МЕТОДИ БІОФІЗИЧНИХ ДОСЛІДЖЕНЬ

УДК 577.37

FLUORESCENT PROBE STUDY OF MODEL PHOSPHATIDYLCHOLINE/CARDIOLIPIN MEMBRANES

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Received 27 October, 2004

The effect of negatively charged phospholipid cardiolipin (CL) on the structural state of phosphatidylcholine (PC) model membranes was studied using fluorescent probes 4-(n-dimethylaminostyryl)-1-methylpyridinium (DSM) and 3-methoxybenzanthrone (MBA). Electrostatic and non-electrostatic contributions to stabilization of DSM-lipid complexes were evaluated. MBA bilayer distribution was found to change on increasing CL content from 5 to 10 mol % suggesting that incorporation of small amounts of CL into PC bilayer is followed by pronounced modification of the membrane structure.

KEY WORDS: cardiolipin, liposomes, fluorescent probes, bilayer structure

Functional properties of biological membranes are known to be largely determined by structural state of the protein and lipid membrane components. The latter, in turn, strongly depends on chemical nature and content of the lipid membrane constituents. More specifically, variations in the bilayer lipid composition can bring about the changes in membrane structure and physical properties, such as electrostatic potential, polarity, phase behavior, etc. To gain insight into the mechanisms underlying these changes model membrane systems whose lipid composition can be widely varied are extensively studied with a number of powerful physical techniques. One of them is based on monitoring the changes in spectral characteristics of membrane-bound fluorescent probes [1]. In the present study fluorescent probes 4-(n-dimethylaminostyryl)-1-methylpyridinium and 3-methoxybenzanthrone have been employed to characterize physical properties of the model membranes composed of zwitterionic (PC) and negatively charged (CL) phospholipids. Cardiolipin is a structurally unique phospholipid, containing four fatty acyl chains and two phosphate groups [2,3]. A great deal of evidence suggests significant role of CL in the coupling of phosphorylation and electron transport, regulation of membrane enzyme activity, programmed cell death (apoptosis) [4-7]. It seems probable that one mechanism by which CL modulates membrane functional properties involves modification of the bilayer structural state. In view of this, our main goal was to elucidate how structural characteristics of PC lipid bilayer are influenced by CL.

MATERIALS AND METHODS

Egg yolk phosphatidylcholine and beef heart cardiolipin were purchased from Biolek (Kharkov, Ukraine). A stock suspension of unilamellar phospholipid vesicles was prepared by the method of Batzri and Korn [8]. The ethanol lipid solution containing appropriate amounts of PC and CL was injected into 13 ml of 5 mM sodium-phosphate buffer, pH 7.4, under continuous stirring. Ethanol was then removed by dialysis. Several types of liposomes have been obtained, containing 0, 5, 10, 20 or 40 mol % of CL. Fluorescence measurements were performed with CM-2203 spectrofluorimeter using a 10 mm pathlength cuvette.

RESULTS AND DISCUSSION

Characterization of fluorescent probes. DSM is a pyridine derivative (Fig. 1) carrying at neutral pH positive charge. This probe resides preferentially in the polar membrane region being sensitive to electrostatic surface potential of a lipid bilayer. MBA (Fig. 2) is an uncharged probe of hydrophobic nature located in the region of glycerol backbone and upper acyl chain carbons [9]. Its fluorescence parameters do not depend on membrane potential but may be sensitive to the bilayer structural rearrangement.

