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Effect of Zeaxanthin on the Structure and Dynamics of Dipalmitoylphosphatidylcholine Bilayers

INTRODUCTION

The unique mechanical as well as dynamic properties of lipid membranes account for an experimental relevance of these self organizing structures in physiological processes [1]. Either the lipid membrane structure or its dynamic properties are sensitive to amphiphilic additives like cholesterol [2,3]. Cholesterol is believed to be the most important lipid membrane modifying agent in Eukaryota cells. The effect of cholesterol on mechanical properties of lipid membranes was studied by means of several techniques like electron paramagnetic resonance (EPR) [4], nuclear magnetic resonance (NMR) [5], differential scanning calorimetry (DSC) [6], diffractometry [7] and ultrasound absorption [8]. In the present work the carotenoid pigment — zeaxanthin is examined as a modifier of dipalmitoylphosphatidylcholine membranes. This study is consistent with the hypothesis that not only cholesterol but also other terpenoids [9] and carotenoid pigments in particular [9,10,11] are physiologically important in the regulation of mechanical properties of biological membranes. This mechanism seems to be particularly important in Prokaryota lacking cholesterol but also in the thylakoid membranes possessing not cholesterol but being very rich in carotenoid pigments (see discussion below).

MATERIALS AND METHODS

Zeaxanthin (β,β-carotene, 3,3'-diol) was obtained from Hoffmann-La-Roche. Pigment was repurified by means of thin layer chromatography directly before use. L-β,γ-dipalmitoyl, α-phosphatidylcholine (DPPC) was obtained from Sigma Chem.

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Co. DPPC membranes were pigmented with 5 mol% of zeaxanthin. This molar percentage of the pigment was shown to form the homogeneous system with DPPC without dependence of the physical state of the membrane [12]. Oriented multibilayers of DPPC analyzed by means of diffractometry were prepared according to the method described previously [13]. Multilamellar liposome suspension containing 1 mol% of 5-doxylstearic acid spin label (5-SASL, Sigma Chem. Co.) or 12-doxylstearic acid spin label (12-SASL, Sigma Chem. Co.) was prepared following the method described elsewhere [14]. Small unilamellar vesicles of DPPC were prepared in the same buffer as in the case of EPR samples (0.1 M borate, pH 9.5) according to the general procedure described elsewhere [15]. Sonicated suspension contained 0.5 mM DPPC. X-ray diffraction spectra were recorded on a HZG-4 apparatus with the Cu K_α radiation (λ = 1.54 Å) and a nickel filter. Data were transferred to the computer and stored on a diskette. Electron paramagnetic resonance spectra were recorded with X-band SE/X-28B spectrometer supplied with a temperature control unit. The measurement details and the temperature control parameters are described previously [16]. The excess ultrasound absorption ($\Delta\alpha$) was measured on the basis of the pulse method with the apparatus (UNIPAN) designed similarly to that described in Ref. 15.

RESULTS AND DISCUSSION

Fig. 1 presents the X-ray diffraction spectra of multibilayers prepared with DPPC and DPPC containing 5 mol% of zeaxanthin. The Bragg's spacing being a periodicity of the multilayer calculated on the basis of these spectra are equal to 62.8 Å and 60.3 Å, respectively. These data show that the thickness of the single bilayer is reduced by 2.5 Å upon the zeaxanthin presence within a DPPC membrane. Such an effect is just contrary to the one found recently in the case of xanthophyll pigment modification with respect to dimyristoylphosphatidylcholine (DMPC) bilayers [13]. It appeared that relatively small addition of zeaxanthin increased the periodicity of DMPC multilayer by 2.2 Å. This result was interpreted in terms of the increased thickness of the hydrophobic core of a single DMPC bilayer. Such interpretation is based on the comparison of the thickness of the hydrophobic core of DMPC estimated to be as high as 25.4 Å with the distance between the two opposite hydroxyl groups of the zeaxanthin molecule (L=30.2 Å, [9,10,11]) and on the finding that the linear pigment chromophore is located within a lipid phase and oriented with an angle of about 24° with respect to the axis normal to the plane of the membrane [13]. The thickness of the hydrophobic core of DPPC bilayer estimated following the method described previously [13] is as high as 32.0 Å at 22°C. It is wider by about 2 Å than the distance between the polar groups of zeaxanthin. The result of such a comparison means that both hydroxyl groups of the pigment molecule could not be pushed out towards the polar zones of the bilayer with the unpolar pigment chromophore being located within hydrophobic membrane interior. The finding that zeaxanthin addition decreases the DPPC multilayer periodicity is an indication that the requirement of the direct contact of the pigment polar groups with hydrophilic regions of the bilayer and

