Physical and Mathematical Sciences

2014, № 2, p. 60-63

Physics

CYCLIC CURRENT-VOLTAGE CHARACTERISTICS OF A BILAYER LIPID MEMBRANE IN THE PRESENCE OF PORPHYRIN

V. B. ARAKELYAN¹, A. L. TOROSYAN^{1*}, R. K. GHAZARYAN²

¹ Chair of Molecular Physics YSU, Armenia ² Chair of Organic Chemistry YSMU, Armenia

The influence of Co-metalloporphyrins on electrical parameters of planar bilayer lipid membranes (BLM) has been investigated. The cyclic current-voltage characteristics (CVC) of BLM have been measured both in the absence and presence of porphyrin in the solution surrounding the membrane. Based on the measured cyclic current-voltage characteristics the specific conductivity and capacity of BLM were calculated. In the presence of porphyrins the conductivity and capacity of BLM were shown to be increased.

Keywords: bilayer lipid membrane, cyclic current-voltage characteristic, specific conductivity and capacity.

Introduction. In the last decade the possibility of utilization of porphyrins in various fields of medical science is actively discussed. Of most interest in this respect are the following fields of the medicine: the photodynamic therapy of tumors, the therapy of hematological diseases and of different forms of jaundice [1-3]. The research carried out during several years has shown that some porphyrins were accumulated in tumors [4] and the photodynamic therapy was based on the photosensitization property of porphyrins [5]. The antiviral and antibacterial properties of porphyrins are also well known [6, 7]. It was established that the injuries caused by porphyrins in malignant cells occur for the most part in the plasma and organelle membranes [8]. However, though the porphyrins are known to be absorbed on the membrane surfaces, are embeddable into a membrane, penetrate the membrane, the mechanism of their interaction with the cellular membrane is unknown. It is noteworthy that as both the structural organization and the functioning of cellular membranes are highly complicated, the investigation of the interaction of porphyrin with cellular membrane is a rather intricate problem. For this reason it seems appropriate to conduct experiments with a model object, such as a planar bilayer lipid membrane (BLM), that would serve as a structural base of biological membranes.

In the present work the influence of Co-metalloporphyrin on the conductivity and capacity of BLM has been studied. The investigation of current-voltage

_

^{*} E-mail: atorosyan@ysu.am

characteristics of BLM has been executed both in the absence and presence of porphyrin. The method of cyclic voltametry, that has been successfully employed in electrochemical studies, proved highly convenient also for investigation of electric properties of BLM [9, 10]. With the help of this method of research it turned out well to obtain important information on the influence of various factors, such as the lipid content of BLM, the concentration of porphyrins or of other substances, of pH etc., on the transport properties of BLM.

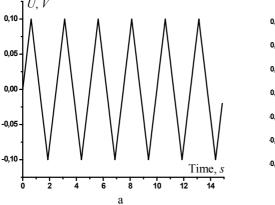
Materials and Methods. The experiments have been conducted with BLM obtained from the mixture of phosphatidylserine and phosphatidylethanolamine (1:1), which are suspended in *n*-decan.

Lipids were purchased from "Avanti Polar Lipids". Co-meso-tetra-[4-N-(2'-oxyethyl)pyridyl]porphyrins (CoTOEtPyP4) have been synthesized in the Dept. of Pharmacological Chemistry of the YSMU [1]. BLM was formed according to the Mueller–Rudin method given in [11] at an aperture with diameter of 1 *mm* on a polytetrafluorrethylene (teflon) cell. The electrolite solution contains 0.1 *M* NaCl with pH 6.1. In the case of investigation of porphyrin influence on the electrical parameters of BLM CoTOEtPyP4 porphyrin with concentration 10^{-5} *M* was added in the electrolitic solution. The experiments have been conducted at room temperature ($20-25^{\circ}C$) using silver-chloride electrodes, located immediately in the compartments of the teflon cell. The BLM conductivity and capacity have been measured with the Keithley 427 amplifier, according to the procedure described in [12]. For measurement of cyclic CVC of BLM the computer package LabVIEW has been used.

