PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2013, № 3, p. 33–37

Biology

DETERMINATION METHOD OF PERMEABILITY COEFFICIENT OF BILAYER LIPID MEMBRANE FOR CATIONIC PORPHYRINS

A. L. TOROSYAN*

Chair of Molecular Physics YSU, Armenia

In this paper a method is presented for determining the permeability coefficient of bilayer lipid membrane (BLM) for porphyrins by UV/Vis spectrophotometry. BLM is formed on the hole separating two compartments of the cell. The coefficient permeability of BLM is calculated for meso-tetra-[4-N-(2'-hydroxyethyl)pyridyl] porphyrin. The proposed method can be used to determine the permeability coefficient of BLM for other substances.

Keywords: bilayer lipid membrane, TOEPyP4 porphyrin, permeability coefficient, Soret band.

Introduction. In the past decade the interest in supramolecular interactions of water-soluble cationic porphyrins with biological molecules has increased steadily. Porphyrins and especially their metal derivatives are biologically active substances widely spread in nature. They are included in many biological compounds like hemoglobin, chlorophyll, vitamins B and so on. Both natural and synthetic porphyrins are widely used in medicine and biology, mainly because they have low toxicity, are highly soluble in water and quickly removed from the organism, have low effective concentration, etc. Porphyrins have a wide range of applications in oncology, radiobiology, they are also used as antiviral, antifungal, antibacterial drugs.

It is shown that porphyrins accumulate selectively in tumor cells. They are used as a diagnostic test in oncology practice to detect the form of tumor. Photosensitizing property of porphyrins is successfully used in photodynamic therapy of tumors [1, 2]. It was found that most of the damage caused by porphyrins in tumor cells, including electric depolarization, increased permeability, membrane rupture and cell lysis, occurred in the plasma and organelle membranes [3–5]. Because of these, many researchers are interested in synthesis of novel porphyrins aiming to create drugs based on them. Although the selectivity of porphyrins for tumors has been recognized for about 60 years, many questions about the interaction mechanisms remain.

* E-mail: atorosyan@ysu.am

During many years in many laboratories of the world the interaction of porphyrins with DNA and proteins has been studied *in vitro* [6–8]. It is well known that the photobiological activity of porphyrins appears to be mainly governed by their ability to locate in the plasmic membrane or to cross it and reach DNA and other intracellular targets. That's why it is very important to study the interaction of porphyrins with membranes and to check, if this interaction has any effect on the physicochemical properties of membranes. The transport of porphyrins through the membrane can also take place. Such a process, as well as localization of porphyrins within subcellular membrane structures, must involve passive diffusion [9, 10]. Thus, in this case also, the final biological effect of porphyrins on the cells essentially depends on its interaction with the cell membrane.

It appears from the above that the physicochemical properties of porphyrins and, in particular, their interactions with membranes are important determining factors of their biological activity. Despite several papers devoted to the interaction of porphyrins with model membranes [11–15], yet there are no direct experimental studies describing the permeability of bilayer lipid membrane (BLM) for porphyrins. That's why it is very important to study the interaction of porphyrins with membranes, as well as to examine membrane permeability in the presence of porphyrins.

Materials and Methods. Because of the complicated organization and functioning of cellular membranes, the study of permeability of membranes is very difficult. Thus, it is appropriate to conduct experiments on model object, as BLM, which is the structural basis of biological membranes. Characteristic parameters of BLM are close to parameters of biological membranes. As a model, BLM is constructed very simply than biological membrane, and results obtained using BLM, in most cases can be unequivocally interpreted.

Experiments are conducted on BLM derived from phosphatydilserine and phosphatidylethanolamine, which are suspended in decane. BLM is formed by the method of Muller et al. [16] on a hole with 1 *mm* diameter in the 0.1 *M* solution of NaCl. The penetration of meso-tetra-[4-N-(2`-oxyethyl)pyridyl] porphyrin (TOEtPyP4) synthesized in the department of pharmacological chemistry at YSMU [17] through the bilayer lipid membrane is studied.

