

Influence of H₂TOEtPyP₄ porphyrin on the stability and conductivity of bilayer lipid membranes

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Abstract Many water-soluble cationic porphyrins are known to be prospective chemotherapeutics and photosensitizers for cancer treatment and diagnosis. The physicochemical properties of porphyrins, in particular their interactions with membranes, are important determining factors of their biological activity. The influence of cationic meso-tetra-[4-*N*-(2'-hydroxyethyl) pyridyl] porphyrin (H₂TOEtPyP) on the stability and conductivity of bilayer lipid membranes (BLMs) was studied. H₂TOEtPyP₄ porphyrin was shown to decrease the stability of BLMs made of a mixture of DOPS and DPPE (1:1) in an electric field because of a reduction of line tension of spontaneously formed pore edges in the BLM. The presence of cationic porphyrin was found to reduce BLM surface tension. This effect was enhanced with increasing porphyrin concentration. H₂TOEtPyP₄ increased the probability of spontaneous pore formation. Further investigating the cyclic current-voltage characteristics of BLMs allowed determining the electrical capacity and conductivity of BLMs in the presence of H₂TOEtPyP₄ porphyrin. It was shown that in the presence of cationic porphyrin the electrical capacity as well as conductivity of the BLM increases.

Keywords Porphyrin · Bilayer lipid membrane · Stability · Line tension

Introduction

Porphyrins and their analogs are of considerable interest for researchers in the fields of biology, medicine, chemistry, optics and materials science because of their unique physical and chemical properties (Rovaldi et al. 2000; Monti et al. 2009; Wongsinkongman et al. 2002). Different catalysts, sensors, drugs, organic semiconductors, liquid crystals and materials for nonlinear optics are created on the base of porphyrins (Drain et al. 2002; Okura 2002; Guldi and Fukuzumi 2002). Some porphyrin derivatives are of great interest in the context of cancer therapy. They preferentially accumulate in cancerous tissues, followed by exposure of the tumor area to visible light (Pandey 2000). Thus, depending on the porphyrin structure, different variants of their localization are possible—intracellularly, on the surface of the membrane or inside the membrane. Often porphyrins become biologically active after binding to DNA, proteins and other intracellular structures (Grand et al. 2002; Gyulkhandanyan et al. 2013). In this case, naturally, the step of porphyrins reacting with cellular binding partners must be preceded by steps in which porphyrins enter the cell by passing through the membrane (Poh-Fitzpatrick 1986). Thus, how effectively porphyrins influence the cells significantly depends on their interaction with the cell membrane. Due to the complex organization of biological membranes, research on porphyrin's interaction with cell membranes faces a variety of complications, while interpretation of the results is often ambiguous. For this reason, it seems appropriate to carry out experiments on model systems such as bilayer lipid membranes (BLMs), which are known to be good models of the bilayer part of biological membranes.

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The effect of various substances on the membrane may lead to a change of its stability and conductivity (Kramar et al. 2009). For this reason, an important aspect of the action of porphyrins on the membrane is to study their effect on the stability and conductivity of the membrane. The present work is devoted to studying the influence of meso-tetra-[4-*N*-(2'-hydroxyethyl) pyridyl] (H2TOEtPyP4) porphyrin on the stability and conductivity of BLMs. The surface tension, linear tension along the pore edge in BLM and probability of pore formation, as well the electrical conductivity and capacity of BLMs, are examined in the work in both the absence and presence of H2TOEtPyP4. This porphyrin has a high efficiency against microorganisms without the need for additional agents enhancing their effectivity and therefore offers the perspective of a wide range of applications in medicine and microbiology (Gyulkhandanyan et al. 2009). Additionally, it has phototoxic and photodynamic properties, which are widely used in anticancer therapy (Tovmasyan et al. 2008).

Materials and methods

In this work a water-soluble cationic meso-tetra-[4-*N*-(2'-hydroxyethyl) pyridyl] (H2TOEtPyP4) porphyrin was used; its structure is presented in Fig. 1. The porphyrin was synthesized as described in Tovmasyan et al. (2008).

