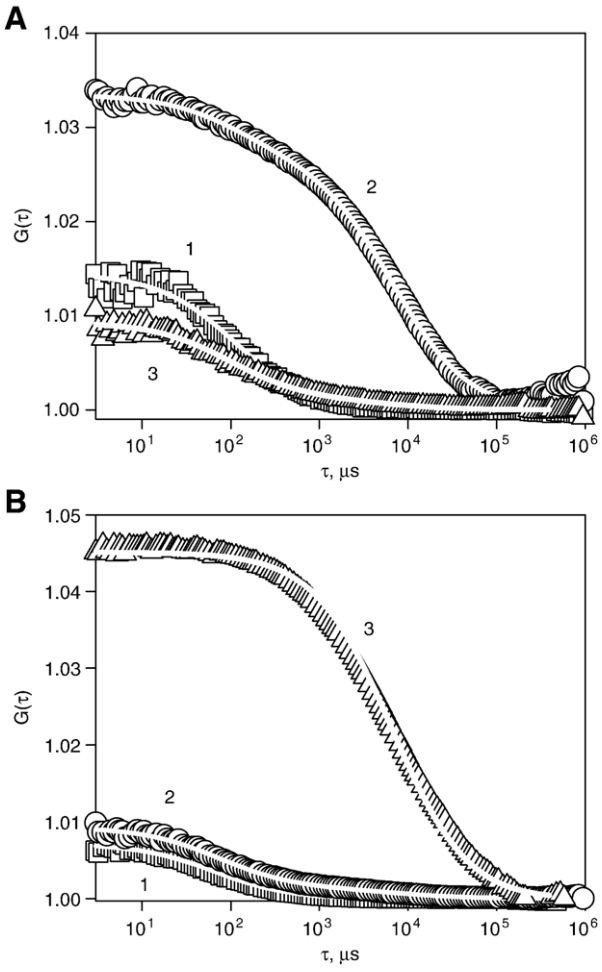


Fig. 5. Binding of $AlPcN_4$ (panel A) and $AlPcS_4$ (panel B) to phosphatidylcholine liposomes measured by FCS. Curve 1 is the autocorrelation function for a control without liposomes with 300 nM $AlPcN_4$; curve 2: in the presence of 0.67 mg/ml of liposomes; curve 3: after the addition of 200 μM CTAB. White curves are best fits by the sum of the components described by Eq. (1) with the following values of τ_D: panel A ($AlPcN_4$) 114 μs; 116 μs (19%), 6900 μs (81%); 100 μs (87%), 6200 μs (13%); panel B ($AlPcS_4$) 66 μs; 74 μs (85%), 7700 μs (15%); 6600 μs.

