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DIFFERENTIATION OF MEMBRANE ACTIVE CROWN ETHERS ON THE MULTILAMELLAR LAYERS

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Abstract

Investigation of the interaction of crown ethers, which are have different structure and mechanism of membrane action, with multilamellar dispersions makes it possible to establish a difference in their effect on the rearrangement of the initial structure of lipid bilayers. Investigation of the restructuring of lipid bilayers during interaction of complexons, ionophores and channel-formers with a membrane allows obtaining important information on the mechanisms of transmembrane ion transport.

Keywords: *crown ether, differential scanning calorimetry, dipalmitoilphosphatidylcholine (DPPC), dimyristoylphosphotidylcholine (DMPC).*

1 Introduction

The crown ethers and their analogues described so far differ both in complexing properties and in membrane action. A detailed study of the dependence of the effect of crown ethers on membranes on their molecular structure serves as a prerequisite for revealing patterns between structure and membrane activity, substantiating the directed synthesis of crown ethers with predetermined properties. Such studies are an inevitable stage in the search for ways of directed synthesis of substances inducing in the membranes permeability of a given type.

It was found that crown ethers differ from their natural prototypes by exceptionally high resistance to thermal and chemical influences [8]. In compounds of this type, by choosing the "chemical skeleton", it is possible to vary widely both the cation selectivity and the lipophilicity of the complexes.

In molecules of cyclopolyethers, especially those containing aromatic residues, practically any functional groups can easily be introduced [1, 2, 4, 5, 6, 7, 11, 12, 22].

2 Materials and methods

The differential scanning calorimetry (DSC) method is one of the effective methods for detecting the features of structural transformations induced by membrane active molecules in lipid bilayers [16]. The results obtained by the DSC method reflects the nature of perturbations introduced by membrane-tropic molecules into the regular spatial packing of the bilayer and allows one to judge the probable portion of the molecule interacting with the membrane. Increasing the mobility of phospholipids in temperature scanning is associated with the appearance of gauche conformers in the acyl chains, and the transition of trans-gosh conformation occurs simultaneously for a large number of lipid molecules, associated in one of the cooperative units. The processes of collective transition of the conformation of trans-gauche are cooperative melting of lipids in the bilayers.

The thermodynamic quantitive parameters measured in the experiments are: the total enthalpy of melting ($\triangle H$) [9] and the cooperativity of melting, determined from the half-width of the peak of the main phase transition ($\triangle T_{1/2}$), reflecting the processes of increasing the mobility of hydrocarbon chains of the phospholipid bilayers, trans configurations. The molecules of membrane-active compounds can change the dynamic state of the lipid bilayer and thereby influence the phase transition of phospholipids from the gel state (when all the carbon-hydrogen chains are in the trans-conformation) to the liquid crystalline state (increase in mobility of phospholipid chains due to an increase in the probability of gosh conformers) [10]. The degree of influence of membranotropic molecules depends on the effectiveness of their interaction with lipid molecules, the level of localization and lateral mobility.

In this connection, by the DSC method, we conducted a comparative study of the effect of a number of crown ethers differing in the mechanisms of membrane action for determining the principles of their functioning in lipid bilayers [16].

The curves of microcalorimetric measurements were obtained with a 5-fold repetition (n=5). These data were subjected to statistical processing using a software package of statistical analysis on a computer with the calculation of the standard deviation (S), the Student's criterion (t), the dispersion of the normal distribution (δ) using the formula:

$$\delta = t^* \triangle S_s$$

where δ is the variance of the normal distribution t is the Student's ratio, $\triangle S_s$ - root-mean-square deviation [16]. For statistically significant changes, a confidence level of $P < 0.05$ was assumed.

3 Results

The study of the interaction of Ca^{2+} -complexons with multilamellar layers of dipalmitoilphosphatidylcholine

The thermogram of melting of the multilamellar dispersion of DPPC is a narrow peak with a maximum at a temperature Tm, called the temperature of the main

phase transition into a gel-liquid crystal [14] equal to $42.2°C$, and a less pronounced pre-transition peak at a temperature T_m equal to (35-36)$°C$ (Fig.1, control). The pretransition is associated with a change in the slope of the oriented hydrocarbon chains of phospholipid molecules in the gel phase, which requires little energy in the transition of the hydrocarbon chains of lipids from $L\beta$ to the $P\beta$ configuration. Upon melting, the phospholipid molecules pass from the gel phase (when the lipids are packed in the trans configuration) into the liquid crystal phase. The main contribution to the change in the entropy of this process introduces the possibility of new configurations, resulting from trans-gosh isomerization in hydrocarbon chains of the lipid. Thus, in the liquid-crystalline state ($T > T_m$), the low viscosity (high fluidity) of the carbon-hydrogen region of the membrane is explained by the increase in the amplitude of the rotational oscillations around the C-C bonds that arise from the Gosh configuration and their rapid isomerization to the next position [15].

The half-width of the main melting peak determines the cooperativity of the phase transition process, while the area under the peak is directly proportional to the melting enthalpy $\triangle H$ of the plume or the thermal effect of this process.

If membrane active crown ethers penetrate deep into the hydrophobic region of the lipid bilayer, they usually lead to a disruption of the initial structural lipid packing in the gel phase. In this case, the nature of the change in lipid packing depends on the structural features of crown ether molecules. Fig.1 shows experimental data on the effect of crown ethers exhibiting Ca^{2+}-complexons properties on multilamellar dispersions from DPPC.