In all experiments BLMs were prepared at room temperature (20–25 °C) according to the technique described in Mueller et al. (1963). Experiments were performed on BLM obtained from a mixture of 1,2-dioleoyl-*sn*-glycero-3-[phospho-*L*-serine] (DOPS) and 1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) (1:1), which were dissolved in *n*-decane. Lipids were purchased from Avanti

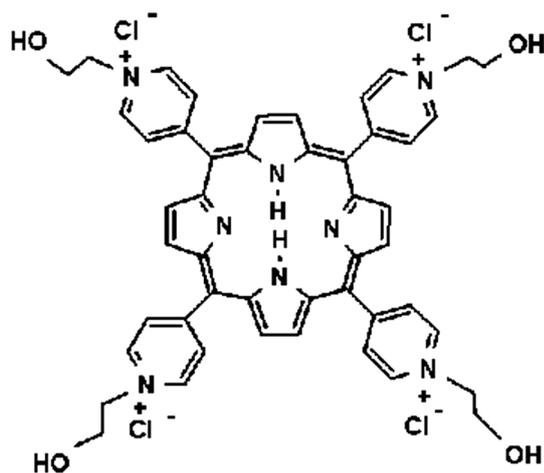


Fig. 1 The structure of meso-tetra-[4-*N*-(2'-hydroxyethyl) pyridyl] porphyrin (H2TOEtPyP4)

Polar Lipids. BLMs were produced in a 0.1 M NaCl solution. The diameter of the hole on which BLM was formed was 1 mm. In view of the torus on the border of the hole, the area of the planar part of the BLM was 0.65 mm². For measurements of the electrical parameters of the BLM, a current amplifier Keithley 427 was used according to the procedure described in Gu et al. (1996). Voltage was applied to the BLM by means of chlorine-silver electrodes connected to the ADC (E14-140-M) and controlled by a computer in a range of 0.20–0.55 V. The geometrical parameters of BLMs were checked by measuring their electrical capacity by means of their cyclic current-voltage characteristics (CVC) implemented in a custom-made LabVIEW program.

The influence of H2TOEtPyP4 on the stability and conductivity of the BLM was investigated at porphyrin concentrations of 1, 5, 10 and 50 μM.

Results and discussion

The loss of BLM stability in an electric field is associated with the formation of hydrophilic pores (Chizmadzev et al. 1979). Pores are formed spontaneously in BLMs, then, because of their random change in size, they reach some critical size, just above which loss of BLM stability occurs. The formation of these pores in the membrane is enhanced in the presence of an electrical field, which thereby leads to the loss of BLM stability.

A main parameter characterizing the degree of stability of BLMs is their mean lifetime at a given voltage across the BLM (Pastushenko et al. 1979). Hence, it is natural to characterize the stability of the membrane by investigating the mean BLM lifetime as a function of the applied voltage.

At first, we studied the variation of the mean lifetime of BLMs at increasing levels of voltage in the absence of porphyrin (Fig. 2, curve 1).

We performed a detailed analysis of the dependence of the BLM mean lifetime \bar{t} on the voltage φ , using the theoretical dependence $\bar{t}(\varphi)$ obtained in a study by Pastushenko et al. (1979):

$$\bar{t} = \frac{(k_B T)^{3/2}}{4\pi D c_0 S \gamma \left(\sigma + \frac{C\varphi^2}{2}\right)^{1/2}} \cdot \exp\left(\frac{\pi \gamma^2}{\left(\sigma + \frac{C\varphi^2}{2}\right) k_B T}\right) \quad (1)$$

where σ is the surface tension of the BLM, γ the linear tension along the pore edge in the BLM, D the diffusion coefficient of defects in the space of radii, which describes the rate of change of pore sizes (in cm²/s), φ the voltage on the membrane, k_B the Boltzmann constant, T the temperature, c_0 the concentration of pores in the BLM, i.e., the number of pores per unit area of BLM, and S the BLM area. C is the change

