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THE INFLUENCE OF CALCIUM ON THE MOLECULAR MOBILITY OF FATTY ACID SPIN LABELS IN PHOSPHATIDYLSERINE AND PHOSPHATIDYLINOSITOL STRUCTURES*

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SUMMARY

The temperature-dependent fluidity of naturally occurring phosphatidylserine and phosphatidylinositol in lamellar structures and in their Ca^{2+} complexes was investigated within the temperature range from 2 to 60 °C using the stearic acid spin label I(12.3) and I(1.14). Both phospholipids show neither in the presence nor in the absence of Ca^{2+} thermotropic phase changes. A considerable difference, however, was observed in the temperature-dependent fluidity of the two lipids in the polar and apolar region of the lamellar structure reflected by label I(12.3) and I(1.14).

INTRODUCTION

The physically important role of Ca^{2+} on biological membranes and in particular on excitable ones is well documented [1, 2]. The effect of Ca^{2+} on artificial membranes and biomembranes includes both functional and structural aspects, the latter especially with regard to changes in the physical state of membrane lipids [3, 4]. The membrane components preferred as binding sites of Ca^{2+} are the anionic groups of the acidic lipids. By studying mono- and bilayer models it was shown that the interaction of Ca^{2+} with phosphatidylserine and phosphatidylinositol results in a simple condensing of the lamellar phase [5, 6], whereby the adsorption of Ca^{2+} on pure phospholipids is controlled by coulombic forces and is directly related to the net excess of negative charges of the phospholipid molecules rather than by their chemical nature [7]. In recent studies on phosphatidylserine membranes containing spin-labeled lecithin it has shown that Ca^{2+} induces a phase separation between the phosphatidylserine and the labeled molecules by forming phosphatidylserine–$Ca^{3}{}_{+}$ clusters [8]. Cardiolipin, however, is converted by Ca^{2+} to hexagonal structures [6], and this effect on the liquid state of the hydrocarbon chains is temperature de-

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pendent [9]. To evaluate further details about the way Ca^{2+} is changing the physical properties of acidic phospholipids in the lamellar phase we tried to clear up the following questions using the spin label technique:

(a) Is the effect of Ca^{2+} on the molecular packing of phosphatidylserine and phosphatidylinositol in bilayer membranes both in the polar and the apolar region temperature dependent and can phase changes from the crystalline to the liquid crystalline state reflected by the stearic acid spin labels I(12.3) and I(1.14) be observed in a temperature range of physiological interest?

(b) Do Ca^{2+}-complexed lipids show a temperature-dependent difference in the van der Waals attraction of the lipid chains in the polar region as compared with the apolar one?

The present study shows that the stearic acid spin labels I(12.3) and I(1.14) reflect distinct temperature-dependent changes in the membrane fluidity of natural phosphatidylserine and phosphatidylinositol and their Ca^{2+}-complexed structures.

MATERIALS AND METHODS

Stearic acid spin labels I(12.3) and I(1.14) (Fig. 1) were purchased from Syva Corp., Palo Alto, Calif. Phosphatidylserine (bovine) and phosphatidylinositol (plant) were obtained from Applied Science Laboratories, State College, Pa. and stored at −20 °C. The purity of the lipids estimated by thin-layer chromatography was greater than 96 %. They were, therefore, used without further purification. The other reagents used for the preparation were of analytical grade, the water was double glass distilled. All preparations were carried out at room temperature under an atmosphere of N_2. Samples for ESR measurements containing aqueous dispersions of lipid structures were prepared as follows: 7.5 mg lipid and 150 μg spin label were dissolved in chloroform at a molar ratio of approximately 1 : 10.

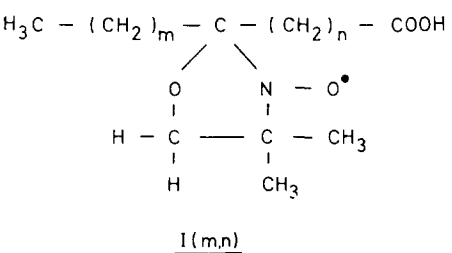

Fig. 1. The structure of the stearic acid spin label.