It is known that the Ca^{2+}-complexons studied by us do not induce a transmembrane transport of Ca^{2+} ions. However, when Ca^{2+}-complexons are added to lipid samples, the main melting peak is broadened in comparison with the control and the total enthalpy of the lipid melting process is increased by 54.9%, 36.7% and 36.9%, a decrease in the cooperativity of the process by 77. 8%, 44.4% and 44.4%, respectively (Fig.1, Table 1).

In condition of increasing in the relative concentration of crown ether to lipid for 4',4"(5")-dipropionyl-DB18C6 and 4',4"(5")-dioctanoyl-DB18C6, the Tm values remain practically unchanged, while for 4',4"- diacetyl- DB18C6, a slight decrease in the T_m value by 1.4% is observed (Table 1). An increase in the total enthalpy with increasing relative concentration of any of the Ca^{2+}-complexons to the lipid indicates the interaction of these crown ethers with the polar part of the lipid bilayer without their deep penetration into the hydrophobic region. For 4',4"(5")-dipropionyl-DB18C6 and 4',4"(5")-dioctanoyl-DB18C6, penetration into the insignificant depth of the interchain space of the hydrocarbon chain of lipids is possible. In the case of 4',4"-diacetyl-DB18C6, its molecule interacts only with the polar part of the lipid bilayer. If the penetration of the hydrocarbon residues of the aforementioned crown ethers 4',4"(5")-dipropionyl-DB18C6 and 4',4"(5")-dioctanoyl-DB18C6 occurs, they should hinder trans-isomerization of hydrocarbon chains of lipids in the melting process at temperatures close to the temperature of T_m at the level of localization of hydrocarbon residues of crown ethers. In this case, the displacement of T_m is observed in the direction of high temperatures (Table 1). 4',4"-diacetyl- DB18C6 introduces a

perturbation entirely on the surface of the lipid bilayer, and such a perturbation leads to a decrease in the value of T_m with a simultaneous increase in the total enthalpy of the phase transition process.

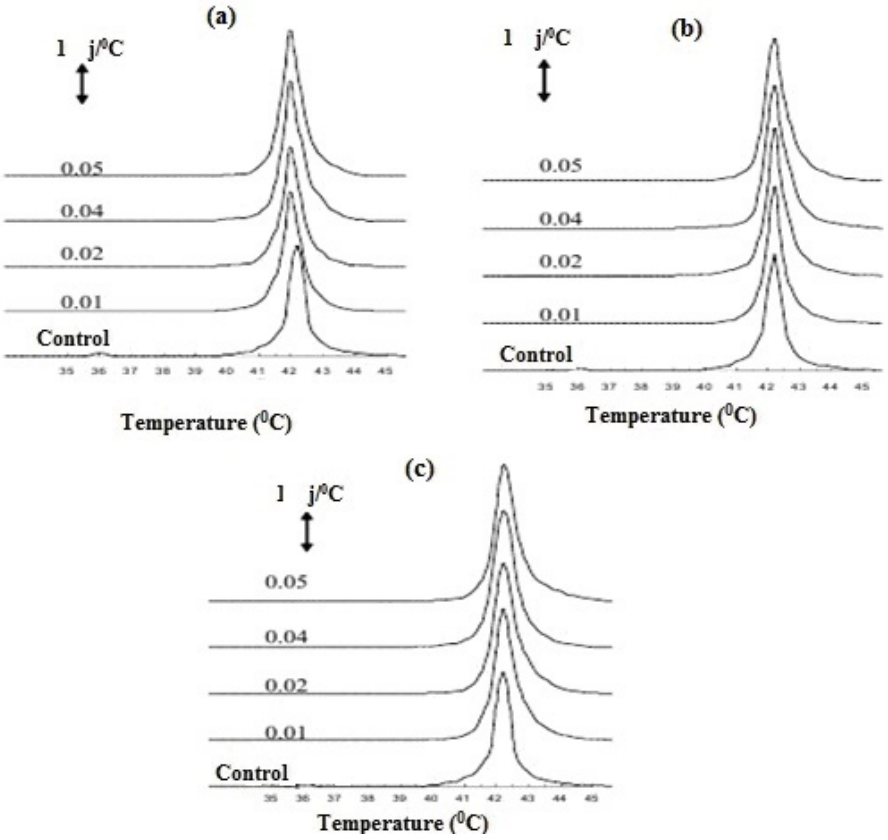

Fig. 1. Thermograms of the melting of multilamellar dispersions from DPPC as a function of the relative concentration of Ca2+-complexons to the lipid: a) 4',4" - diacetyl-DB18C6; b) 4',4"(5")-dipropionyl-DB18C6; c) 4',4"(5")-dioctanoyl-DB18C6.

The cooperativity of the lipid phase transition usually depends on the cluster size, determined from the experimental values of the half-width of the main melting peak. The cooperative evaluation is performed by calculating the value of the parameter $\delta = (\triangle H_{exp}/\triangle H_{VG})$, where $\triangle H_{exp}$ is the total enthalpy of the lipid melting process, $\triangle H_{VG}$ is the Van't Hoff enthalpy, which is calculated by the formula $\triangle H_{VG} \sim 7$. $T_m^2/\triangle T_{1/2}$, where T_m and $\triangle T_{1/2}$ are the temperature and half-width of the main melting peak, respectively. In the absence of cooperativity of the process $\delta = 1$ and for high cooperativity $\delta << 1$.

