

INFLUENCE OF SODIUM DODECYL SULFATE ON STABILITY  
OF BILAYER LIPID MEMBRANES

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In the paper influence of Sodium dodecyl sulfate (SDS) on planar bilayer lipid membranes (BLM) was investigated. It was shown that the presence of SDS and its concentration increasing leads to the loss of stability of the BLM, which is associated with the decrease in the value of the linear tension of pore edge in BLM, because of positive spontaneous curvature of the SDS molecule. It is also shown that the number of lipid pores on the BLM increases as a result of increase in probability of the pore formation, with reduction of the value of linear tension.

**Keywords:** bilayer lipid membrane, sodium dodecyl sulfate, cyclic current-voltage characteristic, BLM stability.

**Introduction.** The membrane is an essential and vital selective barrier, which separates the cell contents and its organelles from the surrounding solution. In the life activity process for various reasons such as the electrical breakdown, the osmotic pressure, the phase transition from liquid crystalline structure to gel, continuity of bilayer membrane can be violated, because of formation of structural defects such as through hydrophilic pores [1]. In this case it is quiet natural to expect changes in many functions of cellular membrane, including permeability and stability [2–4]. In this work the influence of sodium dodecyl sulfate (SDS) on the bilayer lipid membrane (BLM) has been investigated. SDS is a commonly used detergent to solubilize membranes and to isolate and purify membrane proteins and membrane lipids [5–8]. Choice of SDS as a modifier of membranes is due to the fact that, firstly, the volume of water pollution by SDS comes with great speed, because of its intensive use (in industry, virtually all detergents, in preparing various cosmetics) and release into the environment [9]. SDS has the ability to absorb and accumulate in the body, causing disorders in the central nervous system, cardiovascular system, digestive system damages to the organs of the excretory system. It has allergenic properties, even when a small amount of it gets into the body [9]. Secondly, there

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are some molecules (such as fatty acids, lysophospholipids) in the cell, which have similar structures like SDS molecule, and it's necessary to know their influence on the membrane stability and conductance. According to the aforesaid reasons, it is important to investigate the influence of SDS on the cell membrane. But as the study of stability and conductivity on the living cell is too hard, it seems more expedient to do a detailed study on the widely used experimental system for modeling of cell membranes, which are bilayer lipid membranes [10, 11].

**Materials and Methods.** The mixture of phosphatidylserine (1,2-dioleoyl-sn-glycero-3-phospho-L-serine, DOPS) and phosphatidylethanolamine (diphytanoyl-sn-glycero-3-phosphoethanolamine, DPPE) (1:1) was used to make BLM. Phospholipids were purchased from "Avanti Polar Lipids" (USA) and SDS from "Sigma". For BLM formation 3 – 4% freshly prepared lipid solutions were used in *n*-decane. As a buffer solutions were used 0.1M NaCl in distilled water. The membranes were formed according to the Muller–Rudin method in the solution of electrolyte on the hole with 1 mm diameter in a teflon cell's thin partition [12]. All experiments were done at room temperature (20 – 25°C). The influence of SDS on the electrical parameters of the BLM was investigated at SDS concentration of  $10^{-4}$  and  $10^{-5}M$ .

For measurements of the electrical parameters (the conductivity and capacity) of the BLM, two silver-chloride electrodes were located in two compartments of the experimental cell, which were separated by a membrane. The BLM conductivity and capacity were measured by Keitley 427 amplifier. The electrodes were connected to the ADC (E14–140M) and controlled by a computer, using the computer program LabVIEW, according to the procedure described in [11, 13]. Voltage applied to BLM was in range 0.20 – 0.45 V.

**Results and Discussion.** From the electrical point of view, a planar lipid bilayer can be considered as an imperfect condenser with capacity ( $C$ ) and shunted with resistance ( $R$ ), the plates of condenser are electrolyte solutions in both sides of the membrane with polar head groups of the lipid molecules immersed in it. The conductors are separated by a dielectric bilayer formed from non-polar "tails" of lipid molecules [14].