The mixture was poured into a round bottom flask and a thin film was formed by evaporating the chloroform under a N_2 atmosphere using a rotary evaporator corresponding to the method described by Weissmann and Sessa [10]. Subsequently, 1 ml of NaCl/KCl solution (0.145 M total molarity) adjusted to pH 7.0 with 50 mM Tris–HCl buffer was added. Lipid lamellae were formed by shaking the mixture on a Köttermann rotation mixer for 1 h. Spin-labeled Ca^{2+}-complexed lipid structures were formed in a solution containing a final concentration of 50 mM $CaCl_2$ and 50 mM Tris–HCl buffer, pH 7.0. The precipitate was washed for three times in this solution.

The membrane preparations were controlled by electron microscopy. Thin sections of the pellets of our membrane preparations showed myelin-like vesicular structives of different sizes.

ESR measurements were carried out with a Varian E-9 spectrometer (Varian, Palo Alto, Calif.) operating in the X band. The spectra were recorded as first derivatives of the absorption curves with a field modulation of 100 kHz using a rectangular H_{102} cavity. To avoid saturation effects, the incident microwave power was 1 mW. The samples were placed in sealed, calibrated microhematocrit tubes as described previously [11]. The spectra were recorded at increasing and decreasing temperatures using the Varian Variable Temperature Accessory Unit and the sample temperature was measured within an accuracy of ± 1 °C, using a small Fe–constantan thermocouple. Changes of the spectra within the temperature range from 2 to 60 °C were investigated. The hyperfine splitting $2\,T_{\parallel}$ and $2\,T_{\perp}$ of the spectra could be measured within ± 0.5 Oe. The accuracy of the rotational correlation time τ_c is $\pm 5\,\%$.

RESULTS

Fig. 2 shows the outer hyperfine splitting $T_{\parallel}$ of the spectra as a measure of the membrane fluidity as dependent on the temperature. Phosphatidylserine structures in the presence of a concentration of 50 mM Ca^{2+} and controls without Ca^{2+} were paramagnetically marked with spin label I(12.3). The presence of Ca^{2+} causes an increase in the amount of the hyperfine splitting $T_{\parallel}$ in the investigated temperature range from 2 to 60 °C. That implies a decrease of the motional freedom of the stearic acid spin label [12] and a restriction of the motion of the fatty acid chains in the region near the polar head groups. In the investigated temperature range the two curves in Fig. 2 have nearly the same distance from one another. A point of thermotropic phase change is not detectable in these structures neither in the presence of Ca^{2+} nor in its absence.

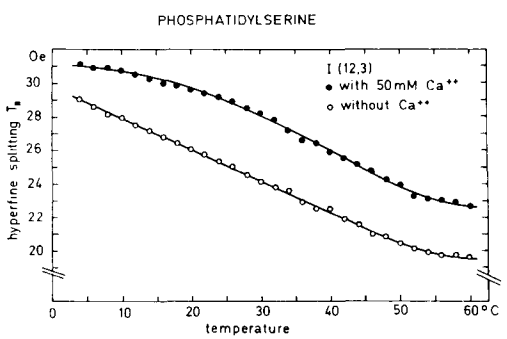

Fig. 2. The temperature dependence of the hyperfine splitting $T_{\parallel}$ of spin label I (12.3) in phosphatidylserine structures with and without Ca^{2+}.