An increase or decrease in the value of δ for lipid samples with the addition of crown ethers indicates a decrease or increase in the cooperativity of the lipid phase transition process, respectively.

From the experimental data it follows that an increase in the relative concentration of Ca^{2+}-complexons leads to an increase in the value of δ in comparison with the control (Table 1) or to a decrease in the cooperativity of the process.

Table 1

Thermodynamic parameters of melting process of multilamellar dispersion from DPPC by Ca^{2+}-complexons at different ratios of crown ether to lipid ($C_{crown\ ethers}/C_{lipids}$).

$C_{crown\ ethers}/\ C_{lipids}$	H_m (kj/mol)	$T_m(^\circ C)^*$	$\triangle T_{1/2}(^\circ C)^*$	σ
Control	35.98±0.87	42.20	0.70	0.036±0.0008
4',4"-diacetyl-DB18C6				
1:100	39.33±1.71	42.00	0.72	0.041±0.0009
1:50	38.96±1.74	41.80	0.72	0.040±0.0009
1:25	48.12±1.84	41.80	0.74	0.051±0.0012
1:20	49.97±1.87	41.80	0.80	0.058±0.0014
1:10	55.75±1.93	41.60	0.80	0.064±0.0015
4',4"(5")-dipropionyl-DB18C6				
1:100	39.13±1.83	42.20	0.72	0.041±0.0009
1:50	46.14±1.80	42.20	0.72	0.048±0.0010
1:25	47.02±1.77	42.20	0.74	0.050±0.0012
1:20	49.07±1.83	42.25	0.74	0.052±0.0012
1:10	49.17±1.80	42.25	0.74	0.052±0.0012
4',5"-dioktanoyl-DB18C6				
1:100	46.47±1.77	42.20	0.72	0.048±0.0011
1:50	48.53±1.84	42.20	0.72	0.050±0.0012
1:25	51.65±1.25	42.20	0.74	0.055±0.0013
1:20	51.00±1.24	42.25	0.74	0.054±0.0013
1:10	49.26±1.19	42.25	0.74	0.052±0.0013

*Temperature measuring uncertainty value about $0.01^\circ C$

All the studied Ca^{2+}-complexons increase the total enthalpy and half-width decrease the cooperativity of the lipid phase transition. The Ca^{2+}-complexons 4',4"(5")-dipropionil-DB18C6 and 4',4"(5")-dioctanoil-DB18C6 penetrate into the insignificant depth of the interchain space of the hydrocarbon chains of lipids. In the case of deep penetration of the hydrocarbon substituents of these compounds, and in the presence of electrostatic interaction between the crown ether molecules and the polar part of the phospholipids, the phenomenon of interdigitation in the lipid phase can be observed, which is usually detected as a high-temperature shoulder on a melting thermogram [16].

The study of the interaction of Ca^{2+}-ionophores with multilamellar layers of dipalmitoilphosphatidylcholine

Ionophoric and channel-former properties of crown ethers depend also on their affinity for highly organized lipid systems, one of which are bilayer lipid membranes. Crown ethers, acting as ionophores, must, in the course of the introduction in the bilayer, completely differently affect the structural and dynamic properties of lipid bilayers, in contrast to the compounds that form ion conduction channels in bilayers. In addition, crown ethers with low lipophilicity and, accordingly, with high polarity can interact only with the polar part of the lipid bilayer without appreciable penetration deep into the hydrophobic region [15]. Therefore, it is very important to identify differences in structural transformations in the lipid matrix that can induce crown ethers with different mechanisms of transmembrane ion transport.

If the crown ether shows ionophore properties, then its molecules are distributed inside the lipid matrix and exclude part of the lipid molecules from the cooperative melting process, since the initial ordering of the lipid molecules in the gel phase changes. In this case, the disappearance of the pre-transition peak, the increase in the half-width of the main melting peak and the decrease in the total enthalpy of the phase transition will be observed. A similar phenomenon is observed for most membrane-active molecules that penetrate deep into the hydrophobic region of lipid membranes [3].

In contrast to Ca^{2+}-complexons, 4',4"-diacetyl-DB18C6, - dipropionyl-DB18C6, 4',4"(5")-dioctanoyl-DB18C6, crown the ethers of 4',4"(5")-dibutyryl-DB18C6 and 4',4"(5") -divaleryl-DB18C6 exhibit Ca^{2+}-ionophore activity [18]. Consequently, these compounds are completely localized within the bilayer space. Ionophor molecules disrupt the strict packing of lipid hydro¬carbon chains at a temperature below the temperature of the main phase transition of the DPPC.In this connection, some of the lipid molecules perturbed by ionophore molecules should be excluded from the phase transition process, which leads to a decrease in the intensity of the main melting peak by 0.94% (Fig. 2). This means that the total enthalpy of the phase transition process decreases by 26.2% and 17.5%, respectively (Table 2).

When introduced into the lipid bilayer, ionophore molecules disrupt the inter-chain hydrophobic interaction between the lipid molecules, which is explained by the de¬crease in the value of Tm and the increase in the half-width $\triangle T_{1/2}$, which depends on the cooperativity of the phase transition process of the lipid dispersion. The parameter δ which estimates the degree of cooperativity in the case of Ca^{2+}-ionophores, in contrast to Ca^{2+}-complexons, decreases insignificantly.

