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INTERACTION OF NEW FLUORESCENT ICT-DYES WITH LIPID MEMBRANES

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The present study was undertaken to evaluate the sensitivity of newly synthesized ICT dyes to the changes in physicochemical properties of lipid bilayer. Partitioning of ICT4 into lipid phase of the model membranes composed of zwitterionic lipid phosphatidylcholine (PC) and its mixtures with anionic lipid cardiolipin and cholesterol was followed by the decrease of fluorescence quantum yield and short-wavelength shift of emission maximum. On the contrary, ICT2 exhibited tenfold increase of the quantum yield upon interaction with liposomes, without any shift of the emission maximum. Analysis of the partition coefficients showed that inclusion of cardiolipin and choleterol into phosphatidylcholine bilayer gives rise to increase of the ICT2 incorporation into lipid phase compared to the neat phosphatidylcholine membrane.

KEY WORDS: ICT dyes, liposomes, phosphatidylcholine, cardiolipin, cholesterol

ВЗАИМОДЕЙСТВИЕ НОВЫХ ICT-КРАСИТЕЛЕЙ С ЛИПИДНЫМИ МЕМБРАНАМИ

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В данной работе была проведена оценка чувствительности новых ICT-красителей к изменению физико-химических свойств липидного бислоя. Распределение красителя ICT4 в липидную фазу модельных мембран состоящих из цвиттерионного липида фосфатидилхолина и его смесей с анионным липидом кардиолипином и холестерином сопровождалось уменьшением квантового выхода флуоресценции и коротковолновым сдвигом максимума эмиссии. Напротив, для красителя ICT2 наблюдалось десятикратное возрастание квантового выхода при взаимодействии с липосомами, без сдвига максимума эмиссии. Анализ коэффициентов распределения показал, что включение кардиолипина и холестерина в фосфатидилхолиновый бислой увеличивает эффективность встраивания ICT2 в липосомальные мембраны по сравнению с фосфатидилхолиновыми липосомами.

KEY WORDS: ICT- красители, липосомы, фосфатидилхолин, кардиолипин, холестерин

ВЗАЄМОДІЯ НОВИХ ICT-БАРВНИКІВ З ЛІПІДНИМИ МЕМБРАНАМИ

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В даній роботі була проведена оцінка чутливості нових ICT-барвників до зміни фізико-хімічних властивостей ліпідного бішару. Розподіл барвника ICT4 в ліпідну фазу модельних мембран, що складались із цвіттеріонного ліпіда фосфатидилхоліна і його сумішей із аніонним ліпідом кардіоліпіном та холестерином супроводжувався зменшенням квантового виходу флуоресценції та короткохвильовим зсувом максимума емісії. Навпроти, для барвника ICT2 спостерігалось десятикратне зростання квантового виходу, без зсуву максимума емісії. Аналіз коефіцієнтів розподілу показав, що включення кардіоліпіну та холестерину в фосфатидилхоліновий бішар підвищує ефективність вбудовування ICT2 в ліпосомальні мембрани у порівнянні з фосфатидилхоліновими ліпосомами.

КЛЮЧОВІ СЛОВА: ICT- барвники, ліпосоми, , кардіоліпін, фосфатидилхолін , холестерин