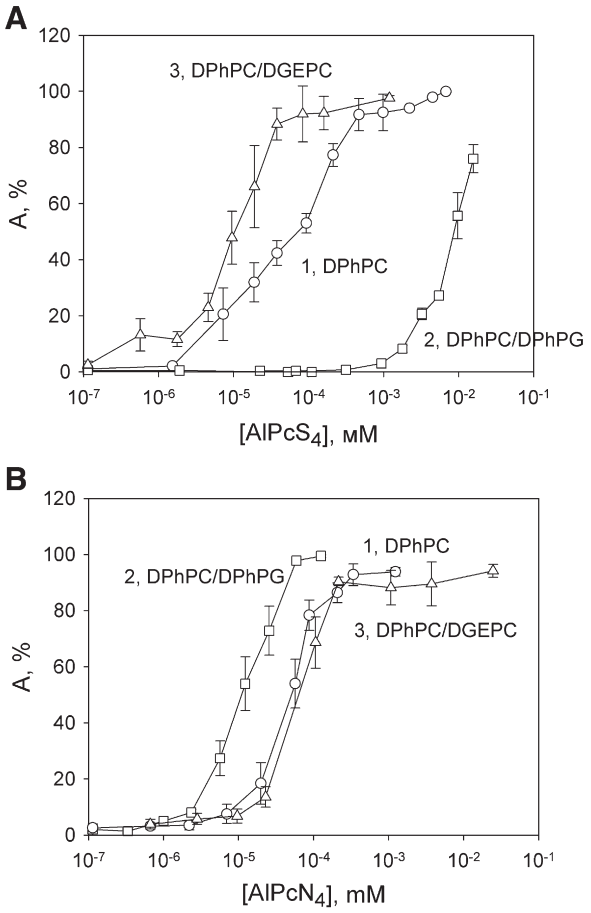


Fig. 6. The dependence of the decrease in the gramicidin-mediated current across a planar lipid membrane formed of different lipids as a result of illumination with visible light during 20 s on the concentration of $AlPcS_4$ (panel A) and $AlPcN_4$ (panel B). The membrane was formed of DPhPC (curves 1), a mixture of DPhPC/DPhPG (curves 2) and a mixture of DPhPC/DGEPC (curves 3). The solution was 100 mM KCl, 10 mM Tris, 10 mM MES, pH 7.

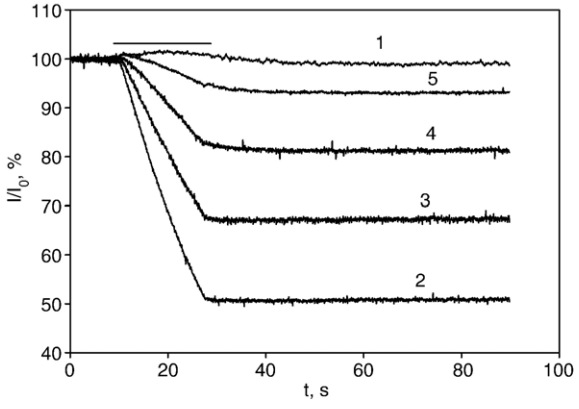


Fig. 7. Effect of different concentrations of fluoride anions on the decrease in the gramicidin-mediated current across a planar lipid membrane as a result of illumination with visible light during 20 s (marked by a bar). Curve 1 is a control, curve 2 was recorded after the addition of 0.15 μM $AlPcN_4$. KF additions were 0.5 mM (curve 3), 1.5 mM (curve 4), and 7.5 mM (curve 5). The membrane was made of DPhPC. The solution was 100 mM KCl, 10 mM Tris, 10 mM MES, pH 7.

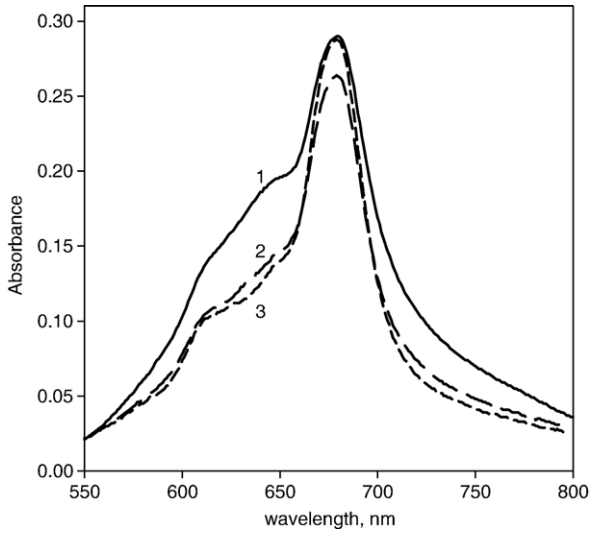


Fig. 8. Absorbance spectra of $AlPcN_4$ in the buffer solution in the presence of different concentrations of egg yolk phosphatidylcholine liposomes (0, 20, and 80 μg/ml for curves 1, 2, and 3, respectively). $AlPcN_4$ concentration was 6 μM. The solution was 100 mM KCl, 10 mM MES, 10 mM Tris, pH 7.0.

in agreement with the binding data (Figs. 1 and 2). The addition of potassium fluoride to the bathing solution prior to illumination inhibited the photodynamic activity of $AlPcN_4$ (Fig. 7), similarly to that of $AlPcS_4$ [26]. On the whole, the concentration dependences of the photodynamic activity of $AlPcN_4$ and $AlPcS_4$ (Fig. 6) correlated with the membrane surface charge, except for one deviation: $AlPcN_4$ had close activity with neutral and positively charged lipid membranes (panel B, curves 1 and 3), though IFC measurements showed that inclusion of positively charged lipids into the membrane resulted in the decrease in adsorption of $AlPcN_4$ on the BLM (data not shown).