Consequently, on the basis of experimental data, it can be concluded that unlike Ca^{2+}-complexons, Ca^{2+}-ionophore molecules slightly increase the size of cooperative clusters.

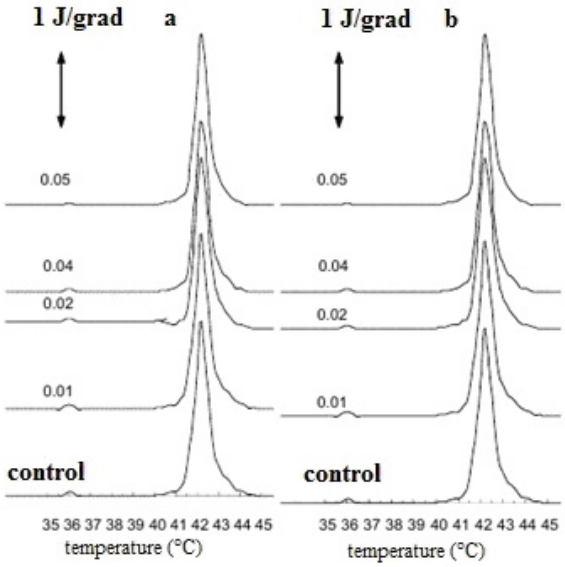

Fig. 2. Thermograms of the melting of multilamellar dispersions from DPPC as a function of the relative concentration of Ca^{2+}-ionophores to the lipid: a) 4',4"(5") -dibutyryl-DB18C6; b) 4',4"(5")-divaleryl-DB18C6.

Table 2

Thermodynamic parameters of the melting process of multilamellar dispersions from DPPC under action of Ca^{2+}-ionophores at different molar ratios crown ether to lipid ($C_{crown\ ethers}/C_{lipids}$.)

$C_{crown\ ethers}/\ C_{lipids}$	H_m (kj/mol)	$T_m(°C)^*$	$\triangle T_{1/2}(°C)^*$	σ
Control	35.98±0.87	42.20	0.70	0.036±0.0008
4',4"(5")-dibuthyryl–DB18C6				
1:100	29.65±0.72	42.00	0.74	0.035±0.0008
1:50	29.17±0.70	41.80	0.80	0.034±0.0008
1:25	28.48±0.69	41.80	0.80	0.033±0.0008
1:20	28.02±0.68	41.80	0.80	0.032±0.0008
1:10	26.55±0.65	41.80	0.80	0.031±0.0007
4',4"(5")-divaleryl-DB18C6				
1:100	35.28±0.86	42.20	0.72	0.036±0.0008
1:25	29.98±0.73	41.05	0.76	0.033±0.0008
1:20	29.95±0.73	41.80	0.80	0.033±0.0008
1:10	29.68±0.72	41.80	0.82	0.032±0.0008

*Temperature measuring uncertainty value about $0.01°C$

The study of the interaction of Ca^{2+}-channel formers with multilamellar layers of dipalmitoilphosphatidylcholine

Experimental data on the interaction of crown ethers exhibiting channel-shaped properties: 4',4"(5")-dimethylaminoethanol-DB18C6 and the bis-o-methoxyphenoxy-diethyl ether with multilamellar lipid dispersions from DPPC are shown in Fig.3. These Ca^{2+}-channel formers, in contrast to ionophore molecules, have limited freedom of movement and can directly affect only the lipid molecules surrounding them. It turned out that Ca^{2+}-channel formers reduce the total enthalpy of the phase transition of the lipid melting process by 39.9% and 15.9%, increasing the cooperativity of the process by 38.9% and 16.7%, respectively.

Thus, the investigated crown ethers show a principally different effect on the structure of lipid dispersions. This is seen in Fig. 4.where the changes in the total enthalpy of the phase transition process are shown for multilamellar dispersions from DPPC with the addition of increasing concentrations of three types of crown ethers: Ca^{2+}-complexons, Ca^{2+}-ionophores and Ca^{2+}-channel formers. For the CCa^{2+}-complexons studied, the total enthalpy increases, whereas forCa^{2+}-ionophores and Ca^{2+} -channel formers, the values of thermal effects decrease.

