

Rocznik Świętokrzyski. Ser. B – Nauki Przyr. 32: 113–118, 2011

Polska Akademia Nauk – Oddział w Krakowie, Kieleckie Towarzystwo Naukowe,
Katedra Ochrony i Kształtowania Środowiska
Uniwersytetu Jana Kochanowskiego w Kielcach

Interaction between membrane-active crown-ethers and multilamellar lipid dispersion

Oddziaływanie między błonoaktywnymi eterami koronowymi
a multilamellarną dyspersją lipidową

U.T. SHARAFUTDINOVA, B.A. SALAKHUTDINOV,
A.K. TASHMUKHAMEDOVA, U.Z. MIRKHODJAEV

Streszczenie. Badania eterów koronowych, różniących się strukturą i mechanizmem aktywności błonowej, pozwalają na określenie różnic wpływu tych związków na strukturę dwuwarstwy lipidowej. Niniejsza praca przedstawia wyniki badań nad określeniem zasad funkcjonowania otrzymanych przez autorów związków makrocyklicznych (Diacylopochoodnych DB18C6, sulfopochodnych DB18C6, 4',4''(5'')-dimetyloetanolamino-DB18C6 oraz ich pseudocyklicznych analogów) w dwuwarstwach lipidowych. Badania były prowadzone za pomocą metody DSC, która pozwala na określenie zmian strukturalnych powodowanych przez cząsteczki błonoaktywne w dwuwarstwie lipidowej. Określono zmiany entalpii przejść fazowych oraz zmiany parametru σ .

Słowa kluczowe: błona aktywna, związki makrocykliczne, podwójna warstwa lipidu.

*U.T. Sharafutdinova, B.A. Salakhutdinov, A.K. Tashmukhamedova, U.Z. Mirkhodjaev,
Department of Biophysics, National University of Uzbekistan, 700174 Tashkent, VUZ Goro-
dok Uzbekistan, uktamnur@mail.ru*

INTRODUCTION

Crown-ethers are the important group of compounds which reveals complex, ionophoric and channel forming activity. Transmembrane ion transfer can be realized by crown ethers due to appearance of ionophoric activities and formation of ionic channels by them. Ionophoric and channel-forming properties of crown ethers depend on their affinity to the high-organized lipid systems, one of them is bilayer lipid membrane. Crown ethers functioning as ionophores due to their embody into bilayer, in contrast to compounds forming ionic channels in bilayers, must influence absolutely differently on structural-dynamic properties of lipid bilayers. Furthermore, crown ethers with a low lipophilic features and with a high polarity, accordingly, can interact only with the polar part of lipid bilayer without appreciable penetration deep into hydrophobic region. Therefore, a revealing of the difference in structural reorganizations in lipid matrix, which can be induced by these crown ethers, is very important.

Study of interaction of crown ethers differing in structure and membrane action mechanisms with the multilamellar lipid dispersion enables to establish the difference in their influences on change of the initial structure of lipid bilayers. In this regard, the comparative study of influence of the series of DB18C6 derivatives and its pseudocyclic analogues differing in membrane action mechanisms on multilamellar dispersion was carried out to determine of the principles of their function in lipid bilayers.

MATERIALS AND METHODS

Diacylderivatives of DB18C6, sulfoderivatives of DB18C6, 4',4''(5'')-dimethylethanolamin-DB18C6 and pseudocyclic analogues of DB18C6 or *bis*-*o*-methoxy-phenoxy-diethyl ether (fig. 1) were synthesized in Laboratory of Macrocyclic Compounds of the Chemical Faculty of the National University of Uzbekistan (Grebenyuk et al. 2001).

Action of crown ethers on a phase transition process of the multilamellar dispersions for DPPC and DMPC was studied by DSC method, because this method is effective and allows us to reveal features of the structural reorganizations in lipid bilayers induced by the membrane-active molecules (Gennis 1997). The multilamellar dispersions using DPPC and DMPC were prepared in 10 mmol *tris*-HCl buffer solution (pH 7.5) at the concentration of lipids 3×10^{-4} mol. The samples of the multilamellar lipid dispersions combined with the pure crown ethers were prepared by mixing of appropriate concentration of lipids

with adding crown ether solutions in ethanol. After drying of these samples, the buffer solution was added to them and shaken for 5 minutes at temperature 25-30°C. The obtained dispersion was put into camera of differential adiabatic scanning microcalorimeter DASM-4 (Russia). Estimation of the thermodynamic parameters of a phase transition of the multilamellar lipid dispersions was carried out at the recording speed 1°C/min. The obtained experimental curves were digitized and data were transferred into computer to analysis and mathematical treatment using of application package of statistical analysis computing probability of error (P). The level of data validity $P < 0.05$ has been taken for the statistically significant change.

RESULTS AND DISCUSSION

The interaction of DB18C6-sulfoderivatives with multilamellar dispersions formed from DMPC (dimiristoylphosphatidylcholine) and DPPC (dipalmitoylphosphatidylcholine) was investigated by us previously (Sharafutdinova et al. 2007). The investigation of how crown-ethers of different membrane activity interact with multilamellar dispersions allows us to establish their unique effect on reorganization of initial membrane packing.

