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The interaction of phthalocyanine with planar lipid bilayers

Photodynamic inactivation of gramicidin channels

Tatiana I. Rokitskaya, Yuri N. Antonenko and Elena A. Kotova

A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russian Federation

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The effect of phthalocyanines, the potent photodynamic sensitizers, on the electric properties of the bilayer lipid membrane (BLM) is studied. It is shown, that tetrasulfonated, as well as trisulfonated, aluminium phthalocyanine do not alter the conductance of BLM, but elicit certain changes in the boundary potential difference, which points in favor of dye adsorption on BLM. Under the conditions of intense visible light irradiation, the phthalocyanines cause an increase in the conductance, resulting in the irreversible breakdown of BLM, formed from soy bean phosphatidylcholine, but fail to change the conductance of BLM, formed from diphytanoilphosphatidylcholine. The phthalocyanine-sensitized inactivation of gramicidin channels incorporated into BLM is observed under the conditions of weak visible light irradiation using an He-Ne laser. The photodynamic blockage of model ionic channels is considerably suppressed after oxygen depletion. The phenomenon consists of a marked reduction of a number of open channels, probably due to photomodification of tryptophan residues, essential for gramicidin functioning. The mechanism of the channel inactivation, involving the photosensitized reaction of the II type, and the relevance to the interaction of sensitizers with biomembranes, is discussed.

Photodynamic action; Phthalocyanine; Planar bilayer lipid membrane; Gramicidin; Ionic channel

1. INTRODUCTION

During the last years phthalocyanines have been intensively studied as potent near-infrared photodynamic sensitizers capable of being retained in tumors, and are thus very promising phototherapeutic agents [1,2]. So far the question of the main targets remains one of the key problems in studying the mechanisms of photodynamic cell inactivation. In a great number of cases, membranes have been identified as critical targets for photomodification leading to cell killing [3]. Both lipid peroxidation [4] and protein modification [5] can be induced by illumination of cells with visible light in the presence of phthalocyanines. To our knowledge there are no data in the literature concerning interaction of phthalocyanines with artificial bilayer lipid membranes (BLMs). Meanwhile BLMs per se, and those containing incorporated proteins, represent a good model for studying the mechanisms of phthalocyanine action on lipid and protein components of cell membranes. Here we present the results of measurements of phthalocyanine effects on the conductance and the boundary potential difference of BLM, the latter being indicative of dye adsorption on the membrane. Photodynamic inactivation of the ionic channel, gramicidin A, in BLM sensitized by aluminium tetra- and trisulfophthalocyanine was discovered and characterized. Preliminary data on the similar effect of another photosensitizer, Bengal rose, were published recently by Strässle and Stark [6].

Correspondence address. E.A. Kotova, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russian Federation.

Abbreviations: BLM, planar bilayer lipid membrane; $\Delta\phi_b$, boundary potential difference; $AlPcS_3$, trisulfonated aluminium phthalocyanine; $AlPcS_4$, tetrasulfonated aluminium phthalocyanine.

2. MATERIALS AND METHODS

BLM were formed by a conventional technique [7] on a 0.4-mm diameter hole in a Teflon partition separating two aqueous compartments from a decane solution of 2% diphytanoylphosphatidylcholine (Avanti Polar Lipids). In the experiment shown in Fig. 1, phosphatidylcholine from soy beans (Sigma) is used instead of a synthetic one. Typically bathing aqueous solutions in the cell contained 1 M KCl, 1 mM KH_2PO_4, pH 7.2. In the experiment presented in Fig. 6 the solution was 10 mM KCl, 10 mM KH_2PO_4, pH 7.2. Currents were amplified by a patch-clamp amplifier (OPUS, Moscow) with an output to a recorder. BLM were illuminated with an He-Ne laser with an incident power density (I) of 30 mW/cm^2. In the experiment of Fig. 1 a slide projector lamp providing I = 250 mW/cm^2 was used for irradiation of BLM. In this case the actinic light passes through a glass filter transmitting light with $\lambda > 500$ nm. The difference of boundary potentials ($\Delta\phi_b$) was measured by the method of inner membrane field compensation by recording a capacitive current of double frequency according to [8].

Gramicidin A was a gift from Dr. S. Zakharov (Institute of Soil Science and Photosynthesis, Pushchino). Tetrasulfo- and trisulfophthalocyanines ($AlPcS_4$ and $AlPcS_3$, respectively) were kindly provided by Dr. M.G. Galpern (Research Institute of Organic Intermediates and Dyes, Moscow). The degree of sulfonation was verified by HPLC

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similarly to [9], and the elemental composition is confirmed by microanalysis of C, H, N, O and S content.

