Biochimica et Biophysica Acta, 307 (1973) 452–458

BBA 76322

THE EFFECT OF CALCIUM ON TEMPERATURE-INDUCED PHASE CHANGES IN LIQUID-CRYSTALLINE CARDIOLIPIN STRUCTURE*

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(Received December 1st, 1972)

SUMMARY

The temperature-dependent fluidity of lamellar and Ca^{2+}-precipitated cardiolipin structures was investigated over the temperature range 5–55 °C, using the stearic acid spin labels I(12.3), I(5.10), and I(1.14). In the lamellar phase the I(12.3) label reflects an abrupt thermotropic change of the membrane fluidity at 37 °C. The I(5.10) and I(1.14) labels show two points of phase changes located at 14, 36 °C and 10, 38 °C, respectively. The Ca^{2+}-complexed cardiolipin structures provoke a retraction of the hydrocarbon chains, preferentially in the polar region, and at the same time a loss of the phase transitions.

INTRODUCTION

The possible functional importance of thermotropic phase transitions in lipid bilayers of artificial and biological membranes has been discussed by several authors[1–3]. It has been shown, that these transitions are determined by several factors for example the length and the degree of saturation of the hydrocarbon chains[4], by electrostatic interactions of the polar head groups of the membrane lipids, the presence of bivalent cations, cholesterol, peptides, or proteins[3,5–7]. Such phase changes within the lipid core of membranes effected by the above-mentioned biochemical or biophysical conditions might have physiological functions in the regulation of membrane permeability and kinetic properties of membrane-associated enzymes of thermosensitive bacteria, plants and animals[8]. It is well known that artificial membranes made up of acid phospholipids react with the Ca^{2+} to form a complex. Such membranes change their physical properties by retraction of their lamellar organization[9,10]. Taking into consideration these facts and the special accumulative properties of the inner mitochondrial membranes[11], with respect to Ca^{2+}, it could be of interest whether Ca^{2+} in cardiolipin structures influences the temperature dependence of phase transitions.

Our present report shows that stearic acid spin labels with different distances between the carboxyl group and the nitroxide radical reflect distinct, reversible thermotropic changes in membrane fluidity of cardiolipin structures. Ca^{2+} affects

* A part of this paper was reported at a congresss in Erlangen (Germany) organized by the Deutsche Gesellschaft für Biophysik and Deutsche Gesellschaft für Medizinische Physik, October 4–6, 1972.

a retraction of the hydrocarbon chains, which is associated with a loss of phase transitions within the temperature range investigated.

MATERIALS AND METHODS

Stearic acid spin labels I(12.3), I(5.10), and I(1.14) were purchased from SYVA Associated Palo Alto, Calif., U.S.A. Cardiolipin (beef heart) dissolved in ethanol was obtained from Applied Science Laboratories and stored at −20 °C. The purity of cardiolipin estimated by thin-layer chromatography was greater than 96%. The cardiolipin, therefore, was used without further purification. The other reagents used were of analytical grade, the water was double glass distilled. All preparations were carried out at room temperature under an atmosphere of N_2. ESR samples containing aqueous dispersions of lipid lamellae or Ca^{2+}-complexed structures of cardiolipin were prepared as follows: 7.5 mg cardiolipin and 150 μg spin label were dissolved in ethanol at a molar ratio of approximately 100:4. (The mol. wt of one half of a cardiolipin molecule is assumed to be 750.)

The mixture was poured into a round bottom flask and a thin film was formed by evaporating the ethanol under a N_2 atmosphere using a rotary evaporator, corresponding to the method described by Weissman and Sessa[12]. Subsequently, 1 ml of a NaCl/KCl solution (0.145 M total molarity) adjusted to pH 7.0, was added. Lipid lamellae were formed by shaking the mixture on a Köttermann rotation mixer for 1 h. Similar samples were prepared in a medium containing NaCl/KCl (0.145 M) and 50 mM Tris–HCl, pH 7.0. Spin-labeled Ca^{2+}-complexed cardiolipin structures were formed in a precipitating solution containing a final concentration of 0.05 M $CaCl_2$ or 0.05 M $CaCl_2$ and 50 mM Tris–HCl, pH 7.0, respectively.

ESR spectra were recorded using a Varian E-9 spectrometer. The samples were placed in sealed, calibrated microhematocrit capillaries as described previously[13]. The spectra were recorded at increasing and decreasing temperatures and the sample temperature measured within an accuracy of ±0.5 °C, using a small Fe–constantan thermocouple. Changes of the spectra at temperatures between 5 and 55 °C were investigated. Care was taken to avoid saturation effects, the microwave power being about 1 mW. The hyperfine splitting $2T_{\parallel}$ and $2T_{\perp}$ of the spectra could be measured within ±0.5 G. The accuracy of the rotational correlation time τ_c is ±5%.