Measurements of phosphatidylserine membranes containing stearic acid spin label I(1.14) can demonstrate changes of the molecular mobility in the more hydrophobic region of the membrane. For this spin label the rotational correlation time τ_c as a measure of the motional freedom can be calculated according to the equation

$$\tau_c = 6.5 \cdot 10^{-10} \cdot \Delta B \cdot (\sqrt{h_0/h_{-1}} - 1) \text{ s/Oe} \qquad \text{(refs 15, 16).}$$

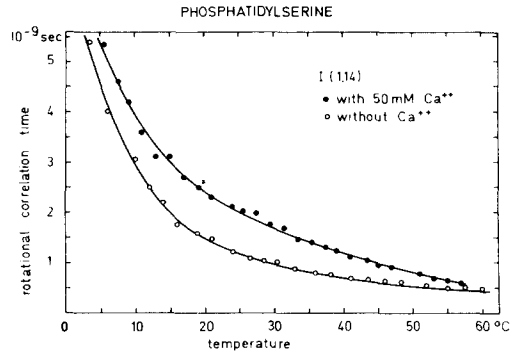

Fig. 3. The temperature dependence of the rotational correlation time τ_c of spin label I(1.14) in phosphatidylserine structures with and without Ca^{2+}.

In this equation ΔB is the width of the middle line of the triplet in the ESR spectrum in Oersted and h_0/h_{-1} is the ratio of the amplitudes of the second and third line. τ_c gives a result in seconds. This equation is valid when τ_c is of the order of 10^{-9} s. For qualitative studies we analysed the spectra in terms of the values up to $5 \cdot 10^{-9}$ s according to other authors [13, 14].

In Fig. 3 the rotational correlation time is given as dependent upon the temperature for structures with and without Ca^{2+}. The presence of Ca^{2+} causes a decrease of the fluidity of the structures even in the region near the apolar end of the fatty acid chains. Temperature-dependent phase changes are not detectable.

The results obtained by the two spin labels with their paramagnetic centers at different positions in the stearic acid chain indicate that Ca^{2+} essentially influences the molecular mobility in the polar region of the fatty acids as well as in the apolar one. This fact is remarkable because it can be supposed that the interaction of Ca^{2+} and phosphatidylserine takes place only in the polar head groups of the phospholipid molecules.

The local Ca^{2+} concentration in biological membranes can be very different. We have, therefore, measured the range of concentration in which Ca^{2+} influences

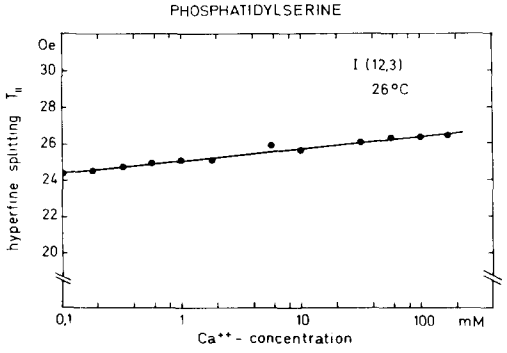

Fig. 4. The hyperfine splitting $T_{\parallel}$ of spin label I(12.3) in phosphatidylserine structures as dependent on the Ca^{2+} concentration. 10 μl of different concentrations of $CaCl_2$ were added to each test tube to reach the final concentration given at the abscissa.

the fluidity of phosphatidylserine membranes. The results obtained with spin label I(12.3) are given in Fig. 4. The hyperfine splitting $T_{\parallel}$ as a measure of the membrane fluidity is dependent on the Ca^{2+} concentration in the investigated range from 0.1 mM to about 100 mM.

The ESR spectra of spin label I(12.3) show different degrees of anisotropic motion in phosphatidylinositol membranes over a wide temperature range. From these ESR spectra of spin label I(12.3) incorporated in phosphatidylinositol membranes the mean angular deviation α between the middle position of the long molecular axis and the nitroxide 2 pπ orbital axis can be calculated according to the equation