the lipophylic pigment chromophore with lipid core is, in fact, fulfilled. This effect was noticed here at 22°C (see caption to Fig. 1), the temperature corresponding to the $L_{\beta'}$, gel phase of DPPC, where the natural tendency of lipid acyl chains is to be maximum ordered. The extended conformation of acyl chains being expressed by a high value of the order parameter S is directly related to the thickness of the lipid membrane [17]. The fact that zeaxanthin reduces the thickness of the membrane in its ordered state indicates a strong modifying ability of the pigment. Zeaxanthin molecule possessing the rigid rod-like structure of conjugated double bonds is anchored by its two hydroxyl groups in the opposite hydrophilic zones of the lipid membrane. The fact of the location of the rigid hydrocarbon chain of zeaxanthin in the close vicinity of the lipid acyl chains should result in modification of the mechanical properties of the membrane since they are directly related to the conformational changes of acyl fatty acids.

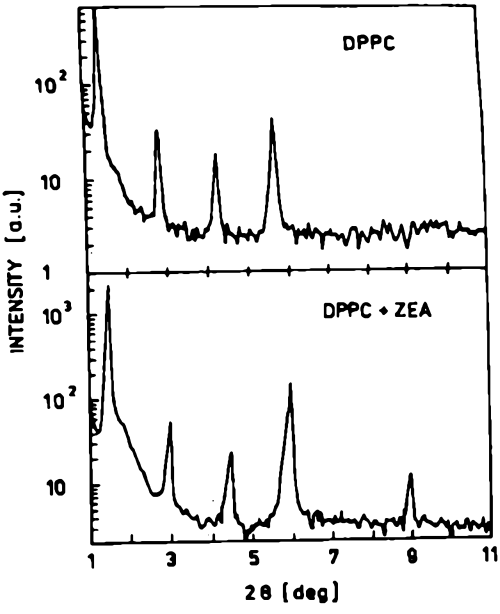

Fig. 1 X-ray diffraction spectra of multibilayers prepared with DPPC and with DPPC containing 5 mol% of zeaxanthin (DPPC+ZEA). The number of bilayers — 200, the scan temperature 22°C

Figs. 2 and 3 present the temperature dependence of the order parameter S calculated on the basis of EPR spectra [18] of spin labels located within the hydrophobic lipid core of DPPC in its depth corresponding to the fifth (5-SASL) and twelfth (12-SASL) carbon atom of the membrane doped ionized stearic acid. As it can be seen in these figures zeaxanthin decreases the DPPC membrane fluidity in its liquid-crystalline state (L_α) in the region of the hydrophobic core adjacent to the headgroup region (as demonstrated with 5-SASL) as well as in the membrane interior (as demonstrated with 12-SASL). The order parameter in the bilayer interior is higher in the pigmented membrane independently of the physical state of the bilayer different in various temperature regions. The rigidifying effect of the pigment is particularly evident in the $L_{\beta'}$ state of the membrane in this membrane

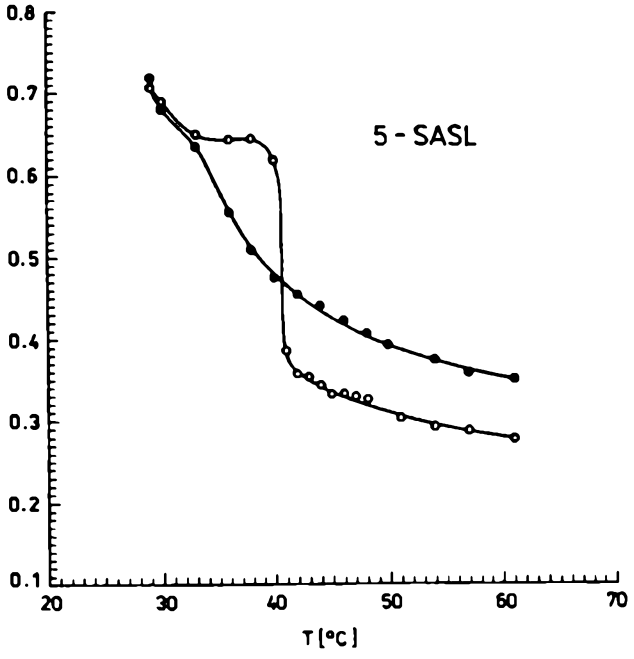

Fig. 2 Temperature dependence of the order parameter S of the 5-SASL spin label incorporated into DPPC liposomes (o) and liposomes of DPPC containing 5 mol% of zeaxanthin (•)