Results and Discussion. The measurement of BLM electrical capacity (*C*) would enable to control important parameters of BLM geometry such as the thickness and area of BLM.

The capacity was measured by applying symmetrical triangular voltage with the sweep rate $0.2 \ V/s$ [9, 13] (Fig. 1, a).

By applying triangular voltage on BLM (Fig. 1, a), we register the voltage, which is shown in Fig. 1, b.



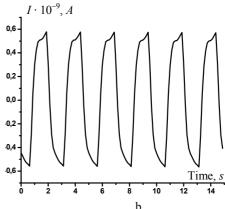


Fig. 1. Applied triangular voltage with amplitude from -100mV to +100mV with the sweep rate 0.2 V/s (a); measured current that passes through BLM (b).

From Fig. 1 it is easy to get a cyclic CVC of BLM (Fig. 2), which allows simultaneously determine the conductance (g) of BLM and its electrical capacity: $g = \Delta U/\Delta I$, $C = \Delta I/2\alpha$,

where ΔI and ΔU are the increment of voltage and current respectively; α is the rate of voltage sweep.

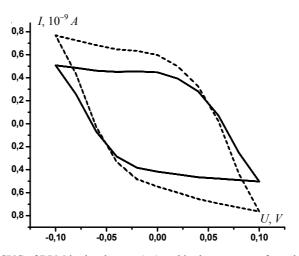


Fig. 2. Cyclic CVC of BLM in the absence (—) and in the presence of porphyrins (---).

From current-voltage characteristics were calculated C and g of BLM in the presence and absence of the porphyrin. In the absence of porphyrin the obtained values of C and g are $2.1 \, nF$ and $0.8 \cdot 10^{-9} \, Om^{-1}$ respectively, and in the presence of porphyrin are 2.85 nF and $2.1 \cdot 10^{-9} Om^{-1}$. In all experiments the thickness of BLM was 40-50 Å in conformity with data cited in [14]. The estimates for the values of specific conductivity and fixed electric capacity of BLM (case of porphyrin absence) are in a good fit with data in literature: $g_s=1.25\cdot10^{-7}~Om^{-1}\cdot cm^{-2}$, $C_s=0.33~\mu F/cm^2$ [10, 14–16]. It is seen that in the presence of porphyrin in NaCl solution both the conductivity and electric capacity of BLM grow. It admits the following explanation: in case of fixed thickness of BLM an increase in the capacity in the presence of porphyrin may be due to the increase of BLM area for account of the reduction of the size tore. Simple estimates in confirmation of this assertion are given below. In case of measured value of capacity (2.1 nF) and 45 Å thickness of BLM, one can estimate the area of BLM and, consequently, also the diameter of the planar part of BLM that is equal to 0.82 mm. Under action of porphyrin the value of capacity increases in 1.36 times, owing to which the diameter of the planar part of BLM would reach 0.95 mm. Since the diameter of BLM formation aperture is 1 mm, such an interpretation of the measured result appears to be acceptable. The experimentally observed increase in the conductivity of BLM in the presence of porphyrin may be also due to the increase in BLM area. It is known, that the conductivity of BLM is effected with the help of spontaneously emerging pores. The increase in conductivity of BLM in the presence of Co-porphyrin testifies to the increase in the number of pores. This means that Co-porphyrin destabilizes the bilayer membrane.

Conclusion. In the present work the influence of Co-metalloporphyrin on electrical parameters of BLM has been investigated by means of the method of CVC. It was shown, that in the presence of Co-metalloporphyrin both the conductivity and the electrical capacity of BLM increase, which is related with the growth of BLM area, for account of decreased sizes of tore.

This work was supported by State Committee of Science MES RA, in frame of research project № SCS "13-1F127".