3. RESULTS AND DISCUSSION

When added to the bathing solution of BLM, the phthalocyanines do not produce any effect on the membrane conductance in the dark (data not shown) nor during exposure of BLM to weak red light produced by the He-Ne laser. However, at the onset of illumination of BLM with a slide projector lamp providing an incident power density (I) of about 250 mW/cm^2, increasing fluctuations of the electric current appear which results in membrane rupture in approx. 10 min (Fig. 1). The BLM disruption is observed only with membranes formed from soy bean phosphatidylcholine and not with those from diphytanoilphosphatidylcholine, in agreement with data obtained on hematoporphyrins [10] where membrane rupture was ascribed to the process of photodynamic lipid peroxidation.

When gramicidin A is incorporated into BLM the induced conductance is almost completely blocked by irradiation of BLM with the He-Ne laser in the presence of phthalocyanine (Fig. 2). This inactivation of ionic

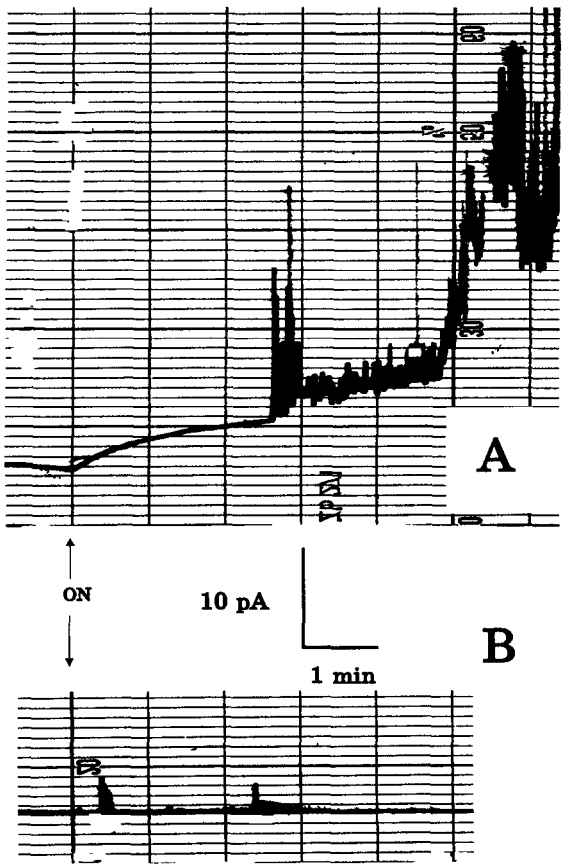

Fig. 1. The effect of visible light irradiation (250 mW/cm^2) on the conductance of BLM in the presence of 1 μM $AlPcS_4$. (A) BLM formed from phosphatidylcholine from soy beans. (B) BLM formed from diphytanoylphosphatidylcholine.