During the past decades fluorophores which can undergo photoinduced itramolecular charge transfer (ICT dyes) find increasing application in biological sciences. Excitation of these fluorophores induces the motion of electron from electron-donating group to an electron-accepting group which are separated in space. In this state, which is called locally excited state (LE), fluorophore dissolved in polar solution is not in an equilibrium with the surrounding solvent molecules. It rotates during the lifetime of the excited state, thereby reaching equilibrium with its environment. The resulting ICT state is characterized by the higher emission wavelengths. Such fluorophore-solvent interactions explain the increase in red-shift of the emission maximum with increasing the solvent polarity [1,2]. Fluorophores which exhibit ICT state are widely used in different areas, including the development of solar cells and chemosensing [3,4]. High sensitivity of these compounds to the environmental polarity provokes their use as fluorescent microenvironmental sensors, particularly, in the studies of membrane structure [5-7] and protein-lipid interactions [8,9]. For example, two widely used membrane probes Prodan and Laurdan display high sensitivity to the environmental polarity, exhibiting large red shift from 420 to 480 nm as the probe moves from the nonpolar membrane region to the bilayer surface. Likewise, some ICT dyes demonstrated sensitivity to the viscosity and rigidity of their surroundings [10]. Special class of ICT dyes is represented by fluorescent molecular rotors, belonging to the group of twisted intramolecular charge transfer complexes (TICT) whose photophysical characteristics depend on their environment. Molecular rotors have been applied to monitor microviscosity changes in polymerization processes [11], phospholipid bilayers [12,13], and cell membranes [14]. Advanced properties of ICT dyes stimulated interest in the development of additional fluorophores for biological application.

The present study was undertaken to evaluate the sensitivity of newly synthesized ICT dyes, whose structural formulas are presented in Fig. 1, to the changes in physicochemical properties of lipid bilayer.

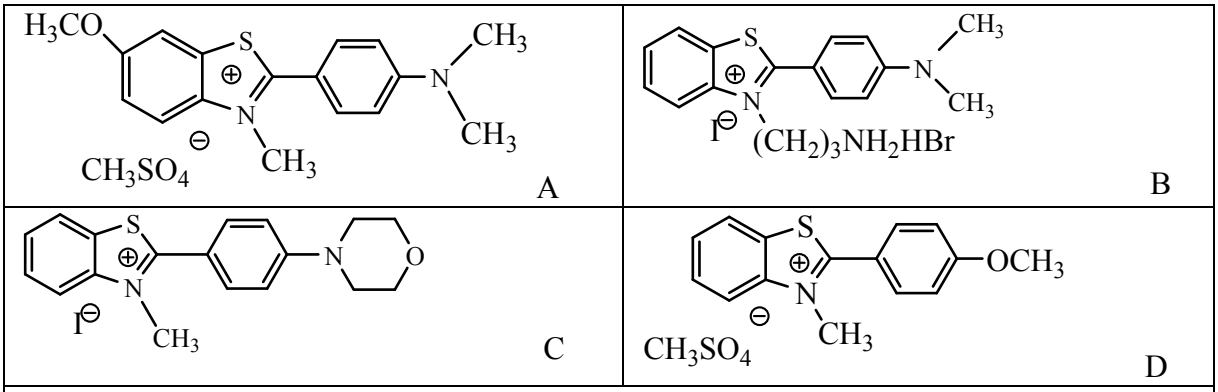


Fig. 1. Structure of the dyes ICT2 (A), ICT4 (B), ICT3(C) and ICT5 (D)