The electrical capacity of the membrane

$$C_0 = \frac{\epsilon_0 \epsilon_m S}{h}, \quad (1)$$

where  $h$  is the thickness of BLM,  $S$  is the area of BLM,  $\epsilon_m$  is the dielectric permeability of BLM and  $\epsilon_0 = 8.85 \cdot 10^{-12} F/m$  is the dielectric constant. From this equation follows, that for controlling the BLM thickness and area, which are important geometrical parameters, it is necessary to measure the electrical capacity. It is easy to determine the cyclic CVC of BLM by applying symmetric triangle voltage with the sweep rate 0.2 V/s [15]. The analysis of CVC of BLM allows to determine simultaneously the conductivity and electrical capacity of BLM:

$$g^{-1} = \frac{\Delta U}{\Delta I}, \quad C = \frac{\Delta I}{2\alpha}, \quad (2)$$

where  $\Delta U$  and  $\Delta I$  are the increments of current and voltage respectively,  $\alpha$  is the rate of voltage sweep. From the analysis of CVC the following values of the specific

capacity and specific conductivity of BLM were obtained in the absence of SDS:  $g_{sp} = (0.42 \pm 0.03) \cdot 10^{-7} \text{Om}^{-1} \text{cm}^{-2}$ ,  $C_{sp} = 0.34 \pm 0.02 \mu\text{Fcm}^{-2}$ . The estimated values of specific electrical capacity and conductivity of BLM in the absence of SDS well agree with the literature data [13, 14]. Estimation of the BLM thickness according to Eq. (1) in all experiments amounted to 4–5 nm, these values are in good correspondence with literature data [14, 16].

Similar measurements were taken in the presence of SDS at concentrations of  $10^{-5}$  and  $10^{-4} \text{M}$ . The results were accordingly such:

$$\begin{aligned} g_{sp} &= (0.77 \pm 0.02) \cdot 10^{-7} \text{Om}^{-1} \text{cm}^{-2}; & C_{sp} &= 0.45 \pm 0.04 \mu\text{Fcm}^{-2}; \\ g_{sp} &= (0.86 \pm 0.03) \cdot 10^{-7} \text{Om}^{-1} \text{cm}^{-2}; & C_{sp} &= 0.37 \pm 0.02 \mu\text{Fcm}^{-2}. \end{aligned}$$

Usually BLM is very unstable in the presence of small quantities of detergents and other impurities [17]. Stability of the BLM can be characterized by the mean lifetime of the membrane. According to [3, 4], the expression for the BLM mean life time in an external electrical field has the following form:

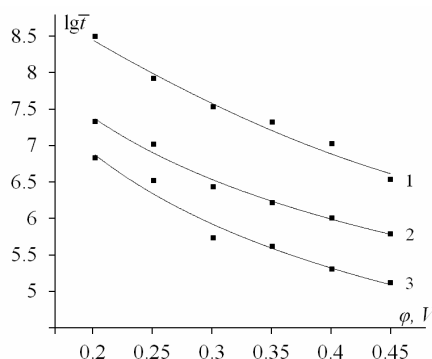
$$\bar{t} = \frac{(k_B T)^{3/2}}{4\pi D c_0 S \gamma \left( \sigma + \frac{C \varphi^2}{2} \right)^{1/2}} \exp \left( \frac{\pi \gamma^2}{\left( \sigma + \frac{C \varphi^2}{2} \right) k_B T} \right), \quad (3)$$

where  $\sigma$  is the BLM surface tension,  $\gamma$  is the linear tension of pore edge in BLM,  $D$  is the diffusion coefficient of defects in the space of radius,  $\varphi$  is the voltage on membrane,  $k_B$  is the Boltzmann constant,  $T$  is the temperature,  $c_0$  is the concentration of pores in BLM and  $S$  is the BLM area.  $C$  is the reduced electrical capacity defined by the relationship  $C = C_0(\epsilon_w/\epsilon_m - 1)$  with  $C_0 = \epsilon_0 \epsilon_m/h$  specific electrical capacity of BLM, where  $\epsilon_w, \epsilon_m$  and  $\epsilon_0$  are the dielectric constants of water, BLM and the vacuum respectively.

The influence of SDS on BLM mean lifetime in an external electrical field at given values of the voltage on BLM was investigated. The moment of current's stepwise increase was attested about BLM destruction as a result of its electrical breakdown. It was plotted dependence of the mean membrane lifetime vs applied voltage. Figure shows the dependence plots of decimal logarithm of the mean lifetime of BLM on the applied voltage, built with different concentrations of SDS.

