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Structural Studies of Selected DSPC-Surfactant Model Systems of Biological Membranes

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Fourier transform infrared spectroscopy was used to analyse the influence of a cationic surfactant from the group of morpholine derivatives on the conformational dynamics of CH_2 group in acyl chain of DSPC. The presence of the surfactant causes a decrease in the DSPC phase transition temperature. This result suggests that the surfactant interactions with phospholipid molecules disturb the lipid layers. The Fourier transform infrared measurements were supplemented with tests of the environmental toxicity of the surfactant used.

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1. Introduction

Functioning of all biological systems (cell organelles, cells, or whole organisms) is strictly dependent on the presence and structure of the cell membranes. The main substances forming the cell membranes are lipids, and among them phosphocholine derivatives [1]. The membranes show amphiphilic properties determining their ability to self-organisation and formation of unique lamellar structures. The temperature of the phase transition in phospholipids depends on the length of the fatty acid chains [2–4]. Fully hydrated 1,2-distearoyl-*sn*-glycero-3--phosphocholine (DSPC) shows two phase transitions: the pretransition ($L'_\beta \rightarrow P'_\beta$) at 321.3 K (325.7 K in D_2O) and the main transition ($P'_\beta \rightarrow L_\alpha$) at 326.5 K (328.5 K in D_2O) [5].

The phospholipids/surfactants systems in water have been recently intensely studied in investigation of phospholipid membranes structure and interactions, drug delivery systems and in many other biological and medical applications [6, 7]. In aqueous solutions of ionic surfactants their molecules can exist in the monomeric form as long as the surfactant concentration is lower than the critical micelle concentration (CMC). At concentrations higher than the value of CMC they can be in the micellar or in a variety of lyotropic liquid crystalline phases being in equilibrium with monomers [8, 9]. Since surfactants can form not only spherical micelles, bilayer membranes ("lamellae") or liposome structures, but also some of them can be used as simple models of biological cells. Therefore the interactions of biological membranes with surfactants seem to be very important in evaluation of the structure of natural systems.

In this work we report results of our experiments with 3-(*N*-tetradecylmorpholine)-1-propansulfonate (zwitterionic surfactant with the sulfobetaine structure, abbreviated as S_3C_{14}). It is a newly synthesised compound being a sulfonate analogue of classical zwitterionic surfactants possessing a betaine structure. The CMC value for this surfactant is 0.52 mM (or 0.02%) at 298 K as determined by surface tension measurements [10].

The study was undertaken to establish the effect of zwitterionic surfactant S_3C_{14} on the stability of the structural phases formed by DSPC in water solutions.

2. Materials and methods

2.1. Sample preparation

The phospholipid 1,2-distearoyl-*sn*-glycero-3--phosphocholine was obtained from Avanti Polar Lipids. The homogeneous 10% solutions of DSPC (in 20 mM K_2HPO_4/D_2O) and 0.1, 0.5, 1, and 5% S_3C_{14} surfactant/DSPC suspensions were prepared by the cyclic sonification at 340 K for 30 min and cooling at 278 K. D_2O was used as a solvent in preparation of the DSPC/surfactant mixture in order to better visualisation of the carbonyl region [11]. In all experiments S_3C_{14} was used at concentrations higher than CMC.

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2.2. FTIR spectroscopic measurements

Fourier transform infrared (FTIR) spectra of DSPC solutions in the temperature range from 288 to 323 K were obtained using BRUKER IFS 66 FTIR-RAMAN spectrometer (Bruker). The spectrometer was equipped with a Globar IR source, KBr beam splitter, DLATGS detector with KBr window and SPECAC Variable Temperature Cell. Phospholipid solutions of 20 μl volume were placed between KRS-5 windows with 0.2 mm teflon spacer and incubated for 15 min at a given temperature before starting the experiment. For each spectrum 128 scans in the spectral range 4000–600 cm^{-1} were collected with the resolution of 1 cm^{-1}. The spectroscopic measurements of the pure DSPC solutions and DSPC/S_3C_{14} systems were performed in the range 275–345 K.

2.3. Toxicity tests

The model water organism *Scenedesmus communis* was cultured on the medium Chu-10 [12] (40 mg/l $Ca(NO_3)_2$, 10 mg/l K_2HPO_4, 25 mg/l $MgSO_4$, 25 mg/l Na_2SiO_3, 20 mg/l Na_2CO_3, 3 mg/l of iron citrate, 0.6 mg/l H_3BO_4, 0.4 mg/l $MnCl_2$, 0.05 mg/l $ZnSO_4$, 0.05 mg/dm_3 $CuSO_4$, and 0.02 mg/l $(NH_4)_2MoO_4$). 200 ml of the medium was placed in conical flasks and inoculated with 2 ml of the suspension of *Scenedesmus*. The concentration of *Scenedesmus communis* cells in the initial culture was from 3000 to 4000 cells/ml. The culture was grown for 11 days at 295 K under artificial illumination of 2900 lx. The concentration of cells was controlled every day of the culture growth in a 2 ml sample, which was fixed by Lugol fluid and analysed in a Buerker chamber under a microscope.