To understand the reason of the reduced photodynamic activity of $AlPcN_4$ in the case of neutral membranes, we measured the absorbance and fluorescence spectra of $AlPcN_4$. As seen from Fig. 8, the absorption of $AlPcN_4$ in the red region of the spectrum peaked at 670 nm with a shoulder at 610–630 nm. The addition of egg yolk phosphatidylcholine liposomes led to a decrease in the absorption, especially around the shoulder. More notable changes were observed in the fluorescence spectrum of $AlPcN_4$. The intensity of fluorescence with the peak position at 688 nm markedly decreased upon the addition of liposomes (Fig. 9A, curves 2 and 3). Further increasing the lipid concentration until it exceeded the dye concentration by an order of magnitude led to the partial recovery of the fluorescence intensity (Fig. 9A, curves 4 and 5). By contrast, the fluorescence of $AlPcS_4$ was completely insensitive to the addition of liposomes (data not shown). Based on the dependence of fluorescence intensity of $AlPcN_4$ on the lipid concentration (Fig. 9B), we calculated that the minimum of fluorescence corresponded to about the 1:1 ratio of the dye to lipid molecules.

4. Discussion

In the present paper we characterized the photodynamic activity and the interaction of the newly synthesized positively

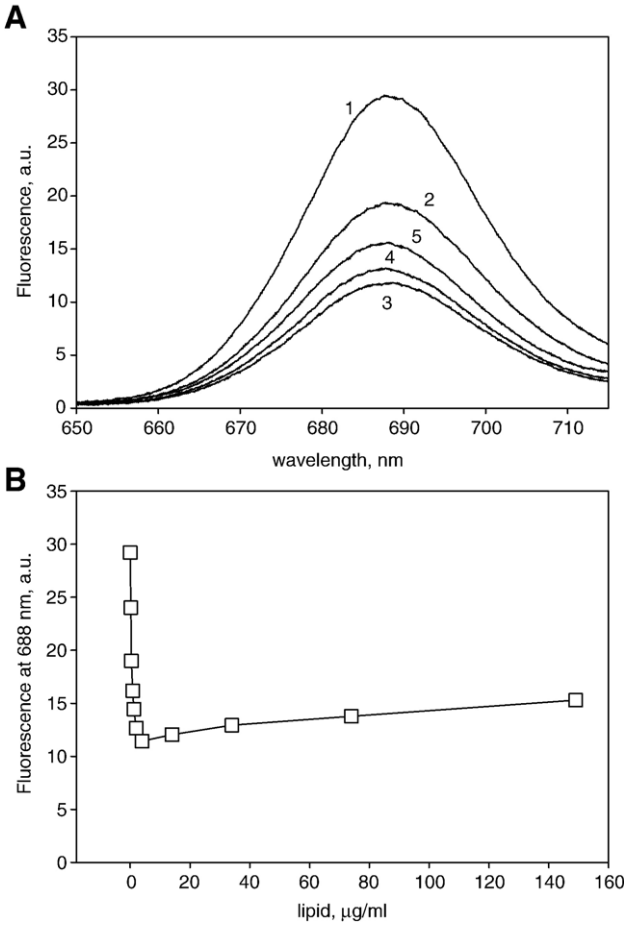


Fig. 9. A. Fluorescence emission spectra of $AlPcN_4$ at different concentrations of egg yolk phosphatidylcholine liposomes (0, 0.4, 4, 34, and 150 μg/ml for curves 1, 2, 3, 4, and 5, respectively). $AlPcN_4$ concentration was 1.7 μM. The increasing concentrations of lipid led to an initial decrease in fluorescence at low lipid concentrations and a subsequent increase in fluorescence at high lipid concentrations. The solution was 100 M KCl, 10 mM MES, 10 mM Tris, pH 7.0. Panel B. The dependence of fluorescence intensity of $AlPcN_4$ at 688 nm on the concentration of egg yolk phosphatidylcholine liposomes. Error bars in panel B were less than the size of the data points.

charged aluminum phthalocyanine $AlPcN_4$ (Fig. 1) with neutral and charged lipid membranes as compared to the properties of the negatively charged tetrasulfonated aluminum phthalocyanine $AlPcS_4$. The structure of $AlPcN_4$ is close to that of cationic substituted phthalocyanine complexes of cobalt reviewed in [28].