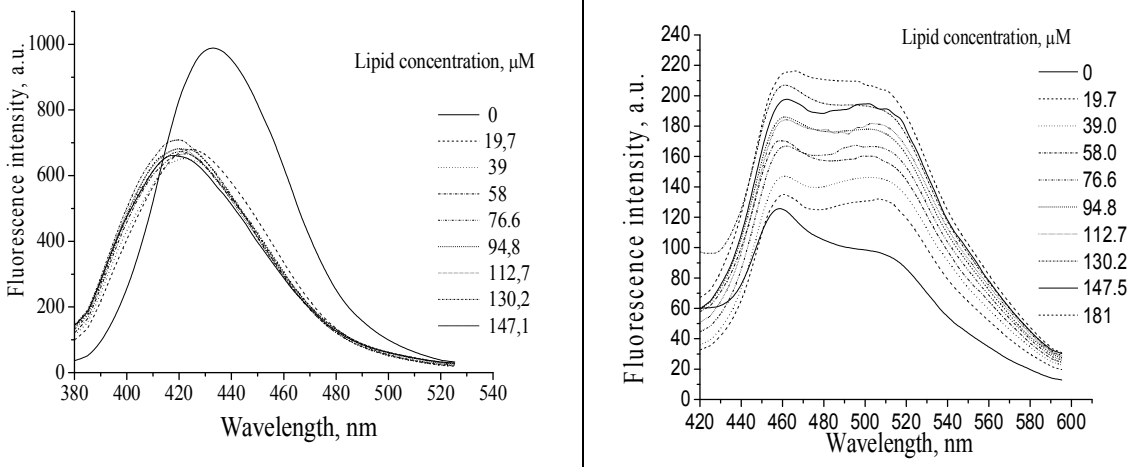
MATERIALS AND METHODS

Egg yolk phosphatidylcholine (PC) and beef heart cardiolipin (CL) were purchased from Biolek (Kharkov, Ukraine), cholesterol was from Sigma. Unilamellar lipid vesicles composed of pure PC and PC mixtures with i) 5 or 10 mol% of CL; and ii) 30 mol% of Chol were prepared by the extrusion method [15]. The phospholipid concentration was determined according to the procedure of Bartlett [16]. The dye-liposome mixtures were prepared by adding the proper amounts of the probe stock solution in ethanol to liposome suspension. The probe concentration was determined spectrophotometrically, using extinction coefficients $\varepsilon_{396} = 7700\ \text{M}^{-1}\text{cm}^{-1}$, $\varepsilon_{373} = 13900\ \text{M}^{-1}\text{cm}^{-1}$, $\varepsilon_{352} = 15100\ \text{M}^{-1}\text{cm}^{-1}$ and $\varepsilon_{332} = 16000\ \text{M}^{-1}\text{cm}^{-1}$

for ICT 2, ICT 3, ICT 4 and ICT 5, respectively. Steady-state fluorescence spectra were recorded with LS-50B spectrofluorimeter (Perkin-Elmer Ltd., Beaconsfield, UK). Fluorescence measurements were performed at 20 °C using 10 mm path-length quartz cuvettes. Quantum yield of ICT dyes in aqueous and lipid phases was determined using 1,8-anilino-naphtalene-sulfonic acid (ANS) as standard (ANS quantum yield in ethanol equals 0.37).

RESULTS AND DISCUSSION

At the first step of the study we compared the lipophilic properties of the ICT-dyes and their sensitivity to the membrane environment. Fluorescence spectra of these dyes were recorded in buffer solution (5 mM Na-phosphate, pH 7.4) and liposomal suspension. It appeared that only ICT2 and ICT4 are capable of fluorescing, while ICT3 and ICT5 are non-fluorescent. As seen in Fig. 2, ICT4 fluorescence decreases, while emission maximum (λ_{max}) is shifted from 435 nm in buffer to about 420 nm on the dye transfer from the aqueous to lipid phase (Fig. 2, A). On the contrary, the binding of ICT2 to the model membranes was followed by the fluorescence increase without any shift of the emission maximum (Fig.2, B).



A B

Fig. 2. Emission spectra of ICT4 in PC:CL (5 %) liposomes (A) and ICT2 in PC:CL (10 %) liposomes (B). ICT2 concentration was 4.3 μM, ICT4 concentration was 9.9 μM

As seen in Table 1, fluorescence quantum yield of ICT2 exhibited tenfold increase in the lipid bilayer compared to the buffer solution, while that of ICT4 decreased about twice. The observed increase of ICT2 fluorescence can be explained by the fluorophore transfer into membrane environment with reduced polarity and higher viscosity with the decreased rate of non-radiative relaxation processes involving excited state dissipation via vibrations, hydrogen bonding to the solvent cage and the probe rotation. The opposite fluorescence intensity changes observed for ICT4 can be interpreted as arising from: i) internal rotation within the fluorophore molecule (for instance, between the dimethylamino group and the phenyl ring) [1]; ii) fluorescence self-quenching occurring upon the dye accumulation in the liposomal membranes [2].

To characterize ICT2-lipid binding quantitatively, at the next step of the study we determined the dye partition coefficient (K_p) for different lipid systems. The relationship between K_p and fluorescence intensity increase (ΔI) can be written as [17]

$$\Delta I = I_L - I_W = \frac{K_p V_L (I_{\max} - I_W)}{1 + K_p V_L} \quad (1)$$

where I_L is the fluorescence intensity observed in the liposomal suspension at a certain lipid concentration C_L, I_W is the probe fluorescence intensity in the buffer, $I_{\max}$ is the limit fluorescence in the lipidic environment.