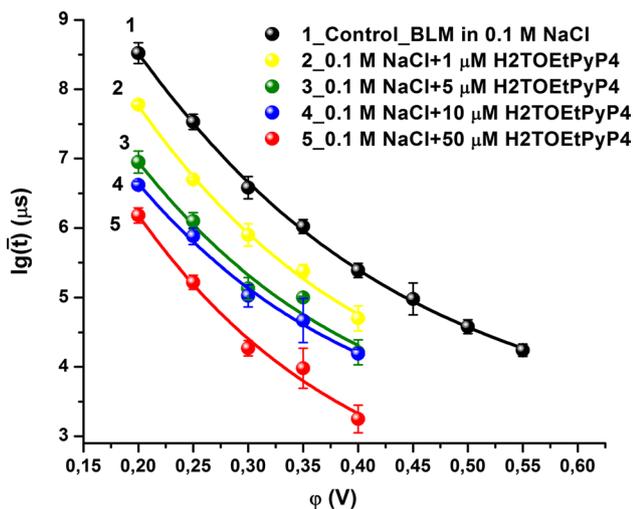


Fig. 2 Decrease in the mean lifetime of BLMs with increasing voltage in the absence of porphyrin (curve 1) and in the presence of H2TOEtPyP4 porphyrin with concentrations of 1 μM (curve 2); 5 μM (curve 3); 10 μM (curve 4); 50 μM (curve 5). Points experimental data (average of five measurements at each potential difference); solid lines theoretical curves obtained by the least-square fitting to Eq. (2) [error bars are standard errors of the mean (±SEM)]

of the specific capacitance in the region of the pore defined by the relation $C = C_0(\epsilon_w/\epsilon_m - 1)$, where $C_0 = \epsilon_0\epsilon_m/h$ is the specific electrical capacity of the BLM, ϵ_w the dielectric constant of water, ϵ_m the dielectric constant of the BLM and ϵ_0 the vacuum dielectric constant.

Comparing the theoretical curve (1) with the experimental points in Fig. 2, it is convenient to introduce Eq. (1) in a logarithmic form:

$$\lg \bar{t} = A - \frac{1}{2} \lg (1 + M\varphi^2) + \frac{B}{1 + M\varphi^2}, \quad (2)$$

$$A = \lg \left(\frac{(k_B T)^{3/2}}{4\pi D C_0 S \gamma \sigma^{1/2}} \right), \quad B = \frac{\pi \gamma^2 \lg e}{\sigma k_B T}, \quad M = \frac{C}{2\sigma}$$

The variation of the mean lifetime of BLMs depending on the applied voltage in the presence of H2TOEtPyP4 porphyrin at four different concentrations is shown in Fig. 2, curves 2–4.

The curves in Fig. 2 are obtained by the least-square method, which allows determining the values of parameters A , B and M in Eq. (2) and hence the values of the BLM surface tension (σ), linear tension of the pore edge in the BLM (γ) and parameter c_0SD , on which the BLM stability mainly depends. First, the change of the specific capacitance of the BLM in the region of pore (C) was determined, then its surface tension without porphyrin, which equals $\sigma = (4.8 \pm 0.3)$ mN/m. Using the value of σ , we can easily obtain the linear tension of the pore edge in the BLM,

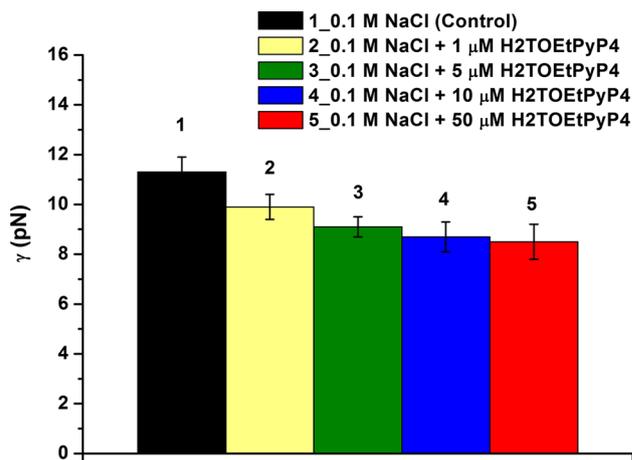


Fig. 3 Linear tensions along the pore edges in the BLM as a function of the H2TOEtPyP4 porphyrin concentration [error bars are standard errors of the mean (±SEM)]

which equals $\gamma = (11.3 \pm 0.6)$ pN. This quantity is in good agreement with the literature data (Abidor et al. 1979; Pastushenko et al. 1979).

The experimental data in Fig. 2 show that the mean lifetime of the BLM decreases in the presence of cationic porphyrin with increasing concentration. Analysis of the curves in Fig. 2 showed that the decrease in the mean lifetime of the BLM is mainly due to a decrease in the linear tension of the BLM in a porphyrin-concentration dependent manner (Fig. 3).

The influence of the H2TOEtPyP4 porphyrin on the stability of the BLM in an electric field can be explained as follows: Cationic porphyrin adsorption on the BLM reduces the linear tension of pore edges, which form spontaneously. This in turn leads to a decrease in the stability of the BLM.