Received 17.04.2014

REFERENCES

- 1. **Tovmasyan A.G., Babayan N.S., Sahakyan L.A.** et al. Synthesis and *in Vitro* Anticancer Activity of Water-Soluble Cationic Pyridyl Porphyrins and Their Metallocomplexes. // Journal of Porphyrins and Phthalocyanines, 2008, v. 12, p. 1100–1110.
- 2. **Neto D.S., Hawe A., Tabak M.** Interaction of Meso-tetrakis (4-N-methylpyridyl)porphyrin in its Free Base and as a Zn(II) Derivative with Large Unilamellar Phospholipid Vesicles. // Eur. Biophys. J., 2013, v. 42, p. 267–279.
- 3. **Makky A., Michel J.P., Maillard Ph., Rosilio V.** Biomimetic Liposomes and Planar Supported Bilayers for the Assessment of Glycodendrimeric Porphyrins Interaction with an Immobilized Lectin. // Biochimica et Biophysica Acta, 2011, № 1808, p. 656–666.
- 4. **Lavi A., Weitman H., Holmes R.** The Depth of Porphyrin in a Membrane and the Membrane's Physical Properties Affect the Photosensitizing Efficiency. // Biophysical Journal, 2002, v. 82, p. 2101–2110.
- Pandey R. Recent Advances in Photodynamic Therapy. // J. Porph. Phthaloc., 2000, № 4, p. 368–373.
- Zeina B., Greenman J., Corry D., Purcell W.M. Antimicrobial Photodynamic Therapy: Assessment of Genotoxic Effects on Keratinocytes in vitro. // British Journal of Dermatology, 2003, № 148, p. 229–232.
- 7. **Grinholc M., Szramka B., Olender K., Graczyk A.** Bactericidal Effect of Photodynamic Therapy Against Methicillin-Resistant Staphylococcus Aureus Strain with the Use of Various Porphyrin Photosensitizers. // Acta Biochimica Polonica, 2007, v. 54, № 3, p. 665–670.
- 8. **Specht K., Rodgers M.** Plasma Membrane Depolarization and Calcium Influx During Cell Injury by Photodynamic Action. // Biochimica et Biophysica Acta, 1991, v. 1070, № 1, p. 60–68.
- 9. **Kutnik J., Tien H.T.** Application of Voltammetric Techniques to Membrane Studies. // Journal Biochemical and Biophysical Methods, 1985, v. 11, p. 317–326.
- 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. // Bioelectochemistry and Bioenergetics, 1996, v. 39, p. 275–283.
- 11. **Mueller P., Rudin D., Tien H., Wescott W.** Methods for the Formation of Single Bimolecular Lipid Membranes in Aqueous Solution. // J. Phys. Chem., 1963, v. 67, № 2, p. 534–535.
- 12. Abidor I.G., Arakelian V.B., Pastushenko V.F., Tarasevich M.R., Chernomordik L.V., Chizmadzhev Yu.A. Electric Breakdown of Bilayer Lipid Membranes: I–VII. // Bioelectrochem. Bioenerg., 1979, v. 6, p. 37–104.
- 13. **Kutnik J., Tien H.T.** Cyclic Voltammetby of Dye-Modified BLMs // Bioelectrochemistry and Bioenergetics, 1986, v. 16, p. 435–447.
- 14. **Fettiplace R., Andrews D.M., Haydon D.A.** The Thickness, Composition and Structure of Some Lipid Bilayers and Natural Membranes. // J. Membrane Biol., 1971, v. 5, p. 277–296.
- 15. **Pant H.C., Rosenberg B.** Electrochemistry on a Bimolecular Lipid Membranes. // Chem. Phys. Lipids, 1971, v. 6, № 1, p. 39–45.
- 16. Alvares O., Latorre R. Voltage-Dependent Capacitance in Lipid Bilayers Mode from Monolayers. // Biophys. J., 1978, v. 21, № 1, p. 1–17.