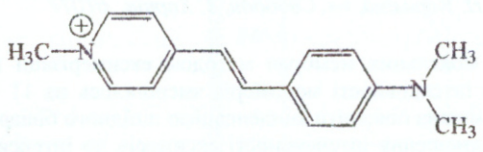

Fig. 1. Structure of DSM

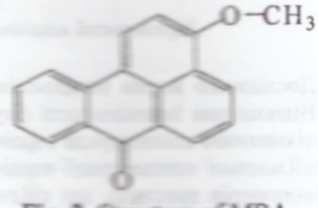

Fig. 2. Structure of MBA

DSM studies. As a first step of the study, we examined DSM binding to PC/CL model membranes. To estimate binding parameters (association constant (K_a) and binding stoichiometry (n_z)), the results of DSM fluorimetric titration with liposomes (Fig. 3) were analyzed quantitatively in terms of the Langmuir binding model [1]. It was assumed that the increase of DSM fluorescence intensity observed at its association with lipids (ΔI_z) is proportional to the concentration of bound probe (B_z):

$$\Delta I_z = a_z B_z \tag{1}$$

where a_z is a coefficient of proportionality, corresponding to the molar fluorescence of bound probe. If the probe binding site contains n_z lipid molecules, association constant is given by:

$$K_a = \frac{B_z}{F_z(L_0/n_z - B_z)} = \frac{B_z}{(Z_0 - B_z)(L_0/n_z - B_z)} \tag{2}$$

where F_z is concentration of free probe, Z_0 is total probe concentration. Given the eq.(2) fluorescence intensity increase ΔI_z can be represented as:

$$\Delta I_z = a_z 0.5\left\{Z_0 + L_0/n_z + 1/K_a - \sqrt{(Z_0 + L_0/n_z + 1/K_a)^2 - 4L_0Z_0/n_z}\right\} \tag{3}$$

Least-squares approximation of the experimental $\Delta I_z(L)$ dependencies by eq. (3) allowed us to estimate K_a, n_z and a_z values. As seen in Table 1, DSM association with model membranes enhances with increasing CL content. Next, it was of interest to evaluate to what extent this effect is controlled by electrostatic phenomena. For this purpose, association constant was represented as consisting of electrostatic (K_{el}) and non-electrostatic (K_0) terms [1]:

$$K_a = K_0 K_{el} = K_0 \cdot \exp\left\{\frac{-zF\psi}{RT}\right\} \tag{4}$$

where z is the probe charge (+1), ψ is electrostatic surface potential which was calculated in terms of Gouy-Chapman theory [10].

Table 1. Parameters of DSM binding to PC/CL liposomes

CL content, %	n_z	K_a, μM^{-1}	K_{el}	K_0, mM^{-1}	ψ, mV	a_z, μM^{-1}
5	11.0 ± 2.4	0.15 ± 0.08	30.8	4.8	-88	0.07 ± 0.007
10	8.9 ± 0.4	0.27 ± 0.06	108	2.7	-120	0.13 ± 0.002
40	8.7 ± 0.2	3.0 ± 0.9	1110	2.5	-180	0.13 ± 0.001

Comparison of the obtained K_0 values (Table 1) suggests that increase of CL content from 5 to 10 mol % gives rise to the changes of both electrostatic and non-electrostatic binding component. This finding led us to assume that incorporation of small amounts of CL into PC bilayer is followed by the changes in the conformational state of lipid groups surrounding DSM in the lipid bilayer (phosphorylcholine group, glycerol backbone and upper acyl chain carbons). Additional arguments in favor of this assumption come from the MBA fluorescence studies.

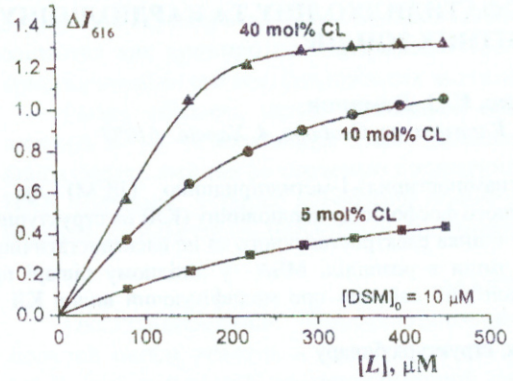

Fig. 3. Change of DSM fluorescence intensity as a function of lipid concentration