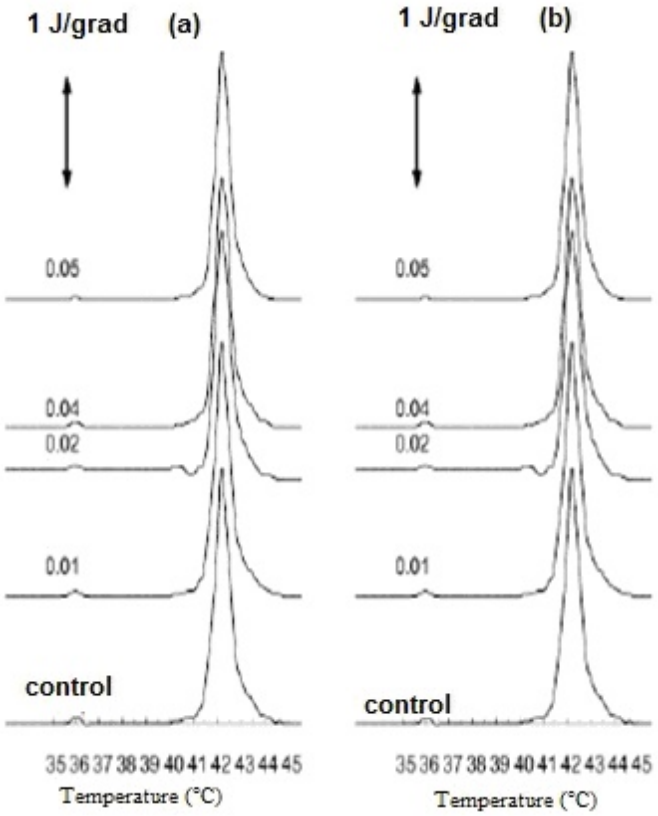

Fig. 3. Thermograms of melting of multilamellar dispersions from DPPC, depending on the relative concentration of Ca^{2+}-channel formers to lipid: a) 4',4"(5") -dimethylaminoethanol-DB18C6; b) bis-o-methoxyphenoxy-diethyl ether.

Table 3

Thermodynamic parameters of the melting process of multilamellar dispersions from DPPC under action Ca^{2+}-channel formers at different molar ratios of crown ether to lipid ($C_{crown\ ethers}/C_{lipids}$.)

$C_{crown\ ethers}/\ C_{lipids}$	H_m (kj/mol)	$T_m(^\circ C)^*$	$\triangle T_{1/2}(^\circ C)^*$	δ
Control	335.98±0.87	42.20	0.70	0.036±0.0008
4',4"(5") - dimethylaminoethanol-DB18C6				
1:100	31.41±0.76	42.20	0.70	0.032±0.0007
1:50	30.55±0.74	42.20	0.70	0.031±0.0007
1:25	29.96±0.73	42.20	0.70	0.030±0.0007
1:20	28.76±0.69	42.20	0.70	0.029±0.0007
1:10	21.62±0.52	42.20	0.70	0.022±0.0005
bis-o-methoxyphenoxy-diethyl ether				
1:100	35.31±0.86	42.20	0.70	0.036±0.0008
1:50	34.46±0.83	42.20	0.70	0.035±0.0008
1:25	34.00±0.83	42.20	0.70	0.034±0.0008
1:20	31.78±0.77	42.20	0.70	0.032±0.0007
1:10	30.25±0.73	42.20	0.70	0.030±0.0007

*Temperature measuring uncertainty value about $0.01^\circ C$

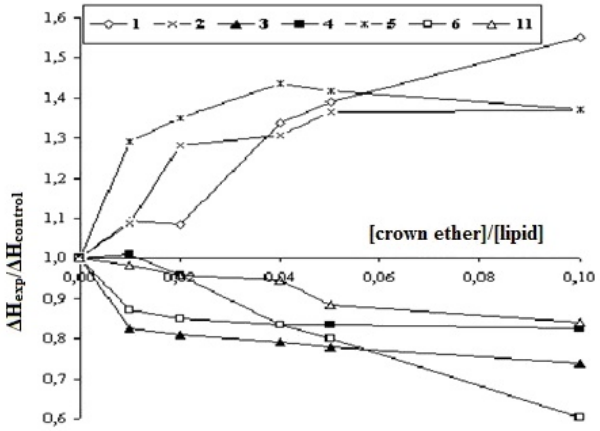

Fig. 4. The change in the relative enthalpy of the melting process of multilamellar dispersions from DPPC as the relative concentration of crown ethers to the lipid increases: 4',4"-diacetyl-DB18C6-(1), 4',4"(5")-dipropynoyl-DB18C6-(2), 4',4"(5")-dioctanoyl-BB18C6-(5)-(Ca^{2+}-complexons); 4',4"(5")-divaleryl-DB18C6-(3), 4',4"(5")-dibutyryl-DB18C6-(4)-(Ca^{2+}-ionophores); 4',4"(5")-dimethylaminoethanol-DB18C6-(6), bis-o-methoxyphenoxy-diethyl ether-(11) (Ca^{2+}-channelformers).

The study of the interaction of K^+– channelformers with multilamellar layers of dipalmitoilphosphatidylcholine

It was previously established that water-soluble sulfo derivatives - DB18C6 (4'-tert butyl-4 "(5") -DB18C6-sulfonic acid, 4'-acetyl-4"(5") - DB18C6-sulfonic acid, 4'-DB18C6-sulfonic acid and 4',4"(5") - DB18C6-disulfonic acid) have the ability to induce the formation of single ionic conduction channels for monovalent cations in lipid bilayers [20]. The channel forming properties of these compounds were significantly dependent on the degree of their lipophilicity and were located in the following series:

(4'-t-butyl-4"(5")- DB18C6-sulfonic acid> 4'-acetyl-4"(5")- DB18C6-sulfonic acid> 4'- DB18C6-sulfonic acid> 4',4"(5")- DB18C6-disulfonic acid [19, 20].

Fig. 5 shows that in the case of both DPPC and DMPC, the thermograms of melting control multilamellar dispersions (DMPC) represent a narrow peak with a maximum at a temperature of T equal to $24.2°C$ and a less pronounced pre-transition peak at a temperature T equal to $(13.5\text{-}14)°C$.