Table 1. Fluorescence quantum yield

System	ICT2	ICT4
Buffer	0.003	0.04
PC	0.03	0.02
PC/CL (5%)	0.04	0.03
PC/CL (10%)	0.03	0.02
PC/Chol (30%)	0.03	0.02

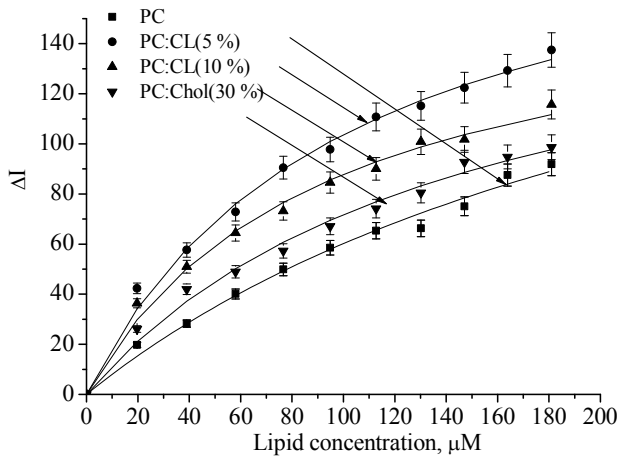


Fig. 3. Fluorescence intensity increase of ICT2 as a function of lipid concentration

To derive the dye partition coefficients for different lipid systems the experimental dependencies $\Delta I(C_L)$ presented in Fig. 3 were approximated by eq. (1). Analysis of the recovered partition coefficients (Table 2) shows that inclusion of anionic CL into PC bilayer gives rise to the increase of partition coefficient relative to the neat PC membrane. This can be explained by electrostatic interactions between the oppositely charged CL and dye molecules.

Table 2. Partition coefficients and limit fluorescence intensity of ICT2 in different lipid systems

System	Partition coefficient	Limit fluorescence intensity
PC	$(4.0 \pm 0.9) \times 10^3$	$(1.7 \pm 0.6) \times 10^2$
PC/CL (5 %)	$(10.0 \pm 1.1) \times 10^3$	$(2.0 \pm 0.1) \times 10^2$
PC/CL (10 %)	$(10.5 \pm 1.3) \times 10^3$	$(1.6 \pm 0.1) \times 10^2$
PC/Chol (30 %)	$(9.8 \pm 1.6) \times 10^3$	$(1.8 \pm 0.2) \times 10^2$

The conical shape of CL molecule induces a negative curvature strain, so that bilayer polar region becomes more accessible to water. Chol is fully embedded between the acyl chains of amphiphilic lipid. It has been assumed that it is more energetically favorable for Chol to have only its hydrophobic moiety buried into the nonpolar membrane core [18]. In such state Chol stretches over one membrane leaflet with its OH-group sticking out into the polar headgroup region of the lipid bilayer. This may lead to appearance of additional packing defects in the interfacial bilayer region on Chol addition, which, in turn, brings about the increase of partition coefficient compared to the neat PC membrane.

To summarize, a comparison of the fluorescence properties of the four newly synthesized ICT compounds revealed that despite being structurally similar, these dyes display different spectral and lipid-associating behavior. The absence of ICT3 and ICT5 fluorescence can be interpreted in terms of a nonemitting twisted state. ICT4 is capable of distributing between the aqueous and lipid phases, but quantum yield of this dye significantly decreases in a membrane environment. ICT2 was featured by the most effective partitioning into lipid phase coupled with considerable increase of the fluorescence quantum yield. Partition coefficient of ICT2 was found to increase upon inclusion of anionic lipid cardiolipin and cholesterol into phosphatidylcholine bilayer. The recovered properties of ICT2 allowed us to recommend this dye for the further use in membrane studies.

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