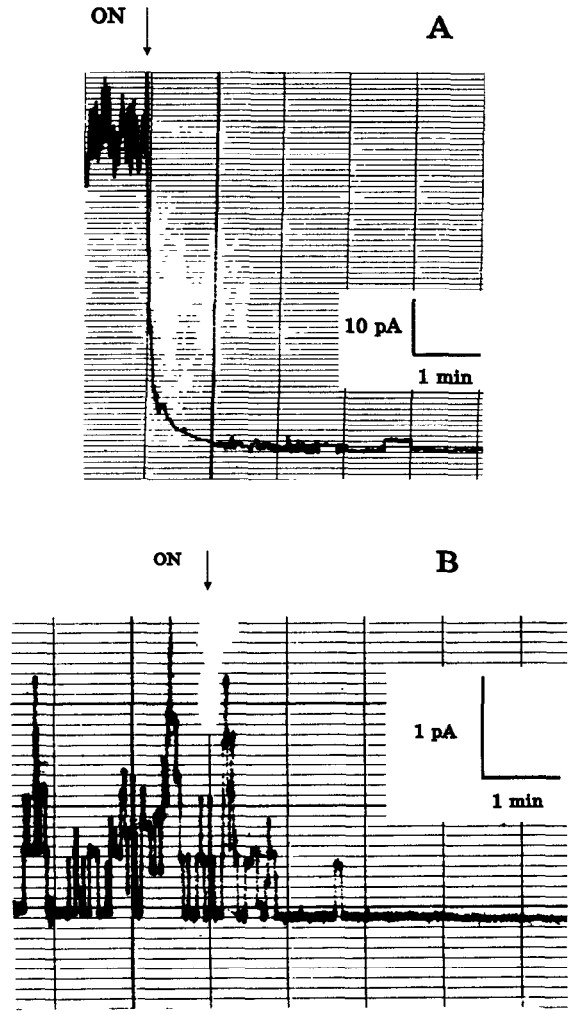

Fig. 2. The effect of visible light irradiation (30 mW/cm^2) on gramicidin-induced BLM conductance in the presence of phthalocyanine. (A) $4 \cdot 10^{-12}$ M gramicidin, 1 μM $AlPcS_3$. (B) $2 \cdot 10^{-13}$ M gramicidin, 10 μM $AlPcS_4$.

channels is accelerated with the increase in phthalocyanine concentration (Fig. 3) and decelerated with the decrease in the light intensity (Fig. 4). It is worth noting that the phenomenon of channel inactivation is observed only when the laser beam is focused exactly on the hole where the BLM are formed (the diameter of the beam cross-section exceeds the diameter of BLM by approx. 5 times). The inactivation process is reversible at high concentrations of gramicidin (data not shown) since new gramicidin molecules incorporate into BLM, whereas at low concentrations of the peptide the process becomes irreversible due to exhaustion of gramicidin in the bathing solution, probably because of its effective incorporation into BLM and adsorption onto the Teflon walls of the cell.

The experiments performed at the level of the single channel conductance recording show that illumination in the presence of phthalocyanines converts the grami-

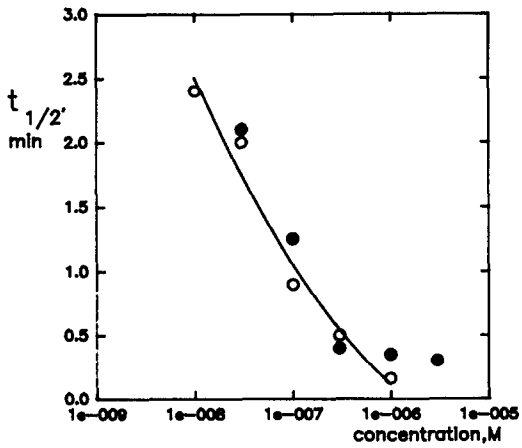

Fig. 3. The dependence of the time to produce a 50% decrease in the gramicidin-induced conductance ($\tau_{1/2}$) on the concentration of phthalocyanine. The incident power density of the light produced by the He-Ne laser is 30 mW/cm^2. (○) $AlPcS_3$; (●) $AlPcS_4$.

cidin channel into a state of virtually zero conductance on a time scale less than the resolution of our experimental setup (≥0.1 s) (Fig. 2B). It is seen that the photodynamic inactivation is due to a marked decrease in the number of open channels rather than to a reduction of the single channel conductance.

Control experiments demonstrate that neither phthalocyanines per se (in the dark) nor visible light irradiation of BLM in the absence of phthalocyanines produce any effect on the gramicidin-induced conductance. Thus, obviously the inactivation of gramicidin channels observed upon illumination of BLM by visible light is sensitized by phthalocyanine.

The mechanism of the photodynamic inactivation of gramicidin channels may be proposed based on the works of Stark et al. [11–14] on the effect of ionizing

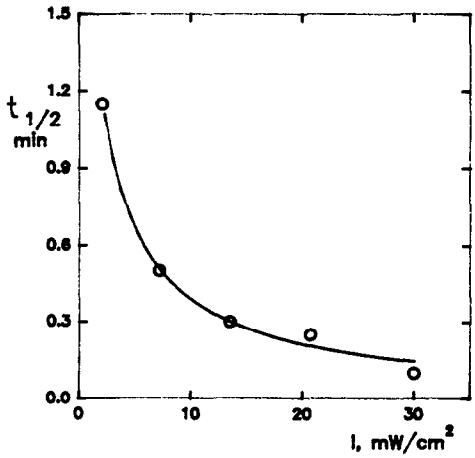

Fig. 4. The dependence of the time to produce a 50% decrease in the gramicidin-induced conductance ($\tau_{1/2}$) on the incident power density (I) of the He-Ne laser light in the presence of phthalocyanine (1 μM $AlPcS_3$).