Learned sulphonated derivatives DB18C6 lead to a change in the initial organization of lipid molecules which primarily reduces the magnitude of the peak pre-transition [14,15]. This is observed when lipid dispersion of DMPC (Fig.5) are added lipophilic DB18C6-sulphonated derivatives (4'-third-butyl-4'(5')-DB18C6-sulfonic acid and 4'-acetyl-4"(5")- DB18C6-sulfonic acid). Practically these crown ethers already at the ratio $C_{crown\ ether}/C_{lip} = 0.02$ peak disappears, whereas in the case of compounds DB18C6-4'-sulfonic acid and 4',4"(5")-DB18C6-disulfonic pre-transition peak is observed at all used concentrations.

Consequently, the crown ethers 4'-third-butyl-4"(5")-DB18C6-sulfonic acid and 4'-acetyl-4"(5") - DB18C6-sulfonic acid lead to a decrease in the total enthalpy of the phase transition process by 17,7%, whereas for 4'-DB18C6-sulfonic acid and 4',4 "(5")- DB18C6-disulfonic acid this decrease is 6.3% and 2.6%, respectively.

Table 4 shows that 4'-third-butyl-4"(5")-DB18C6-sulfonic acid and 4'-acetyl-4"(5")-DB18C6-sulfonic acid lead to a significant expansion of the main melting peak by 50% and shift of the temperature of the maximum of the main phase transition towards low temperatures at $C_{Crown\ ether}/C_{lip} = 0.02$. It is shown that the expansion of the main melting peak indicates an increase in the parameter δ and consequently, a decrease in the cooperativity of the process.

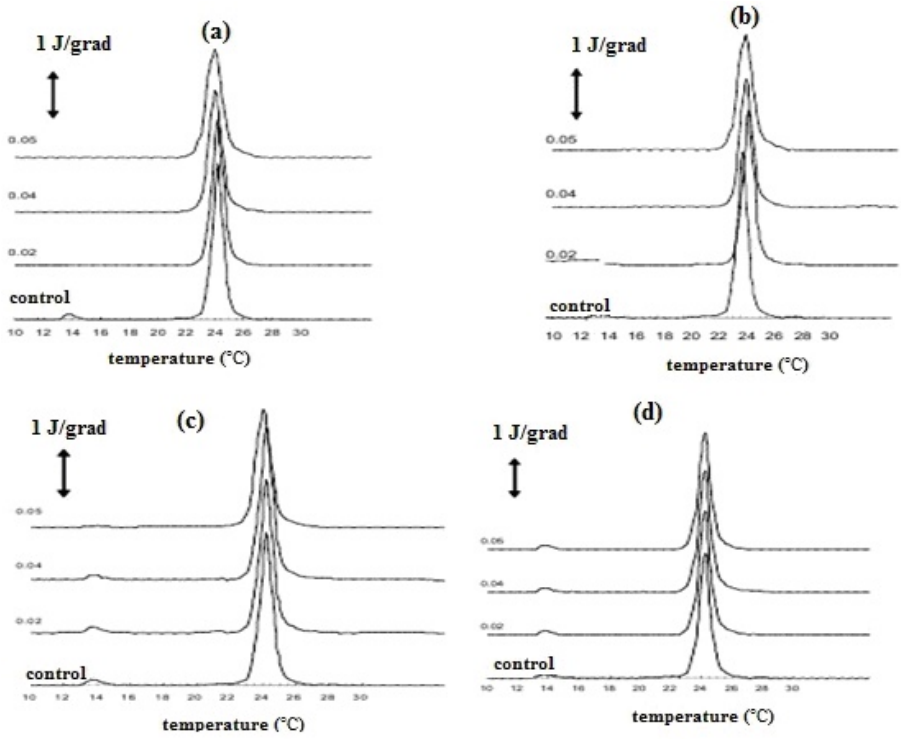

Fig. 5. Thermograms of the melting of multilamellar dispersions from DMPC as a function of the relative concentration K^+ -channel formers to the lipid: a) 4'-third-butyl-4"(5")-DB18C6-sulfonic acid b) 4'-acetyl-4"(5")-DB18C6-sulfonic acid c) 4'- DB18C6-sulfonic acid d) 4',4 "(5")-DB18C6-disulfonic acid K^+-channel formers to the lipid: a) 4'-third-butyl-4"(5")-DB18C6-sulfonic acid, b) 4'-acetyl-4"(5")- DB18C6-sulfonic acid, c) 4'-DB18C6-sulfonic acid, d) 4',4 "(5")-DB18C6-disulfonic acid.

Table 4

Thermodynamic parameters of the melting process of multi-lamellar dispersions from dimyristoylphosphotidylcholine under the action of K^+-channel formers at various molar ratios of crown ether to lipid ($C_{crown\ ethers}/C_{lipids}$.)

$C_{crown\ ethers}/\ C_{lipids}$	H_m (kj/mol)	$T_m(^\circ C)^*$	$\triangle T_{1/2}(^\circ C)^*$	σ
Control	27.34±0.66	24.2	0.80	0.035±0.0008
4'-third-butil-4"(5") –DB18C6-sulfonic acid				
1:50	25.77±0.63	24.2	0.90	0.038±0.0009
1:25	24.38±0.59	24.0	0.98	0.039±0.0009
1:20	22.50±0.54	24.0	1.20	0.044±0.001
4'-acetyl-4"(5")-DB18C6- sulfonic acid				
1:50	25.94±0.62	24.2	0.84	0.035±0.0008
1:25	24.79±0.60	24.0	0.88	0.038±0.0009
1:20	22.50±0.54	24.0	1.20	0.044±0.001
4'-DB18C6-sulfonic acid				
1:50	27.50±0.67	24.2	0.84	0.036±0.0008
1:25	26.88±0.65	24.2	0.84	0.037±0.0009
1:20	25.63±0.62	24.1	0.86	0.037±0.0009
4'-4"(5")-DB18C6- disulfonic acid				
1:50	27.64±0.67	24.2	0.80	0.035±0.0008
1:25	27.13±0.66	24.2	0.82	0.036±0.0008
1:20	26.63±0.64	24.2	0.82	0.036±0.0008