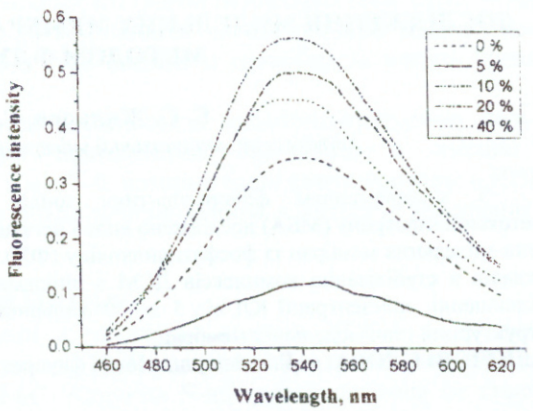

Fig. 4. MBA fluorescence spectra in PC/CL liposomes

MBA studies. Fig. 4 shows MBA fluorescence spectra in liposomes of various composition. Approximation of these spectra by Gaussian $I = A \cdot \exp\left\{-(\lambda - \lambda_{max})^2 / 2w^2\right\}$ revealed two (0, 5 mol % CL) or one (10, 20, 40 mol % CL) spectral components (Table 2). This implies that in liposomes whose CL content does not exceed 5 mol % there exist two spectral components corresponding to the two populations of MBA molecules differing in polarity and relaxation characteristics of the probe microenvironment. In the meantime, increase of CL content to 10-40 mol % is followed by the bilayer structural changes resulting in the MBA location at homogeneous binding sites.

Table 2. Parameters of MBA fluorescence spectra

CL content, %	Component 1			Component 2		
	λ^1_{max}, nm	w_1	A_1	λ^2_{max}, nm	w_2	A_2
0	528.3	32.3	0.05	577.3	48.6	0.20
5	528.9	31.2	0.39	573.2	46.6	0.29
10	546.6	45.7	0.39			
20	546.1	44.1	0.52			
40	546.9	40.5	0.12			

Importantly, the results presented here are in accordance with the data obtained by other authors [4,6]. Particularly, IR spectroscopy studies revealed that incorporation of small amounts of CL in PC bilayer stabilizes intermolecular hydrogen-bonded network including hydrational water molecules [6]. As CL concentration increases, electrostatic repulsion between negatively charged phosphate groups is likely to decrease phospholipid packing density thereby destabilizing hydrogen-bonded network. This, in turn, can facilitate redistribution of MBA molecules within the bilayer.

CONCLUSIONS

Fluorescent probes DSM and MBA appeared to be sensitive to the CL-induced changes in structural properties of PC model membranes. Evaluation of electrostatic and non-electrostatic components of the DSM binding to lipid bilayer showed that CL incorporation can exert influence not only on electrostatic surface potential, but also on the structural state of phospholipid molecules. Analysis of MBA fluorescence spectra is suggestive of the different structure of the bilayers with CL content less than 5 mol % and that exceeding 10 mol %.

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ДОСЛІДЖЕННЯ МОДЕЛЬНИХ МЕМБРАН ІЗ ФОСФАТИДИЛХОЛІНУ ТА КАРДІОЛІПІНУ МЕТОДОМ ФЛУОРЕСЦЕНТНИХ ЗОНДІВ

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З використанням флуоресцентних зондів 4-(n–диметиаміностирил)-1-метилпіридинію (ДСМ) та 3-метоксибензантрону (МБА) досліджено вплив негативно зарядженого фосфоліпіду кардіоліпіну (КЛ) на структурний стан модельних мембран із фосфатидилхоліну (ФХ). Проведена оцінка електростатичного та не електростатичного вкладів в стабілізацію комплексів ДСМ з ліпідами. Виявлені зміни в розподілі МБА у ліпідному бішарі при підвищенні концентрації КЛ від 5 до 10 молярних %. Отримані дані свідчать про модифікуючий вплив КЛ на структурний стан модельних мембран.

КЛЮЧОВІ СЛОВА: кардіоліпін, ліпосоми, флуоресцентні зонди, структура бішару