Special cells adapted to UV/Vis spectrophotometer (Specord M40) are made for investigation of porphyrin penetration through BLM. After forming and complete blackening of the membrane the solution of porphyrin with concentration 10^{-4} *M* is added to one side of the cell. After turning on spectrophotometer the rays of 424 *nm* length (corresponds to the Soret band maximum of TOEtPyP4) [18] pass trough quartz walls of the cells. At every moment the device measures the time dependence of absorbance.

Estimation Method of BLM Permeability. The aim of the present work is to determine the permeability of BLM for cationic porphyrins. The cell for BLM formation consists of two compartments, which volumes are equal to *V*. The porphyrin is added into the compartment I. In compartment II the device records the absorption of porphyrin molecules, which pass through BLM from compartment I to compartment II.

The beginning concentration of the porphyrin in the compartment I is c_{I}. Porphyrin's concentration dynamics in compartment II as a result of molecules' outflow from compartment I to compartment II is described by equation (1):

$$c_{\mathrm{II}}(t) = \frac{c_{\mathrm{I}}}{2}\left(1 - e^{-kt}\right), \tag{1}$$

where $k = P\dfrac{A}{V}$, P is permeability of BLM, $c_{\mathrm{II}}(t)$ is the current concentration of porphyrin in compartment II, A is area of BLM.

It is very convenient to represent the experimental points by the coordinates $\dfrac{c_{\mathrm{II}}(t)}{0.5 \cdot c_{\mathrm{I}}}$ and t:

$$\frac{c_{\mathrm{II}}(t)}{0.5 \cdot c_{\mathrm{I}}} = 1 - e^{-kt} \approx kt. \tag{2}$$

In this case $P\dfrac{A}{V}$ is determined by the method of least-squares, which is equal to tangent of angle of curve slope. If the values of A and V are known the permeability P can be determined.

Results and Discussion. The passage of porphyrin through the membrane is investigated by absorption in the Soret band as a function of time. As a result the time dependence of the porphyrin's concentration in compartment II is obtained. Then, by using obtained results from equation (2), parameter $P\dfrac{A}{V}$ is calculated by the method of least-squares for 3 different experiments (min^{-1}):

$$P_1\frac{A}{V} = 1.8 \cdot 10^{-5}, \quad P_2\frac{A}{V} = 1.1 \cdot 10^{-5}, \quad P_3\frac{A}{V} = 1.1 \cdot 10^{-5}.$$

The volume of the compartments is equal to $V = 10\ mL = 10\ cm^3$. The diameter of the hole is $d = 1\ mm = 0.1\ cm$. In this case the area of the BLM will be $A = 7.85 \cdot 10^{-3}\ cm^2$.

So, for permeability coefficients we obtained these results (cm/s):

$$P_1 = 2.3 \cdot 10^{-4}, \ P_2 = 3.8 \cdot 10^{-4}, \ P_3 = 3.8 \cdot 10^{-4}.$$

The average of them will be the following:

$$P_{av} = \frac{P_1 + P_2 + P_3}{3} = 3.3 \cdot 10^{-4}\ cm/s.$$

Conclusion. In this work it is shown how the permeability coefficient of the BLM can be determined for TOEPyP4 porphyrins by placing the cell for BLM formation into the differential spectrophotometer. This method allows to record the time dependence of substance absorption and to calculate the permeability parameter. The real situation in biological membranes is much more complex than that described in the present model system. The obtained results allow us to find out the mechanisms of porphyrins passage through the lipid bilayer part of the cell

membranes. This approach can be used to optimize the chemical structure and pharmacological properties of porphyrins used in photodynamic therapy.

I am grateful to Prof. V.B. Arakelyan for supervisory leadership and to R.K. Ghazaryan for providing of cationic porphyrins.

This work was supported by State Committee Science MES RA in frames of research project № SCS "11A-1f040".