*Temperature measuring uncertainty value about $0.01^\circ C$

Fig. 6 shows that 4'-DB18C6-sulphonic acid and 4',4"(5")-DB18C6-disulfonic acid influence the thermodynamic parameters of the phase transition process of the DMPC dispersion more weakly. In the case of the interaction of Ca^{2+}-channel formers (4',4"(5")-dimethylaminoethanol-DB18C6 and bis-o-methoxyphenoxy-diethyl ether) with multilamellar dispersions from DPPC, the half-width of the phase transition and the temperature of the main melting peak . remained unchanged, and in the case of the interaction of K^+-channel formers (4'-third-butyl-4"(5")-DB18C6-sulfonic acid, 4'-acetyl-4"(5")-DB18C6-sulfonic acid, 4'-DB18C6-sulfonic acid, 4',4"(5")-DB18C6-disulfonic acid) with multilamellar dispersions from DMPC observed both an expansion of the main melting peak and a shift in the temperature of the main phasetransition toward low temperatures. Thus, a fundamental difference in the character of the interaction of crown ethers differing in the mechanisms of membrane action with multilamellar dispersions from DPPC and DMPC was revealed.

Thus, the interaction of Ca^{2+}-complexons (4',4"(5")-diacetyl- DB18C6, 4',4"(5") -dipropinoyl- DB18C6 and 4',4"(5") -dioctanoyl- DB18C6), Ca^{2+}-ionophores (4',4"(5")-dibutyryl-DB18C6 and 4',4"(5")-divaleryl- DB18C6) and Ca^{2+}-channel formers (4' ,4"(5")-dimethylaminoethanol-DB18C6 and bis-o-methoxy-phenoxy-diethyl ether with

multilamellar dispersions from DPPC, and K^+-channel formers (4'-third-butyl-4"(5") -DB18C6-sulfonic acid, 4'-acetyl-4"(5")-DB18C6-sulfonic acid, 4'-DB18C6-sulfonic acid and 4',4"(5")- DB18C6-disulfonic acid with multilamellar dispersions from DMPC.

It is shown that Ca^{2+}-complexons interact due to electrostatic forces with the polar part of the lipid, which leads to an increase in the energy necessary for the transition of the lipid dispersion from the gel to the liquid-crystalline phase, i.e. the total enthalpy of the process. With an increase in the relative concentration of crown ethers to the lipid in the case of Ca^{2+}- ionophores, the value of the half-width of the phase transition increases.

It has been established that the interaction of K^+-channel formers with multilamellar dispersions from DMPC shows both an expansion of the main melting peak and a shift of the temperature of the main phase transition toward low temperatures.

The determination of the features of the interaction of crown ethers differing by the mechanism of membrane action with multilamellar layers makes it possible to show a difference in their effect on the restructuring of the initial structure of lipid bilayers. This reflects the nature of the perturbations introduced by the membrane molecules into the regular packing of the bilayer and allows us to judge the probable part of the interaction with the molecule.

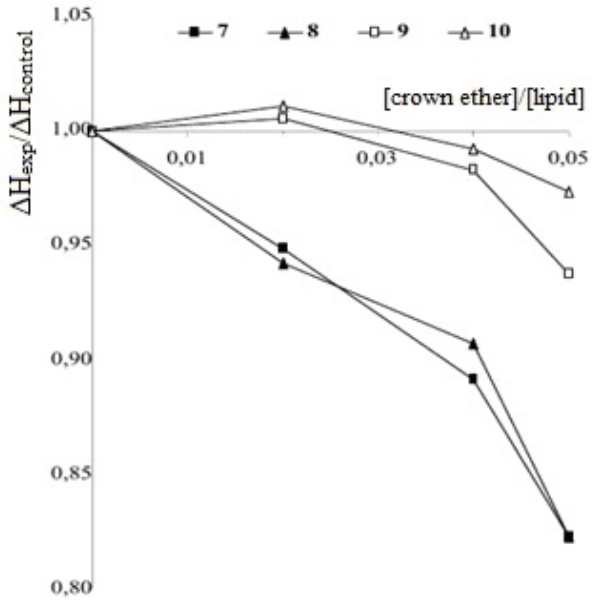

Fig. 6. The change in the relative enthalpy of the melting process of multilamellar dispersions from DMPC with an increase in the relative concentration of crown ethers to the lipid: 4'-third-butyl-4"(5")-DB18C6-sulfonic acid (7), 4'-acetyl-4"(5")-DB18C6 sulfonic acid (8), 4'-DB18C6-sulfonic acid (9), 4',4"(5")-DB18C6-disulfonic acid (10) -K^+-channel formers.