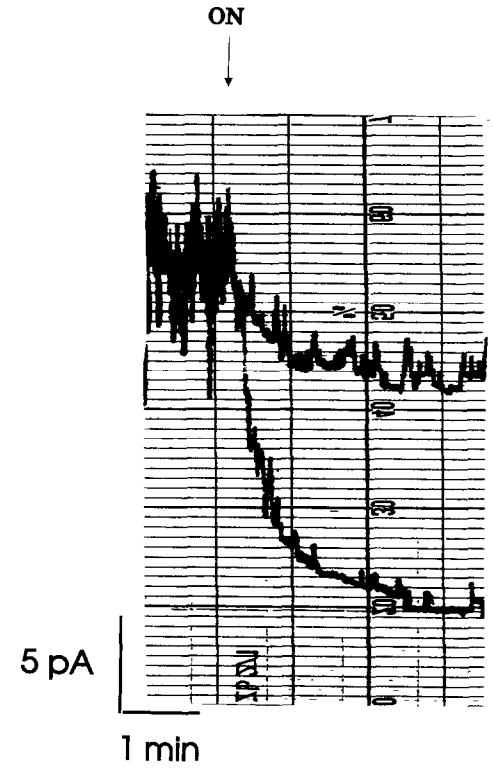

Fig. 5. The effect of oxygen on the photoinactivation of the gramicidin-induced BLM conductance in the presence of phthalocyanine (1 μM $AlPcS_3$). The upper trace is recorded after argon bubbling.

radiation on gramicidin-induced conductance. It was demonstrated that reactive oxygen species generated as a result of water radiolysis cause the inactivation of gramicidin channels due to oxidation of tryptophan residues essential for channel functioning.

As seen from Fig. 5, oxygen depletion by bubbling argon through the bathing solution markedly suppresses the photodynamic inactivation of gramicidin-induced BLM conductance caused by phthalocyanine. Variations in the pH of the bathing solution from 3 to 10 have no influence on the photodynamic blockage of the ionic channels (data not shown). Based on these results it can be supposed that phthalocyanine-induced gramicidin inactivation is mediated by singlet oxygen. Aluminium phthalocyanines are known to be potent sensitizers of singlet oxygen generation [2,15], capable of bringing about tryptophan oxidation in solution [16–18]. However, the involvement of a radical mechanism in the ionic channel blockage, i.e. a photosensitized reaction of the I type according to revised Foote's classification [19], cannot be completely excluded.

It is unclear whether singlet oxygen species participating in BLM photomodification are generated by phthalocyanine molecules dissolved in the bathing solution or those adsorbed on the membrane. Bachowski et al. [4] suggested that membrane-bound molecules of phthalocyanine generate most of the singlet oxygen involved in

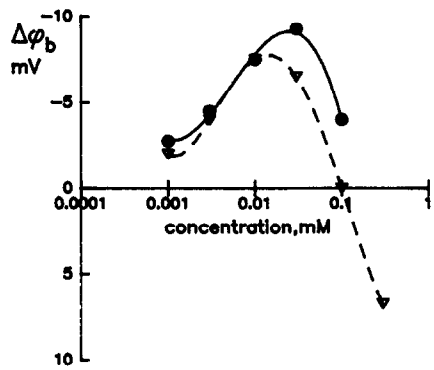

Fig. 6. The dependence of the boundary potential difference ($\Delta\phi_b$) of BLM on the concentration of phthalocyanine (▽, $AlPcS_3$; ○, $AlPcS_4$) added at one side of the membrane. The minus sign indicates the side of the BLM where the dye is added.

the interaction with a lipid membrane. We decided to test the possibility of phthalocyanine adsorption on BLM by measuring the effect of phthalocyanines on the difference of boundary potentials of BLM ($\Delta\phi_b$). It is seen from Fig. 6 that addition of $AlPcS_4$, as well as $AlPcS_3$, leads to changes in $\Delta\phi_b$. These changes unambiguously demonstrate the process of phthalocyanine adsorption on BLM.

In conclusion we can say that the phenomenon of phthalocyanine-mediated photodynamic inactivation of model ionic channels may be of high biological importance. In recent work [20] it was shown that the regulation of potassium channels may be one of the earliest cellular responses to the action of active oxygen species. Furthermore, early works by Pooler and Valenzeno [21,22] demonstrated the photosensitized blockage of integral ionic conductance in excitable cells in the presence of Bengal rose, eosin, etc. The data obtained here with model ionic channels show that the mechanism of the photodynamic suppression of normal physiological ionic currents may be based on the conversion of ionic channels into a zero-conductance state as a result of photomodification of certain amino acid residues.

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