depth. The additional effect accounted for zeaxanthin-modification is the suppression of the abrupt $P_{\beta'} \rightarrow L_{\alpha}$ phase transition clearly visible in the control samples probed with the 5-SASL as well as with the 12-SASL. The temperature-dependent conformational order parameter does not demonstrate any changes in the phase pretransition ($P_{\beta'} \rightarrow L_{\alpha}$, at 35°C [19]) region of the pure DPPC indicating that this structural reorganization has not significant effect on the hydrocarbon packing as recently discussed by Cevc [20]. The DPPC membrane fluidity is markedly increased in the zone of the lipid core adjacent to the headgroup region (Fig. 1) in the phase-pretransition temperature-region upon the zeaxanthin presence within a membrane. This depth of the membrane corresponds to the location of the β-ionone rings of the zeaxanthin molecule and the disorder-effect-related to their presence is most likely responsible for the increased membrane fluidity as compared with the pure DPPC. The rigidifying effect of zeaxanthin is most probably the result of the hydrophobic interactions between the rod-like pigment molecule and acyl chains of the lipid. Such an interaction should obviously increase probability of the extended conformation of acyl chains decreasing probability of the *gauche-trans* isomerisation. The cooperative *gauche-trans* isomerisation taking place in the bulk lipid phase results in the abrupt changes of the mechanical properties of the membrane known as gel to liquid-crystalline ($P_{\beta'} \rightarrow L_{\alpha}$) phase transition. According to the calorimetric measurements the addition of zeaxanthin to DPPC membranes

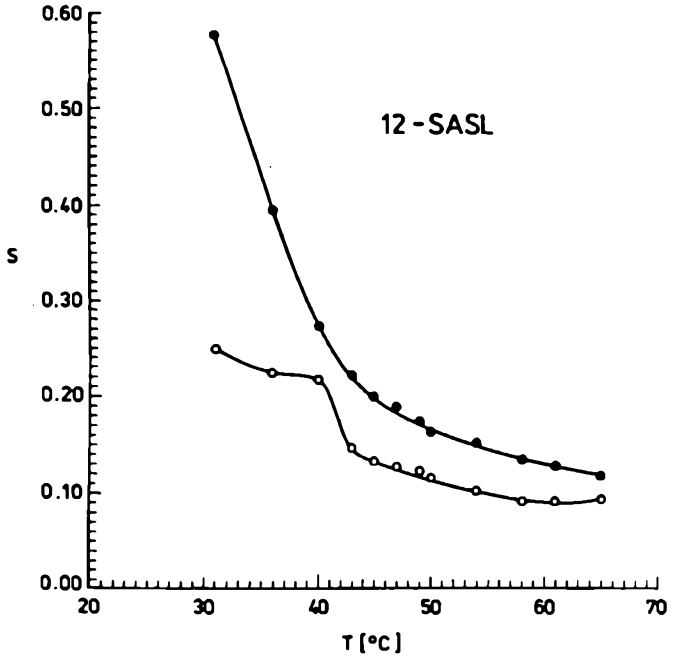

Fig. 3. Temperature dependence of the order parameter S of the 12-SASL spin label incorporated into DPPC liposomes (o) and liposomes of DPPC containing 5 mol% of zeaxanthin (•)

shifts down the $P_{\beta'} \rightarrow L_{\alpha}$ phase transition by about 1°C decreasing markedly its cooperativity [12]. Since the *gauche-trans* isomerisation is a relaxation process [21] its cooperative occurring in the region of the phase transition can be stimulated by the energy coming from ultrasound wave and this process can be followed by observation of the ultrasound absorption ($\Delta\alpha$). The difference and the main advantage of this method as compared to the calorimetric technique is that depending on the ultrasound wave frequency applied (f), we can separate numerous processes taking place during the phase transition by following the particular relaxation process corresponding to the particular relaxation frequency f_o. For instance the characteristic time τ_0 of the *gauche-trans* isomerisation of the terminal part of the acyl chain is about one order of magnitude shorter than in the case of the isomerisation of the upper part of the chain [22].