Furthermore, the surface tension of the BLM (σ) was determined in the presence of porphyrin. In the presence of H2TOEtPyP4 the surface tension of the BLM is lower than in the control (Fig. 4). Moreover, σ decreases with increasing concentrations of H2TOEtPyP4. Note that the qualitative porphyrin concentration-dependence of the coefficient of BLM surface tension is consistent with the concentration-dependence of the linear tension along the BLM pore edges (γ). This should be expected, since a common measure of line tension is the formula $\gamma = \sigma h$ (where h is the thickness of the BLM), from which it follows that the linear tension along the pore edges on the BLM and surface tension σ of the BLM are linearly dependent (Pastushenko et al. 1979).

Knowing the values of σ and γ , the values of c_0SD can be calculated from Eq. (2). From the analysis of the experimental data, the following value of c_0SD was obtained in the absence of the porphyrin: $c_0SD = 2.68 \times 10^{-23}$ m²/s, while in the presence of porphyrin parameter c_0SD

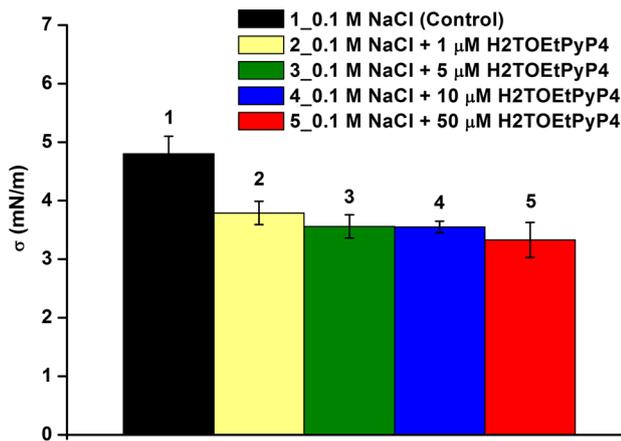


Fig. 4 BLM surface tension σ as a function of H2TOEtPy4 porphyrin concentration [error bars are standard errors of the mean (\pm SEM)]

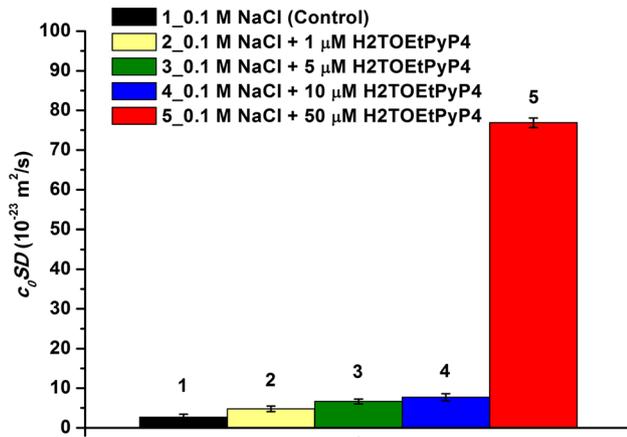
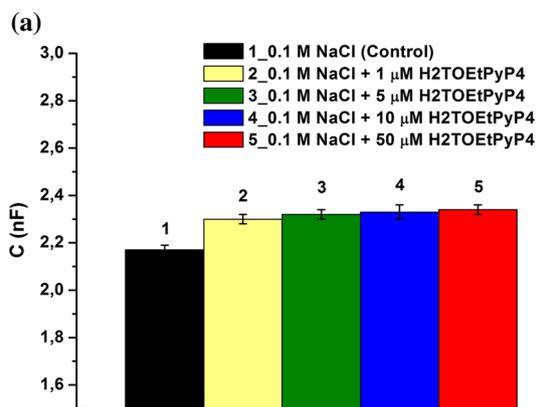


Fig. 5 Dependence of the parameter c_0SD on the concentration of the H2TOEtPy4 porphyrin [error bars are standard errors of the mean (\pm SEM)]



increases (Fig. 5). Since the linear tension of BLMs decreases with increasing concentration of H2TOEtPy4, the increase of the parameter c_0SD with increasing concentration of the porphyrin can be associated with an increased probability of pore formation in the BLM.