The received curves (see Figure) show that the presence of SDS and its increasing in the concentration leads to decrease in the membrane stability. According [3, 4, 11], the BLM electrical breakdown is conditioned by the spontaneous appearance of pores in BLM in the electrical field and by their developing to the critical size. The critical size of the pore corresponds to the critical value of the height of energy barrier  $\Delta\Phi_* = \pi\gamma^2/(\sigma + C\varphi^2/2)$ , and overcoming of which brings to BLM electrical breakdown. From Eq. (1) it is seen, that the mean lifetime of BLM depends exponentially on critical value of the height of energy barrier. Obviously any factor reducing the height of the energy barrier also will reduce the BLM mean life time. As such factors, firstly can be the line tension of pore edge  $\gamma$  and the voltage on membrane  $\varphi$ , because the height of the of the energy barrier is

square-law proportional to them. From the other parameters, which can influence on mean lifetime of BLM, the most important are the tension  $\sigma$  of the BLM and  $D_{c_0}S$  parameter, i.e. the product of all number of defects on BLM to the diffusion coefficient of defects in the space of radius.



Decreasing in the mean lifetime of BLM at the increasing in voltage:

- 1) in the absence of SDS;
- 2) in the presence of SDS with concentration  $10^{-5}M$ ;
- 3) in the presence of SDS with concentration  $10^{-4}M$ .

Points are the experimental data (average of 5 measurements at each potential difference), solid lines are theoretical curves obtained from formula (3) by the least-square method.

For determination of the values of the tension of BLM, the linear tension of pore edge on the BLM and the  $D_{c_0}S$  parameter in the absence and in the presence of SDS, let's to represent the formula form (3):

$$\lg \bar{t} = A - \frac{1}{2} \lg(1 + M\phi^2) + \frac{B}{1 + M\phi^2}, \quad (4)$$

$$A = \lg \left( \frac{(k_B T)^{3/2}}{4\pi D_{c_0} S \gamma \sigma^{1/2}} \right), \quad B = \frac{\pi \gamma^2 \lg e}{\sigma k_B T}, \quad M = \frac{C}{2\sigma}. \quad (5)$$

From comparison of theoretical curves with experimental points the values of parameters  $A, B$  and  $M$  were determined. Obtained values of  $A, B$  and  $M$  allow determining  $\sigma, \gamma$  and  $D_{c_0}S$  from formulas (5). The results are shown in the Table.

*The calculated values of the surface tension ( $\sigma$ ), the linear tension of spontaneously formed pores ( $\gamma$ ) and the  $D_{c_0}S$  parameter*

[SDS], $M$	$\sigma, 10^{-3} N/m$	$\gamma, 10^{-12} N$	$D_{c_0}S, 10^{-25} m^2/s$
0 (control)	$4.3 \pm 0.10$	$6.8 \pm 0.05$	$3.1 \pm 0.7$
$10^{-3}$	$2.6 \pm 0.30$	$5.8 \pm 0.06$	$23.0 \pm 0.9$
$10^{-4}$	$0.7 \pm 0.09$	$3.2 \pm 0.05$	$253.0 \pm 1.2$

From the Table it is seen that the influence of SDS on BLM leads to the reduction of  $\gamma, \sigma$  and increase the  $D_{c_0}S$  parameter in the contrast with the control values,

moreover these changes are more intensify with increasing the SDS concentration. The influence of SDS on specified parameters can be explained as follows. It is known, that the effective form of the SDS molecules can be modeled as an inverted cone (“wedge”) shaped molecule, i.e. this molecules have quite large polar “heads”, and the cross section of the “tail” is small. A monolayer of these molecules tries assume a convex shape and in the natural condition has some positive curvature. The linear tension of spontaneously formed pores, according [18–21] depends from spontaneous curvature of a detergent molecule: a positive spontaneous curvature leads to decrease the coefficient of linear tension, which was observed experimentally (see Table).

Since the tension is proportional to the linear tension [4], so the reduction of  $\gamma$  must lead to reduction of  $\sigma$ , that explain the experimental dependency of the BLM tension from the concentration of SDS. The strong growth of the  $Dc_0S$  parameter with increasing of SDS concentration can be connected with increasing of number of lipid pores on the BLM (see Table), the last fact can be result of increase of probability of the pore formation with reduction of the value of  $\gamma$  [3].

Thus, we have shown that the presence of SDS at the concentrations  $10^{-5}$  and  $10^{-4}M$  leads to the loss of stability of the BLM, which is associated with the decrease in the value of the linear tension of pore edge in BLM, because of positive spontaneous curvature of the SDS molecule.