On the basis of heating thermograms of phospholipid multilamellar dispersions it was established that Ca^{2+} complexones (4',4''(5'')-Diacetyl-DB18C6; 4',4''(5'')-Dipropanoyl-DB18C6; 4',4''(5'')-Dioctanoyl-DB18C6) increase the total enthalpy (H) of lipid phase transition to 54,94%, 36,65% and 36,9%, and increase the σ parameter to 77,77%, 44,44% and 44,44%, respectively.

When crown-ether/lipid ratio increases the width of peak at half-height increases as well while the temperature of main transition (T_m) doesn't change significantly for long-chain complexones, but decreases for short-chain complexones for 1,42%.

In experiments with long-chain complexones (4',4''(5'')-Dioctanoyl-DB18C6; 4',4''(5'')-Dipropanoyl-DB18C6) slight shift of (T_m) towards high temperatures region was observed. It testifies that long-chain complexones are capable to penetrate into a short depth of interchain area. 4',4''(5'')-Diacetyl-DB18C6 perturbs the surface of lipid bilayer causing the decrease of T_m and the increase of the total enthalpy of phase transition (fig. 2a).

Ca^{2+} -ionophores (4',4''(5'')-Dibutyryl-DB18C6; 4',4''(5'')-Divaleryl-DB18C6) decrease the total enthalpy (fig. 2a) of phase transition to 26,2% and 17,5%, decreasing at the same time σ parameter to 13,88% and 11,11% correspondingly. When

crown-ether/lipid ratio increases, the width of peak at half-height increases, while the temperature of T_m decreases to 0,94%.

Ca^{2+} -ionophores localize entirely into bilayer space, disrupt rigid package of hydrocarbon chains at the temperature lower than the temperature of main phase transition, also disturb inter-chain hydrophobic interactions between phospholipid molecules. In this case the certain number of phospholipids excludes from the process of phase transition that leads to decrease of intensity of melting peak and consequently leads to decrease of total enthalpy of phase transition. Cooperativity also decreases to more than 10%. Ca^{2+} -ionophores cause slight perturbations of bilayer surface that leads to decrease of T_m to 0,94%.

Ca^{2+} -channel formers 4',4''(5''')-Dimethylethanolamine-DB18C6 and Bis-O-methoxy-phenoxy-diethyl ether decrease total enthalpy (fig. 2a) of phase transition to 39,91% and 15,92%, respectively. At the same time decreasing σ parameter to 38,88%, and 16,66% respectively. At crown-ether/lipid ratio increase the width of peak at half-height and the temperature of main phase transition T_m remain unchangeable.

Ca^{2+} -channel formers form channel-like structures that incorporate into membrane bilayer space and disrupt the rigid packing of phospholipids' hydrocarbon chains. In this case the disturbances of membrane's surface are not observed. ($T_m - \text{const}$). It leads to decreasing of the total enthalpy of phase transition and cooperativity decreases to more than 10%.

K^+ -channel formers (4''-tert-butyl-4''(5''')-DB18C6-sulfoacid; 4'-acetyl-4''(5''')-DB18C6-sulfoacid; 4'-DB18C6-sulfoacid; 4',4''(5''')-DB18C6-disulfoacid) decrease the total enthalpy (Fig. 2 b) of phase transition to 17,7%; 17,7%; 6,25% and 2,59%, increasing σ parameter to 25,71%; 25,71%; 5,7% and 2,85% correspondingly. When crown-ether/lipid ratio increases the width of peak at half-height increases 50%; 50,7; 5% and 2,5% respectively while the temperature of main phase transition T_m remain unchangeable.

K^+ -channel formers form channel structures that embed into membrane bilayer and disrupt the rigid package of hydrocarbon chains that is manifested in decrease of total enthalpy of phase transition. Apparently there are no any remarkable perturbations caused by these compounds ($T_m - \text{const}$).

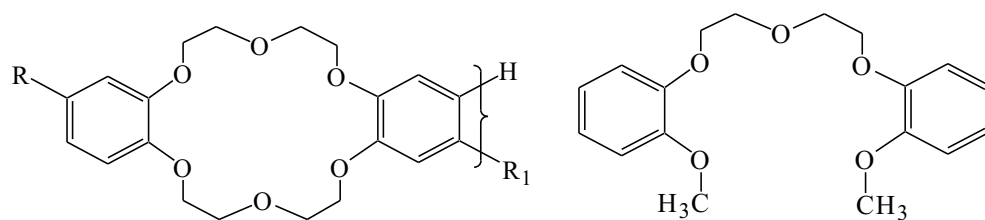
In contrast to Ca^{2+} -ionophores and Ca^{2+} -channel formers, the cooperativity of melting process increased both in case of Ca^{2+} -complexones and the most active K^+ -channel former (4''-tert-butyl-4''(5''')-DB18C6-sulfoacid) and the cooperativity decreased to 26%.

When crown-ether/lipid ratio increases, the width of peak at half-height increases also for all the studied crown-ethers, only one exception was observed in

experiments with Ca^{2+} -channel formers, when the width of peak at half-height remained unchangeable, though the cooperativity of melting decreased.

