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Effect of phase transitions in hydrated 1,2-dipalmitoylphosphatidylethanolamine bilayers on the spin probe order parameter

L. Horniak, E. Kutejová* and P. Balgavý+

Institute of Molecular and Subcellular Biology, + Faculty of Pharmacy and Institute of Physics and Biophysics, J.A. Comenius University, 832 32 Bratislava and **Institute of Molecular Biology, Slovak Academy of Sciences, 842 51 Bratislava, Czechoslovakia*

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The 'main' phase transition $L_\beta \rightarrow L_\alpha$ of hydrated 1,2-dipalmitoylphosphatidylethanolamine (DPPE) bilayers in excess water affects the ESR order parameter S_{33} of *N*-cetyl-*N,N*-dimethyl-*N*-tempoylammonium bromide (CAT-16), 5-doxylstearic acid (5-DSA) and 16-doxylstearic acid (16-DSA) spin probes. The 'pre-transition' and 'subtransition' suggested to occur in hydrated DPPE by Chowdhry et al. [(1984) Biophys. J. 45, 901–904] and Silvius et al. [(1986) Biochemistry 25, 4249–4258], respectively, affect exclusively the S_{33} of CAT-16, but not that of 5-DSA and 16-DSA spin probes. The subtransition occurs about 15 ± 1°C below the main transition.

1,2-Dipalmitoylphosphatidylethanolamine; Phase transition; ESR; Spin probe

1. INTRODUCTION

Phosphatidylcholines (PC) and phosphatidylethanolamines (PE) together constitute the majority of the total phospholipids in most biological membranes, and their lyotropic phases in excess water are widely studied as models of the lipid part of biological membranes. A combination of a wide range of physical techniques has demonstrated that the phase behaviour of hydrated 1,2-diacyl-PEs in excess water can be described according to the form

$$L_\beta \rightleftharpoons L_\alpha \rightleftharpoons H_{II} \quad (1)$$

where L_β is the solid-like lamellar gel phase with extended acyl chains in all-antiplanar conformation, L_α and H_{II} are the fluid liquid-crystalline phases with disordered acyl chains due to antiplanar-*gauche* isomerization, and L_α is the lamellar and H_{II} the inverted hexagonal phase [1–18]. Besides these phases, saturated 1,2-diacyl-PEs form 'high-melting' solid phases, which remain stable up to temperatures well above the $L_\beta \longrightarrow L_\alpha$ phase transition temperature [9–18]. The high-melting phases appear to be nearly anhydrous and form when dispersing a well-dried lipid in cold water or when incubating the hydrated lipid for long periods at temperatures near 0°C. Silvius et al. [17] recently suggested that besides the above-mentioned phases, hydrated 1,2-diacyl-PEs can adopt a 'subgel-like' phase which is different from the anhydrous high-melting phase and which we denote L_c in analogy to PCs [19–23]. The phase transition $L_c \longrightarrow L_\beta$ ('subtransition') occurs in 1,2-dimyristoyl-PE about 15°C below the $L_\beta \longrightarrow$

Correspondence address: P. Balgavý, Faculty of Pharmacy and Institute of Physics and Biophysics, J.A. Comenius University, 83232 Bratislava, Czechoslovakia

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L_α transition and seems to be primarily a lattice rearrangement without substantial absorption of heat or an increase in chain *gauche* conformers [17]. Furthermore, microcalorimetric experiments performed by Chowdhry et al. [13,14] suggest that immediately below the 'main' phase transition temperature of hydrated PEs (within about 1°C), further transition occurs analogously to the 'pretransition' of PCs. Seddon et al. [11] have also reported three phase transitions in 1,2-dilauroyl-PE except $L_\alpha \longrightarrow H_{II}$. It thus seems that the phase behaviour of hydrated PEs in excess water is more extensive than described by the scheme [1].

Here, we have investigated the effects of the phase transitions in hydrated 1,2-dipalmitoyl-PE (DPPE) on the order parameter S_{33} of spin probes located in the hydrophobic or polar part of the DPPE bilayer. The supposed sub- and pretransition affect S_{33} of the probe located in the polar part only, while the $L_\beta \longrightarrow L_\alpha$ main phase transition affects S_{33} of probes located in both the polar and hydrophobic parts of the bilayer.

2. MATERIALS AND METHODS

The spin probe *N*-cetyl-*N*,*N*-dimethyl-*N*-tempoylammonium bromide (CAT-16) was prepared by Dr A. Atanasov (Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia). The spin probes 16-doxylstearic acid (16-DSA) and 5-doxylstearic acid (5-DSA) were purchased from Syva (Palo Alto, USA). DPPE was from Fluka (Buchs, Switzerland). Spin probe and DPPE were mixed in a molar ratio of 1:100 in methanol, and the methanol was evaporated under a stream of nitrogen gas followed by evacuation. Thereafter, lipid was dispersed in redistilled water in a weight ratio of 1:25 using a bath sonicator. Lipid was hydrated by heating at 75°C for 10 min. Hydrated lipid dispersion was filled into a glass capillary, sealed and stored at −30°C. Immediately before the experiment, the lipid was rehydrated by heating at 75°C for 10 min, and cooled to room temperature. ESR spectra were recorded by means of an ERS 230 (ZWG AdW DDR, Berlin, GDR) X-band spectrometer using the 100 kHz modulation technique. Typical instrumental settings were 5 mW microwave power, modulation amplitude 2 G or less, scan rate 15 G·min^{-1} or less, and the accuracy of temperature setting ±0.5°C.