3. Results and discussion

The FTIR results are presented in Figs. 1–3 as a function of temperature. The temperature changes in the wave numbers of the maxima of the bands corresponding to ν_{as} CH_2 ($\approx$ 2917 cm^{-1}) and ν_s CH_2 ($\approx$ 2850 cm^{-1}) can be used for determination of the temperature of the main phase transition (T_m), from the gel to the liquid crystal phase [2]. The gel phase is characterised by a high degree of packing thanks to the *trans* conformers and for this phase the ν_{as} CH_2 and ν_s CH_2 vibrations are characterised by lower values of wave numbers. Increasing temperature of the system induces formation of the *gauche* conformers whose presence reduces the strength of interaction between the alkyl chains of phospholipids. This reduction is manifested as an increase in the wave number values [3]. Figure 1 presents the positions of the ν_{as} CH_2 and ν_s CH_2 bands for pure DSPC and the DSPC/S_3C_{14} systems. The point of the main phase transition for pure DSPC is at 329 K, which is consistent with literature data [5]. Also the shifts of symmetric methylene CH_2 stretching modes ν_s from 2850.4 to 2853.3 cm^{-1} and antisymmetric CH_2 stretching modes ν_{as} from 2918.3 to 2922.7 cm^{-1} (Fig. 2) in the vicinity of the main transition are in good agreement with the data obtained by Huffman et al. [13]. For DSPC/0.1% S_3C_{14} system, the shifts of the bands from 2850.4 to 2853.4 cm^{-1} and from 2919.2 to 2923.0 cm^{-1}, characteristic of the main transition, were noted for ν_s CH_2 and ν_{ss} CH_2, respectively. The analogous shifts of the bands of the symmetric (from 2850.3 to 2852.8 cm^{-1}) and antisymmetric (from 2919.0 to 2922.7 cm^{-1}) methylene CH_2 stretching modes were also observed for DSPC/0.5% S_3C_{14} and DSPC/1% S_3C_{14}. For the DSPC/5% S_3C_{14} system the antisymmetric stretching band was shifted from 2918.9 to 2922.6 cm^{-1} and the symmetric stretching band was displaced from 2850.7 to 2853.0 cm^{-1}.

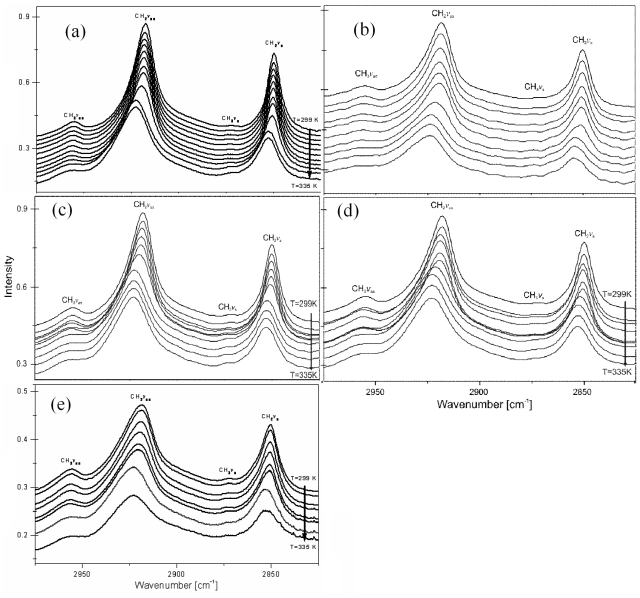

Fig. 1. FTIR spectra recorded for 10% DSPC (a), 10% DSPC/0.1% S_3C_{14} (b), 10% DSPC/0.5% S_3C_{14} (c), 10% DSPC/1% S_3C_{14} (d), and 10% DSPC/5% S_3C_{14} (e), systems.