The results obtained show the correlation between the photodynamic activity as judged by the sensitized photoinactivation of gramicidin channels and the membrane binding of both $AlPcN_4$ and $AlPcS_4$ as measured by the IFC (Fig. 2) and FCS methods. The latter was applied here for the first time to measure the binding of phthalocyanines to lipid bilayers (Figs. 3–5). In one case the data on the gramicidin photoinactivation did not correlate with those on the photosensitizer binding: $AlPcN_4$ had similar efficacy in sensitizing gramicidin photoinactivation in DPhPC membranes and in DPhPC/DGEPC membranes (Fig. 6), though the presence of positively charged DGEPC suppressed the binding. The results of fluorescence spectral measurements (Fig. 9A) enable to explain the fact that strong binding could reduce the photodynamic activity. It appeared that the interaction of $AlPcN_4$ with neutral liposomes led to significant quenching of the photosensitizer fluorescence which could be attributed to the fluorescence concentration quenching because of adsorption of a large number of dye molecules on a single liposome leading to aggregation [29]. According to the literature [30,31], the fluorescence concentration quenching is accompanied by the reduction of the triplet quantum yield and thus by a decrease in the singlet oxygen yield.

As shown in our previous work [25], the binding of $AlPcS_4$ to phospholipid membranes is driven by the interaction of central aluminum with a phosphate group of phospholipid. It was proved, in particular, by the release of $AlPcS_4$ from the membrane upon the addition of fluoride ions forming the coordination bond with aluminum. Here it was demonstrated that fluoride ions also removed $AlPcN_4$ from the membrane surface (Fig. 4) and suppressed the photodynamic activity of this dye (Fig. 7), which favors the common nature of the interaction of the two photosensitizers with a phospholipid membrane. To explain the increased membrane affinity of $AlPcN_4$ as compared to $AlPcS_4$, one should take into account the effect of electric field created by the different charges at the periphery of phthalocyanine molecules. In the case of $AlPcN_4$, the positively charged tributylammoniomethyl groups would enhance the coordination interaction of aluminum with phosphate, whereas in the case of $AlPcS_4$ the negatively charged sulfonic groups would impede this interaction.

To estimate this influence quantitatively, one should calculate a free energy change (ΔG) resulting from Coulombic interaction of two elementary charges q_1 and q_2.

$$\Delta G = q_1 q_2 / \varepsilon r$$

The distance between charges, r, corresponding to the distance from the edge of the phthalocyanine ring to its center can be taken from the dimensions of the phthalocyanine ring. According to [32], it is about 7 Å. The value of the dielectric coefficient (ε) at the water–membrane interface varied from 5 to 40 in different works [33–35]. Following the estimation described in [36] and adjusting ε to 30, we obtained $\Delta G = 0.43$ kcal/mol. To compare the effect of the electrostatic interaction on the metal–phosphate coordination in $AlPcN_4$ and $AlPcS_4$ containing four positive and four negative charges, respectively, this value should be multiplied by 8 which gives $\Delta G = 3.4$ kcal/mol. At room temperature this value of ΔG corresponds to a difference in binding constants of the order of 300 ($\exp(\Delta G/kT)$). This calculation shows that the proposed mechanism can account for the substantial difference in the affinity of $AlPcN_4$ and $AlPcS_4$ to phospholipid membranes observed in our experiments (Fig. 3).

In view of above consideration the effect of the membrane surface charge on the binding of these two dyes can be explained by changes in the nearmembrane concentration of charged molecules due to formation of a Gouy–Chapman electric double layer, though the very process of the dye adsorption can proceed through the metal–phosphate coordination. However, under the conditions of strong electrostatic interaction (low ionic strength), the binding of charged dye molecules to oppositely charged membrane surface may not involve the coordination interaction. This is supported by the absence of the fluoride effect on the

binding of $AlPcN_4$ to liposomes of brain phosphatidylserine at 25 mM KCl, as measured by FCS (data not shown).

The present study of the properties of the positively charged aluminum phthalocyanine is relevant not only to understanding the mechanisms underlying the distribution and photodynamic activity of photosensitizers in different (especially malignant) tissues, but also to elucidation of their interaction with charged cell walls of pathogenic microorganisms.

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