The geometrical parameters of BLMs were checked for each experiment by measuring the BLM capacitance using (CVC). In all experiments the thickness of the BLM was 40–50 Å (Abidor et al. 1979). From CVC the electrical capacitance (C) and conductance (g) of the BLM were calculated according to the following formulas:

$$g = \Delta U / \Delta I, \quad C = \Delta I / 2\alpha$$

where ΔU and ΔI are the increments of voltage and current, respectively, and α is the sweep rate of the voltage. For the specific electrical conductivity and capacitance of the BLM, the following values were obtained in the absence of the porphyrin: $g_s = (1.33 \pm 0.03) \times 10^{-7} \text{ Ohm}^{-1} \cdot \text{cm}^{-2}$, $C_s = 0.34 \pm 0.03 \text{ } \mu\text{F}/\text{cm}^2$ (average values of five measurements) in accordance with Tien and Ottova-Leitmannova (2003).

The experimental values of capacitance and conductance of the BLM in the presence of porphyrin are shown in Fig. 6.

In the presence of porphyrin the electrical capacity of the BLM is higher than in the control and is almost not changed with increasing porphyrin concentration (Fig. 6a). At a fixed BLM thickness the change in BLM capacitance in the presence of porphyrin may be due to a change in the area of the flat part of the BLM, which is confirmed by simple estimates.

In the presence of porphyrin in a solution of NaCl the electrical conductivity of the BLM increases (Fig. 6b). In this case one cannot explain the increase in conductivity by an increase in the BLM area since the increase in the area

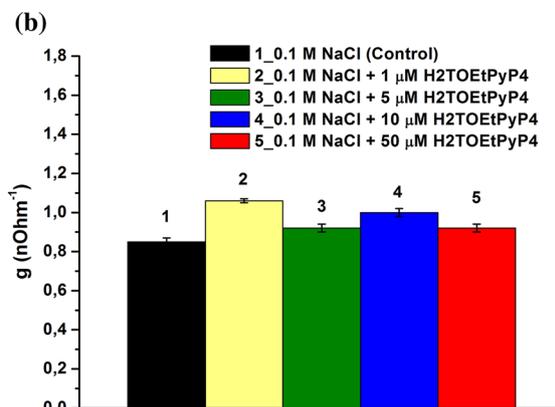


Fig. 6 Capacitance in **a** and conductance in **b** of the BLM in the presence of different concentrations of H2TOEtPy4 porphyrin [error bars are standard errors of the mean (\pm SEM)]

is too small. The changes (increase) in conductivity shown in Fig. 6 are such that they exceed the level of statistical error especially when the concentration of porphyrin is 1 and 10 mM. This increase in conductivity correlates well with the result that follows from Fig. 3, which shows that with increasing porphyrin concentration the linear tension decreases. According to the theory of BLM stability in an electric field (Chizmadzhev et al. 1979) on the one hand the reduction of line tension should increase the probability of formation of pores on the BLM and as a consequence increase the conductivity of the BLM. On the other hand, a monotonic decrease in line tension, which follows from the results of Fig. 3, should lead to a monotonic increase in conductivity. However, as can be seen from Fig. 6b, the experimental data show no monotonic increase in conductivity, since the conductivity decreases at porphyrin concentrations higher than 1 mM, while it remains slightly higher than in the control. We suppose that a reduction of BLM conductivity may be a consequence of the decrease of the pore conductivity with increasing porphyrin concentration. Reducing the linear tension of pores means that in the vicinity of the lipidic pore the number of porphyrin molecules increases, giving rise to a considerable local positive charge. This charge leads to either a high maximum or a deep minimum on the potential energy profile of the ion penetrating through the pore depending on the charge of the ion, and this in turn causes a decrease in the conductivity of the lipidic pore. Thus, with an increasing concentration of porphyrin on the one hand the number of lipid pores will increase; on the other hand the conductivity of the lipid pores will decrease, which qualitatively explains the nonmonotonic dependence of the BLM conductivity on the porphyrin concentration in Fig. 6b.

Conclusions

The obtained results allow drawing several conclusions about the interactions of H₂TOEtPyP₄ porphyrin with BLM and hence suggest interactions with the bilayer part of biological membranes of living cells. Here H₂TOEtPyP₄ porphyrins were found to reduce BLM stability in a concentration-dependent manner because of a decreased line tension along the edges of spontaneously forming pores. This leads to enhanced formation of membrane pores ultimately destabilizing the BLM.

We suggest that facilitation of the formation of transient lipidic pores in the membrane bilayer due to lowered linear tension of the pores disturbs the normal permeability barrier function of biological membranes and allows H₂TOEtPyP and perhaps other porphyrins to cross the membranes and enter cells.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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