$$\alpha = \text{arc cos } \sqrt{(2S+1)}/3$$

at which

$$S = 0.56 \cdot (T_{\parallel} - T_{\perp})/a$$

and

$$a = 1/3 \cdot (T_{\parallel} + 2T_{\perp}) \qquad \text{(refs 17, 18).}$$

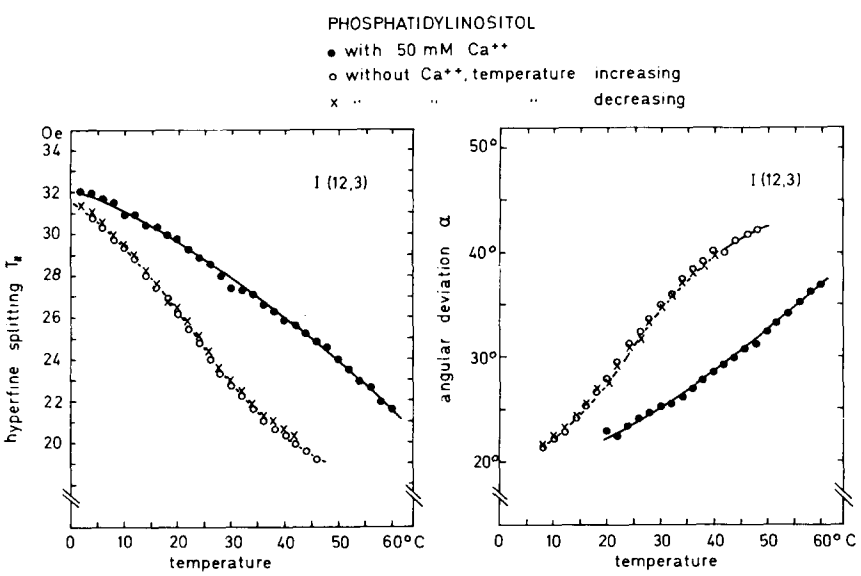

Fig. 5. The temperature dependence of the hyperfine splitting $T_{\parallel}$ and of the angular deviation of spin label I(12.3) in phosphatidylinositol structures with and without Ca^{2+}.

Fig. 5 shows the hyperfine splitting $T_{\parallel}$ and the angular deviation α as dependent upon the temperature for phosphatidylinositol membranes with spin label I(12.3). The results yield a Ca^{2+}-effected restriction of the motion of the fatty acid chains in the region near the polar head groups in the whole investigated range of temperature. The degree of Ca^{2+}-induced polar attraction increases with higher temperatures. A point of thermotropic phase change is likewise not detectable with this phospholipid membrane. The identity of the curves measured at increasing and decreasing temperature points to a reversible change of the membrane fluidity in the investigated temperature range as with the case of phosphatidylserine.

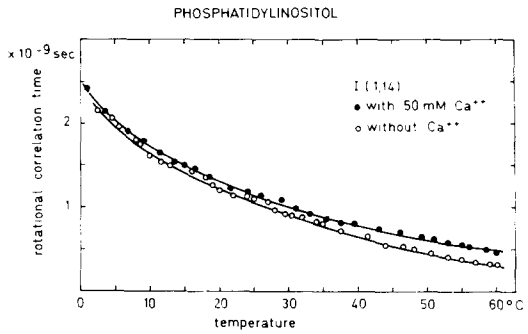

Fig. 6. The temperature dependence of the rotational correlation time τ_c of spin label I(1.14) in phosphatidylinositol structures with and without Ca^{2+}.

Measurements with spin label I(1.14) (Fig. 6) give the result that the rotational correlation time, dependent upon the temperature, does not show such a great difference for phosphatidylinositol structures with and without Ca^{2+} as is the case with phosphatidylserine. The effect of Ca^{2+} is limited to the more polar region of this lipid membrane.