CONCLUSIONS

On the basis of crown-ethers structure, the classification of interactions of Ca^{2+} -complexones, Ca^{2+} -ionophores, Ca^{2+} -channel formers and K^{+} -channel formers with multilamellar dispersions, as well as definition the thermo dynamical characteristics of the interaction process were established.



a

a

- $R = R_1 = -\text{COCH}_3$
- $R = R_1 = -\text{COC}_2\text{H}_5$
- $R = R_1 = -\text{COC}_3\text{H}_7$
- $R = R_1 = -\text{COC}_4\text{H}_9$
- $R = R_1 = -\text{COC}_7\text{H}_{15}$
- $R = R_1 = -\text{CH}_2\text{NH}(\text{CH}_2)_2\text{OH}$
- $R = -\text{C}(\text{CH}_3)_3$; $R_1 = -\text{SO}_3\text{H}$
- $R = -\text{COCH}_3$; $R_1 = -\text{SO}_3\text{H}$
- $R = -\text{H}$; $R_1 = -\text{SO}_3\text{H}$
- $R = -\text{SO}_3\text{H}$; $R_1 = -\text{SO}_3\text{H}$

b

b

- $4',4''(5'')$ -Diacetyl-DB18C6 (1)
- $4',4''(5'')$ -Dipropionyl-DB18C6 (2)
- $4',4''(5'')$ -Dibutyryl-DB18C6 (3)
- $4',4''(5'')$ -Divaleryl-DB18C6 (4)
- $4',4''(5'')$ -Dioctanoyl-DB18C6 (5)
- $4',4''(5'')$ -Dimethylethanolamine-DB18C6 (6)
- $4''$ -tert-butyl- $4''(5'')$ -DB18C6-sulfoacid (7)
- $4'$ -acetyl- $4''(5'')$ -DB18C6-sulfoacid (8)
- $4'$ -DB18C6-sulfoacid (9)
- $4',4''(5'')$ -DB18C6-disulfoacid (10)
- Bis-O-methoxy-phenoxy-diethyl ether (11)

Fig. 1. Chemical structures of studied macrocyclic (a) and pseudocyclic (b) crown ethers

Rys. 1. Struktura chemiczna badanych makrocyklicznych (a) i pseudocyklicznych (b) eterów koronowych

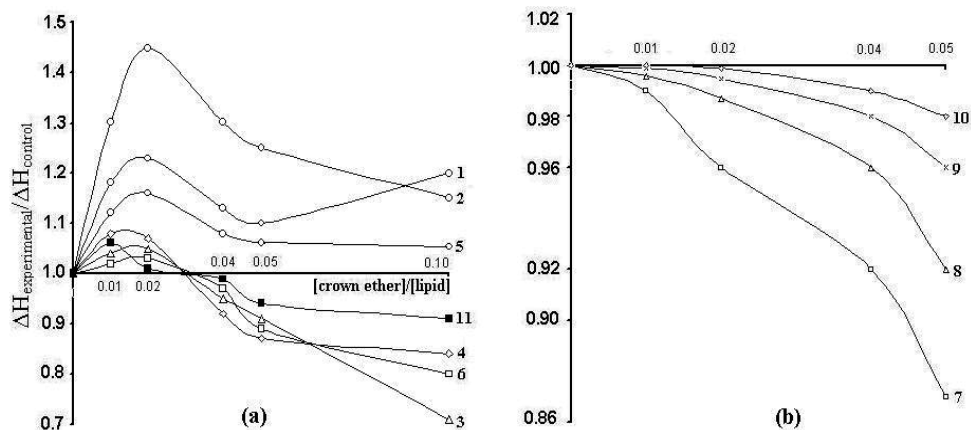


Fig. 2. Changes of the relative enthalpy of melting process of the multilamellar dispersions for DMPC and DPPC at increasing of the ratio of concentrations of crown ether to lipid: a) 1, 2, 5 (Ca^{2+} -complexons); 3, 4 (Ca^{2+} -ionophores); 6, 11 (Ca^{2+} -channel formers); b) 7, 8, 9, 10 (K^{+} -channel formers)

Rys. 2. Zmiany względnej entalpii procesu topnienia multilamellarnej dyspersji dla DMPC i DPCC przy wzrastającym stosunku stężenia eteru do lipidu: a) 1, 2, 5 (Ca^{2+} -kompleksy); 3, 4 (Ca^{2+} -jonofory); 6, 11 (związki tworzące kanały Ca^{2+}); (związki tworzące kanały K^{+})

References

- Gennis R., 1997. Biomembranes: molecular structure and functions//Mir. Moscow: 624-627.
- Grebenyuk A.D., Zotova L.V., Tashmukhamedova A.K. 2001.//Khim. Geterots. Soed. V. 7: 894-899.
- Sharafutdinova U.T., Kosymbetov P.G., Salakhutdinov B.A., Mirkhodjaev U.Z., 2007. Deystvie sulfoproizvodnih DB18C6 na termotropnie svoystva lipidnih dyspersiy. // Uzbekskiy biologicheskiy jurnal. No. 4: 34-39.