All three spin probes in hydrated DPPE display axially symmetric powder pattern spectra. From their inner and outer extrema the experimental inner and outer splittings $A_\parallel'$ and $A_\perp'$, respectively, were evaluated and hence the order parameter S_{33} calculated

$$S_{33} = [f_a(A_\parallel - A_\perp)]:[A_{zz} - \tfrac{1}{2}(A_{xx} + A_{yy})] \quad (2)$$

$$A_\parallel \approx A_\parallel' \quad (3)$$

$$A_\perp \approx A_\perp' + 1.4\{1 - (A_\parallel' - A_\perp'):[A_{zz} - \tfrac{1}{2}(A_{xx} + A_{yy})]\} \quad (4)$$

$$f_a = (A_{xx} + A_{yy} + A_{zz}):(2A_\perp + A_\parallel) \quad (5)$$

where A_{ii} are the cartesian principle values of the diagonalized hyperfine splitting tensor $\bar{A}$, $A_\perp$ is the corrected value of the inner splitting, and f_a is the polarity correction factor (see [24,25] for details). The A_{ii} values used in calculations of S_{33} for the spin probes CAT-16 and m-DSA were those found for tempone [26] and obtained by computer simulation of 5-DSA spectra in oriented PC bilayers [27], respectively. The change in S_{33} indicates a change in the ensemble-averaged direction cosine of the angle between the *z*-axis of the $\bar{A}$ tensor and the direction perpendicular to the lipid lamellae, but it can also indicate a change in the correlation time(s) of the spin probe's rotational motion. These two contributions were not separated in the present communication.

3. RESULTS AND DISCUSSION

The results are presented in fig.1. The spin probe 16-DSA, of which the paramagnetic N-O group is located near the center of the bilayer hydrocarbon core, indicates a sudden change in the hydrated DPPE bilayer at 63.8 ± 0.5°C. This coincides with the $L_\beta \longrightarrow L_\alpha$ phase transition temperature T_c = 63.8 ± 0.02°C found in differential scanning calorimetry experiments [13,14]. The results obtained with the spin probes 5-DSA and CAT-16 indicate that the width of the $L_\beta \longrightarrow L_\alpha$ phase transition region is greater than that detected with 16-DSA. Close inspection of the data in fig.1 or in a plot of $d(\ln S)/d(1/T)$ vs $1/T$ (not shown) shows a more gradual change in S at the beginning of the $L_\beta \longrightarrow L_\alpha$ transition than at its end. This effect is

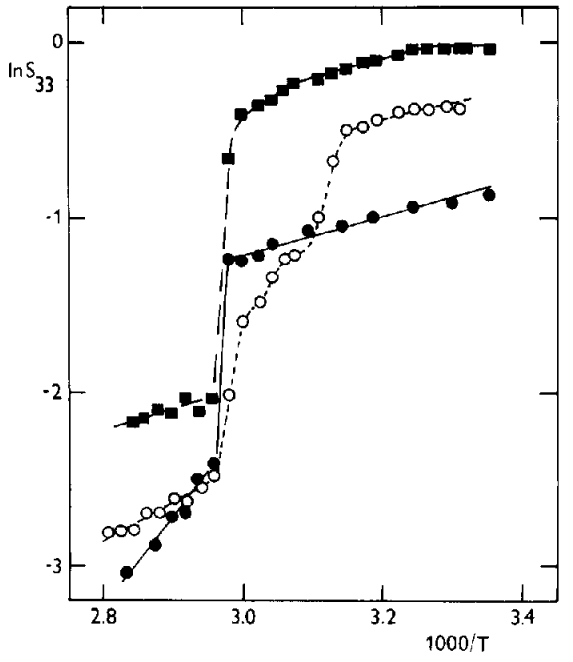

Fig.1. Temperature dependence of the order parameter S_{33} as detected by the spin probes 5-DSA (■), 16-DSA (●) and CAT-16 (○). T is expressed in K.

especially clearly seen in the case of the spin probe CAT-16. As detected by calorimetry [13,14], the $L_\beta \longrightarrow L_\alpha$ phase transition of hydrated 1,2-diacyl-PE is asymmetric on the low-temperature side. This asymmetry is supposed to be caused by phase transition(s) which take(s) place at temperatures immediately below the $L_\beta \longrightarrow L_\alpha$ (main) transition and is(are) analogous to the pretransition of PCs [13,14]. Our results indicate that this supposed 'pretransition' in hydrated DPPE affects mainly the polar region of the bilayer, because it influences the spin probe CAT-16, of which the paramagnetic N-O group is located in the polar region. The spin probe 5-DSA has its N-O group located in the hydrocarbon region but nearer to the polar part of bilayers than that of 16-DSA, therefore its response to the pretransition is slightly more pronounced than that of 16-DSA but much less distinct than that of CAT-16.

The order parameter S_{33} of CAT-16 indicates further changes in the bilayer centered about 15 ± 1°C below the $L_\beta \longrightarrow L_\alpha$ (main) phase transition. This might be the subtransition $L_c \longrightarrow L_\beta$ suggested by Silvius et al. [17]. They also observed distinct changes in Raman spectra of hydrated 1,2-dimyristoyl-PE about 15°C below the $L_\beta \longrightarrow L_\alpha$ transition temperature. Our results indicate that not only the supposed pretransition but also the subtransition affect mainly the polar part of the hydrated DPPE bilayer.

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