4 Discussion

On the basis of the obtained thermograms of melting of multilayered dispersions of phospholipids, it can be stated that Ca^{2+}-complexons: 4',4"-diacetyl-DB18C6, 4',4"(5")-dipropionyl-DB18C6 and -dioctanoyl-DB18C6 - increase the overall enthalpy of the phase transi¬tion of the melting process of lipids, reducing the cooperative nature of the process. As the relative concentration of crown ethers increases, the half-width of the phase transition increases, and the temperature of the main melting peak of Tm lipids increases insignificantly for long-chain complexons and decreases for a short-chain complexon. For long-chain Ca^{2+}-complexons: 4',4"(5")-dipropionyl-DB18C6 and 4',4"(5")-dioctanoyl-DB18C6, a slight shift of T_m to high temperatures is observed. This indicates that for long-chain complexons penetration into the insignificant depth of the interchain space is possible. 4',4"-diacetyl-DB18C6 introduces perturba¬tion entirely on the surface of the lipid bilayer and this leads to a decrease in T_m with a simultaneous increase in the total enthalpy of the phase transition process.

Ca^{2+}-ionophores: 4',4"(5")-dibutyryl-DB18C6 and: 4',4"(5")-divaleryl-DB18C6 lower the overall enthalpy of the phase transition of the melting process of lipids, slightly increasing the cooperativity of the process. The Ca^{2+}-ionophores are completely localized within the bilayer space, they disrupt the strict packing of hydrocarbon chains of phospholipids at a temperature below the temperature of the main phase transition, disrupt the interchain hydrophobic interaction between the various molecules of phospholipids. In this connection, a certain part of the phospholipid molecules perturbed by the molecules of Ca^{2+}-ionophores is excluded from the phospholipid phase transition process, which leads to a decrease in the intensity of the main melting peak and, as a consequence, to a decrease in the total enthalpy of the phase transition process. These Ca2+-ionophores introduce insignificant perturbations of the surface of the lipid bilayer, which leads to a decrease in T_m by 0.94%.

Ca^{2+}-channel formers: 4',4"(5")-dimethylaminoethanol-DB18C6 and bis -o-methoxyphenoxy-diethyl ether lower the total enthaethanollpy of the phase transition of the melting process of lipids, increasing the cooperativity of the process. As the relative concentration of crown ethers to the lipid increases, the value of the half-width of the phase transition and the temperature of the main melting peak of the Tm lipids remain unchanged. Ca^{2+}-channel formers form channel structures, which, embedded in the bilayer, violate the strict packing of carbon-hydrogen chains of phospholipids. This leads to a decrease in the total enthalpy of the phase transition process and an increase in its cooperativity.

K^+-channel formers: 4'-tret-butyl-4"(5")- DB18C6-sulfonic acid, 4'-acetyl-4"(5") - DB18C6-sulfonic acid, 4-DB18C6-monosulphonic acid and 4',4"(5")- DB18C6-disul fonic acid lower the total enthalpy of the phase transition of the melting process of lipids, reducing the cooperativity of the process. When the relative concentration of crown ethers to the lipid increases, the value of the half-width of the phase transition increases, and the temperature of the main melting peak of the lipids Tm remains unchanged. K^+-channel formers, embedded in the bilayer, violate the strict packing of hydrocarbon chains of phospholipids, which leads to a decrease in the total enthalpy

of the phase transition process. Apparently, these channel measures do not cause any noticeable surface disturbances ($T_{m-const}$).

The cooperativity of the lipid phase transition depends on the clusters determined from the experimental values of the half-width of the main melting peak. Areas with conserved short-range order (clusters) are characteristic at each given instant of time for both liquid crystals and ordinary liquids. In such clusters, the closest sections of the chains to the polar heads have a somewhat more dense packing of the gel in the "solid" bilayer. Toward the center of the bilayer, the packing density decreases, as at the boundaries of clusters, which are continuously disintegrated and formed, moving along the bilayer. Moreover, the boundary regions between the clusters are, as it were, dynamic defects in the bilayer. Thus, clusters are dynamic (instantaneous) formations with a lifetime of 10^{-7} seconds, including (40-60) carbon-hydrogen chains (20-30) molecules of phospholipid).

5 Conclusion

Indeed, for crown ethers 4',4"'(5"')-dimethylaminoethanol-DB18C6 and bis-o-methoxyphenoxy-diethyl ether inducing Ca^{2+}-channel conduc¬tivity on bilayers type lipid channel, an increase in the cooperativity of the lipid phase transition is observed to increase by 38.9% and 16.7%, and hence an increase in the process of cluster formation, respectively. For crown ethers (sulfoproductions-DB18C6) inducing K^{+}-channel conductivity of the "molecular associates" type, this trend is not observed, but a decrease in the cooperativity of the lipid phase transition is recorded, although as in the case of the lipid channel we are dealing with the induction of channel structures. This may serve as one more weighty argument in favor of the difference in the mechanisms of these two cases of channeling by crown ethers on bilayers.

In general, for the Ca^{2+}-complexons studied, the total enthalpy increases, whereas for Ca^{2+}-ionophores and Ca^{2+}-channel formers, the values of thermal effects decrease. For K^{+}-channel formers, this trend persists, but unlike Ca^{2+}-channel formers, the half-width of the main melting peak of the lipid dispersion from DMPC is dose-dependently reduced. Thus, for the studied crown ethers it was possible to find a general pattern: the relative enthalpy of the melting process of multilamellar lipid dispersion with increasing ratio of the concentration of crown ethers to the lipid for complexons grows, and for ionophores and channel formers decreases independently of the tolerable cation.

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