Fig. 4 presents the temperature dependence of the $\Delta\alpha/f^2$ value produced on the basis of ultrasound absorption at a fixed frequency of 20 MHz. As it can be seen from the comparison of the two panels of the Fig. 4 the single maximum appearing in the case of pure DPPC is shifted towards lower temperatures and splitted for at least two separate peaks in the case of the pigmented membranes. The latter effect is fairly sure since it was reproducible in all the examined samples. The dependence obtained in the case of the zeaxanthin-pigmented DPPC liposomes can be analyzed as the single, broad maximum. Then, one can find that the half-high

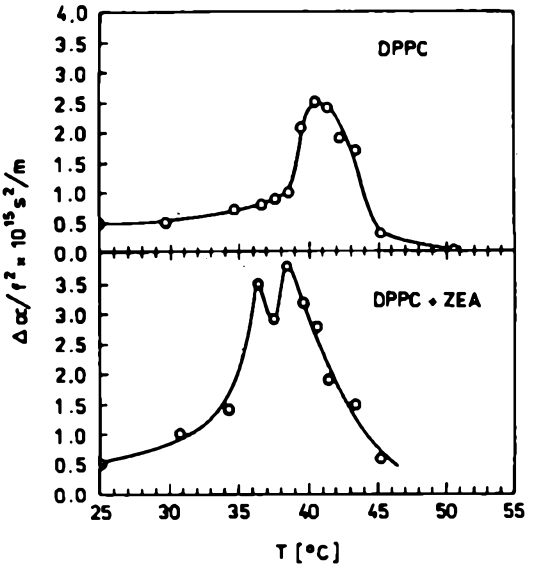

Fig. 4. Temperature dependence of ultrasound absorption per square of frequency ($\Delta\alpha/f^2$) of the DPPC liposomes and liposomes of DPPC containing 5 mol% of zeaxanthin (DPPC+ZEA). The dependencies correspond to the frequency of 20 MHz

width of the maximum increases from 5°C to 7°C and its position is shifted from 41°C to 38°C after the membrane modification with 5 mol% of zeaxanthin. This is an indication of the decreased cooperativity of the phase transition combined with the decreased activation barrier for the acyl chain isomerisation. Such finding seems to be particularly interesting in connection with the presented above conclusion about the existence of interactions between acyl chains and the molecule of the pigment. As a consequence of such interaction one can suppose the increase of the activation barrier to any kind of the molecular motion. The opposite effect can be interpreted in terms of the formation by the pigment molecules rigid compartments providing the favourable energetic conditions for the *gauche-trans* isomerisation. According to the spin-label data this hypothetical compartments decrease the motional freedom by reducing the order parameter-related angle of the cone in which the hydrocarbon chain segmental rotation undergoes [23]. The ultrasound absorption-monitored decreasing of the phase transition demonstrated by the broadening of the maximum is always combined with the decreasing of its intensity. This phenomenon can be observed in the case of the acoustic study of cholesterol effect on a phase transition [7]. The increased intensity of dependence obtained in the present study in the case of zeaxanthin-pigmented DPPC membrane combined with the general broadening of the maximum suggests that this maximum may be a result of the superposition of a certain number of narrow peaks reflecting different relaxation processes of a different activation barrier. The fact of the splitting of the experimental maximum strongly supports the presented above explanation. The source of the different activation barrier can be for example the different energy of a lipid-pigment interaction in the region of β-ionone rings and in the region of conjugated double bond linear system of zeaxanthin. Such difference was demonstrated above by means of spin label technique.

CONCLUSION

As can be revealed on the basis of the findings presented within this paper zeaxanthin — an example of the polar carotenoid pigment is a very efficient agent regulating the membrane structure (thickness) as well as dynamics (fluidity, phase transition). This fact can be of a special importance to consider the possible involvement of zeaxanthin and other carotenoids in regulation of the properties of biological membranes [24,25,26].

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