Received 30.09.2013

REFERENCES

1. **Pandey R.** Recent Advances in Photodynamic Therapy. // J. Porph. Phthaloc., 2000, № 4, p. 368–373.
2. **Wongsinkongman P., Brossi A., Wang H.K., Bastow K.F., Lee K.H.** Antitumor Agents. Part 209: Pheophorbide-a Derivatives as Photo-Independent Cytotoxic Agents. // Bioorg. Med. Chem., 2002, v. 10, № 3, p. 583–591.
3. **Malik Z., Djaldetti M.** Destruction of Erythroleukemia, Myelocytic Leukemia and Burkitt Lymphoma Cells by Photoactivated Protoporphyrin. // Int. J. Cancer, 1980, v. 26, № 4, p. 495–500.
4. **Kessel D.** Hematoporphyrin and HPD: Photophysics, Photochemistry and Phototherapy. // Photochem. Photobiol., 1984, v. 39, № 6, p. 851–859.
5. **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.
6. **Pasternack R., Gibbs E.** Porphyrin and Metalloporphyrin Interactions with Nucleic Acids. // Met. Ions Biol. Syst., 1996, v. 33, p. 367–397.
7. **Aloyan L., Vardanyan V., Dalyan Y.** The Effect of Peripheral Substituent on Interaction of Meso-Tetra-(3N-pyridyl)porphyrins with DNA. // 5th EBSA & 15th IUPAB International Biophysics Congress, Montpellier (France), August 27th – September 1st, European Biophysics Journal, 2005, v. 34, № 6, poster 340, p. 667.
8. **Haroutiunian S., Dalyan Y., Ghazaryan A., Chalikian T.** Brilliant Light in Life and Material Sciences (eds. Tsakanov V., Wiedeman H.). Ch. Thermodynamic Profile of Interaction of Porphyrins with Nucleic Acids. Springer, 2007, p. 213–218.
9. **Deliinger M., Vever-Bizet C., Brault D.** et al. Cellular Uptake of Hydroxyethylvinyl-deuteroporphyrin (HVD) and Photoinactivation of Cultivated Human Leukemia (Reh6) Cells. Photochemistry and Photobiology, 1986, v. 43, № 6, p. 639–647.
10. **Torosyan A., Gevorgyan H.** The Influence of non-Metal Porphyrins on Bilayer Lipid Membrane. 2nd European Symposium on Microbial Lipids "Microbial Lipids: Diversity in Structure & Function". Bern, Switzerland, Abstract Book, 2012, poster № 50.
11. **Brault D., Vever-Bizet C., Le Doan T.** Spectrofluorimetric Study of Porphyrin Incorporation into Membrane Models-Evidence for pH Effects. // Biochimica et Biophysica Acta, 1986, v. 857, № 2, p. 238–250.
12. **Brault D., Vever-Bizet C., Dellinger M.** Fundamental Aspects in Tumor Photochemotherapy: Interactions of Porphyrins with Membrane Model Systems and Cells. // Biochimie, 1986, v. 68, № 6, p. 913–921.
13. **Brault D.** Physical Chemistry of Porphyrins and their Interactions with Membranes: the Importance of pH. // Journal of Photochemistry and Photobiology B Biology, 1990, v. 6, № 1–2, p. 79–86.

14. **Richelli F., Jori G., Gobbo S., Tronchin M.** Liposomes as Models to Study the Distribution of Porphyrins in Cell Membranes. // Biochimica et Biophysica Acta, 1991, v. 1065, p. 42–48.
15. **Rich M., Ferraro G., Brody S.** Effects of pH on the Binding of Hematoporphyrin Derivative to Monolayer and Bilayer Membranes. // Biochimica et Biophysica Acta, 1992, v. 1104, № 2, p. 269–272.
16. **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.
17. **Gyulkhandanyan A., Gyulkhandanyan L., Ghazaryan R., Fleury F.** et al. Assessment of New Cationic Porphyrin Binding to Plasma Proteins by Planar Microarray and Spectroscopic Methods. // Journal of Biomolecular Structure and Dynamics, 2013, v. 31, № 4, p. 363–375.
18. **Gasparyan G., Hovhannisyan G., Ghazaryan R.** et al. *In vitro* Testing of Cyto- and Genotoxicity of New Porphyrin Water-Soluble Metal Derivatives. // International Journal of Toxicology, 2007, v. 26, № 6, p. 497–502.