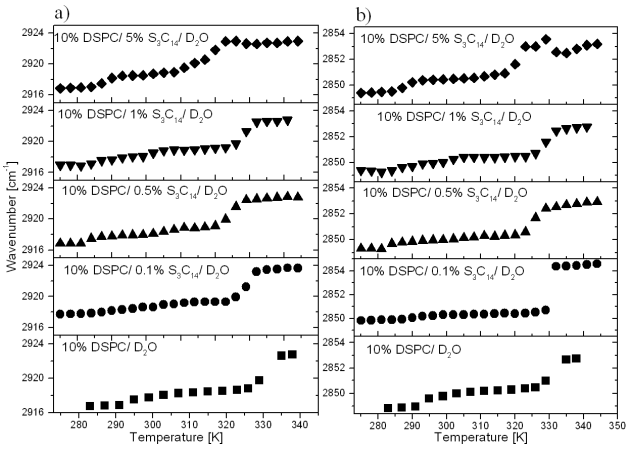

Fig. 2. Temperature variation of the frequency of the CH_2 asymmetric (a) and symmetric (b) stretching modes of DSPC liposomes in the absence and presence of S_3C_{14} surfactant.

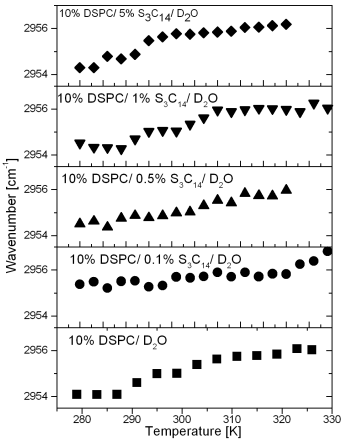

Fig. 3. Temperature dependence of frequency changes at the CH_3 asymmetric modes for pure DSPC and for DSPC liposomes containing S_3C_{14} surfactant.

Introduction of the surfactant to the system results in a decrease in the temperature of the main phase transition, probably as a result of the surfactant molecules grafting into the phospholipid bilayer. The surfactant molecules weaken the interchain interactions in the bilayer and induce the formation of the gauche conformers of alkyl chains. Moreover, depending on the surfactant concentration, the temperature range of the phase transition is broadened. This fact can be caused by a slower isomerisation of the chains related to the weakening of the interchain interactions [14–16]. The main phase transition point (T_{onset}), estimated on the basis of the CH_2 stretching modes is shifted to 328 and 324 K for DSPC/0.1% S_3C_{14} and DSPC/0.5% S_3C_{14} systems, respectively. Incorporation of 1% of S_3C_{14} shifted the main phase transition temperature to 326 K. Further addition of the surfactant S_3C_{14} results in a shift of the main phase transition even to 314 K for DSPC/5% S_3C_{14} system.

Analysis of the changes in the wave numbers of the band assigned to the vibrations of the terminal CH_3 group of alkyl chains provides the information on the conformational changes at the centre of the phospholipid bilayer [17]. The temperature dependence of the wave number of the asymmetric stretching vibrations of CH_3 group is shown in Fig. 3. In the spectra of pure DSPC the wave number continuously increases with temperature, which suggests increasing freedom of the CH_3 group vibrations. In the spectra of the systems DSPC/surfactant, the wave number undergoes jumpwise changes depending on the surfactant concentration. After the main phase transition (in the gel phase), the frequencies of the asymmetric stretching vibrations of CH_3 group of the DSPC/S_3C_{14} systems are higher than those in pure DSPC, indicating that the interactions with the surfactant (or inclusion into bilayers) increases the librational freedom of the acyl chains of DSPC in the central area of the bilayer.

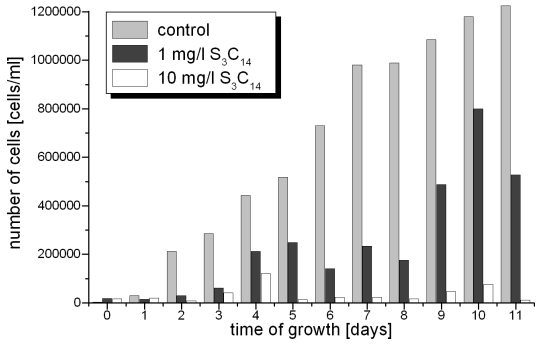

Fig. 4. The effect of the surfactant S_3C_{14} concentration on the growth of the *Scenedesmus communis*.

Figure 4 illustrates the effect of the surfactant on the growth of the model aquatic organism *Scenedesmus communis*. The cultures containing the surfactant in the medium were observed to show a significant inhibition of growth of the organisms studied relative to that in the reference culture (without the surfactant). The surfactant S_3C_{14} had a detectable inhibitory effect on the growth of the organisms studied already at the concentration of 1 mg/l. The total inhibition of growth of the algae was observed in the cultures growing in the medium containing 10 mg/l of the surfactant.

4. Conclusions

- Surfactants built into the phosholipids bilayer introduce the conformational disorder, which causes a decrease in the phase transition temperature of DSPC;
- The differences in the parameters characterising the main phase transitions in the systems DSPC–surfactant, can be related to the surfactant toxicity effect on the algae studied. The toxic effect of the surfactant studied is supposed to be related to disturbances in the cell membrane structure.

Acknowledgments

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