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#### REFERENCES

1. **Chernomordik L.V., Sukharev S.I., Popov S.V., Pastushenko V.F., Sokirko A.V., Abidor I.G., Chizmadzhev Y.A.** The Electrical Breakdown of Cell and Lipid Membranes: the Similarity of Phenomenologies. // *Biochimica et Biophysica Acta*, 1987, v. 902, p. 360–373.
2. **Antonov V.F.** Lipid Pores: Stability and Permeability of Membrane. // *Soros Educational Journal*, 1997, v. 10, p. 10–17.
3. **Abidor I.G., Arakelyan V.B., Pastushenko V.F., Tarasevich M.R.** et al. Electric Breakdown of Bilayer Lipid Membranes. // *DAN SSSR*, 1978, v. 240, p. 733–736 (in Russian).
4. **Abidor I.G., Arakelyan V.B., Pastushenko V.F.** et al. Mechanism of Electric Breakdown of Bilayer Lipid Membranes. // *DAN SSSR*, 1979, v. 245, № 5, p. 1239–1242 (in Russian).
5. **Tan A., Ziegler A., Steinbauer B., Seelig J.** Thermodynamics of Sodium Dodecyl Sulfate Partitioning into Lipid Membrane. // *Biophysical J.*, 2002, v. 83, p. 1547–1556.
6. **Pletnev M.Yu.** Surface-Active Substances and Compositions. Reference book, 2002, 768 p. (in Russian).
7. **Seddon A.M., Curnow P., Booth P.J.** Membrane Proteins, Lipids and Detergents: Not Just a Soap Opera. // *Biochimica et Biophysica Acta*, 2004, v. 1666, p. 105–117.

8. **Micelli S., Meleleo D., Picciarelli V., Stoico M.G., Gallucci E.** Effect of Nanomolar Concentrations of Sodium Dodecyl Sulfate, a Catalytic Inductor of  $\alpha$ -Helices, on Human Calcitonin Incorporation and Channel Formation in Planar Lipid Membranes. // *Biophysical Journal*, 2004, v. 87, p. 1065–1075.
9. **Fetisov V.O.** Abstract of PhD Thesis. Belgorod, 2015 (in Russian).
10. **Fosnarić M., Kralj-Iglic V., Bohinc K., Iglic A., May S.** Stabilization of Pores in Lipid Bilayers by Anisotropic Inclusions. // *Journal of Physical Chemistry*, 2003, v. 107, p. 12519–12526.
11. **Kramar P., Miklavcic D., Macek Lebar A.** Determination of the Lipid Bilayer Breakdown Voltage by means of Linear Rising Signal. // *Bioelectrochemistry*, 2007, v. 70, p. 23–27.
12. **Mueller P., Rudin D.O., Tien H., Wescott W.C.** Methods for the Formation of Single Bimolecular Lipid Membranes in Aqueous Solution. // *Journal of Physical Chemistry*, 1963, v. 67, № 2, p. 534–535.
13. **Gu L., Wang I., Xun J., Ottova-Leitmannova A., Tien H.T.** A New Method for the Determination of Electrical Properties of Supported Bilayer Lipid Membranes by Cyclic Voltammetry. // *Bioelectrochemistry and Bioenergetics*, 1996, v. 39, p. 275–283.
14. **Antonov V.F.** et al. *Biophysics. M.*, 2000, p. 9–11 (in Russian).
15. **Kutnik J., Tien H.T.** Cyclic Voltammetry of Dye-Modified BLMs. // *Bioelectrochemistry and Bioenergetics*, 1986, v. 16, p. 435–447.
16. **Fettiplace R., Andrews D.M., Haydon D.A.** The Thickness, Composition and Structure of Some Lipid Bilayers and Natural Membranes. // *Journal of Membrane Biol.*, 1971, v. 5, p. 277–296.
17. **Bragina N.A., Mironov A.F.** *Membranology. M.*, 2002 (in Russian).
18. **Cohen F.S., Melikyan G.B.** The Energetics of Membrane Fusion from Binding, Through Hemifusion, Pore Formation, and Pore Enlargement. // *Journal Membrane Biol.*, 2004, v. 199, p. 1–14.
19. **Karatekin E., Sandre O., Guitouni H., Borghi N., Puech P., Brochard-Wyart F.** Cascades of Transient Pores in Giant Vesicles: Line Tension and Transport. // *Biophysical Journal*, 2003, v. 84, p. 1734–1749.
20. **Derzhansky A., Petrov A.G., Mitov M.D.** Edge Energy and Pore Stability in Bilayer Membranes. 15-th International Biophys. Congress. Kyoto, Jap. Abstr., 1978, v. 9, № 24.
21. **Markin V.S., Kozlov M.M.** The Pore Statistics in Bilayer Lipid Membranes. // *Journal Biological Membranes*, 1985, v. 2, № 2, p. 205–222.