DISCUSSION

The studies reported here show a temperature-dependent difference in the packing of the hydrocarbon chains of the two naturally occurring acidic lipids. There is also a difference in the arrangement of the polar head groups of the molecules in bilayer membrane particles dependent on the presence or absence of Ca^{2+}. A thermotropic phase change of the crystalline state of the hydrocarbon chains as observed by Papahadjopoulos and Bangham [19] in studies on the temperature-dependent permeability of bimolecular phosphatidylserine particles was not detectable in our model systems because of the inhomogeneity of the fatty acid composition of these natural phospholipids. Phosphatidylserine shows a more condensed packing of the molecules in the polar region than phosphatidylinositol as is reflected by the label I(12.3). This is in agreement with studies determining the limiting areas of molecules in lipid films [7]. At lower temperatures the fluidity of the apolar region of the hydrocarbon chains is greater for phosphatidylinositol than for phosphatidylserine. A remarkable difference was found in the interaction of the two lipids with Ca^{2+}. The polar region of the phosphatidylinositol–Ca^{2+} complex is restricted and the degree of this restriction varies with the temperature (Fig. 5). The apolar region remains nearly unaffected by Ca^{2+} (Fig. 6). For phosphatidylserine–Ca^{2+}, however, the restriction of the polar region is nearly constant over the whole temperature range (Fig. 2), a hindrance of the molecular motion of the apolar core of the fatty acid chains is also detectable (Fig. 3). The explanation of these differences is difficult as the true structure of these Ca^{2+} complexes is still unknown [5]. This difference remains even at Ca^{2+} concentrations far beyond those of biological interest.

The difference between phosphatidylserine–Ca^{2+} and phosphatidylinositol–Ca^{2+} (Figs 2 and 5) should, therefore, be related to the difference in the nature of the two complexes. In the case of phosphatidylserine both the phosphate and the

carboxyl groups interact with Ca^{2+} thus involving six [5] or four [20] coordinating bonds of Ca^{2+}, whereas in phosphatidylinositol molecules the only polar binding site for Ca^{2+} would be the phosphate group. X-ray diffraction studies in general support the assumption of a lamellar structure [6, 21]. ESR exchange spectra recorded from Mn^{2+}–phosphatidylserine complexes were estimated to belong to the hexagonal structures of inverted mycells [22]. Whatever the true nature of the complexes perhaps two explanations for the above described differences in the motion parameters of the spin labels should be mentioned here.

(a) The fatty acid chains of phosphatidylinositol are more unsaturated than those of phosphatidylserine and the chains have a greater mobility because the energy barrier for the rotation around a C–C bond is lowered in the neighborhood of a C=C double bond. This mobility prevents strong attraction of the lipid chains in the apolar core of the membrane, even if Ca^{2+} interacts with the polar head groups (Fig. 6).

(b) The affinity of Ca^{2+} for the lipid is diminished by the C=C double bonds of the fatty acid chains [23] and furthermore the density of the packing of the lamellar lipids influences the amount of Ca^{2+} bound to the polar head groups [24, 25]. Finally it could not be excluded that the inositol rest is a steric hindrance for Ca^{2+} interaction. This could be the reason for the small apolar condensation effect (Fig. 6) and also the temperature dependence of the degree of polar attraction in the phosphatidylinositol–Ca^{2+} complex.

The physiological relevance of pure phosphatidylserine and phosphatidylinositol with respect to biological membrane function is extensively discussed by several authors [5, 7, 26]. The difference in the physical properties between naturally occurring phosphatidylserine and phosphatidylinositol in lamellar liquid crystalline membrane particles as well as the difference between their Ca^{2+} complexes observed in this study may contribute to the explanation of the differences in the electrical [5] (surface potential) and permeability properties [26] of these membrane models.

In monolayers of phosphatidylserine, Ca^{2+} produces a higher increase of surface potential ΔV compared with phosphatidylinositol–Ca^{2+} [5].

This corresponds to our findings that phosphatidylserine shows a closer packing of the hydrocarbon chains which are further condensed by Ca^{2+}, whereas phosphatidylinositol is only condensed by Ca^{2+} in the polar region. This difference in the intermolecular spacing produces a difference in the surface area concentration of the molecules and should also change the surface dipole moments [24].

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