RESULTS

Fig. 1 shows ESR spectra of the different spin-labeled stearic acids incorporated in cardiolipin structures. When these spectra were recorded the temperature of the assay was 20 °C. I(5.10) and I(12.3) labels incorporated in cardiolipin vesicles (dotted line spectra) and in Ca^{2+}-complexed cardiolipin precipitates (solid line spectra), show different degrees of anisotropic motion around the long molecular axis μ (ref. 14). The presence of Ca^{2+} causes an increase in the amount of hyperfine splitting $T_{\parallel}$. That signifies a decrease in the motional freedom of the label and a restriction of the motion of the fatty acid chains in the region near the polar head groups and also in the more apolar core of the lipid bilayers. For a more detailed analysis of the spectra, refer to Table I. The different degree of anisotropic motion

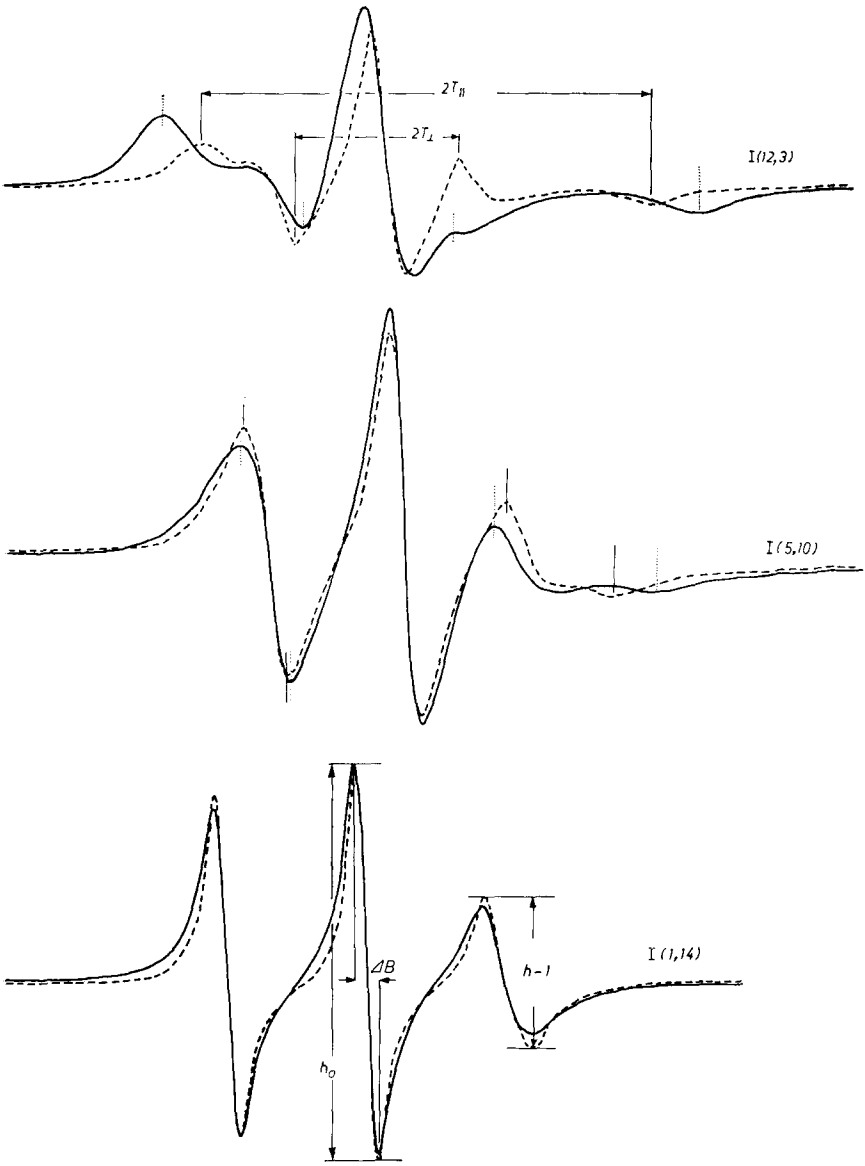

Fig. 1. ESR spectra of the spin labels I (12.3), I(5.10) and I(1.14) in cardiolipin structures recorded at 20 °C, dotted-line spectra without and solid-line spectra with calcium-precipitated structures.

is described by the parameter S, which can be calculated from the equation $S=0.56\cdot(T_{\parallel}-T_{\perp})/a$[15,16]. $T_{\perp}$ and $T_{\parallel}$ represent the inner and outer hyperfine splitting of the spectra, respectively, and a is the hyperfine splitting constant. The angular deviation α between μ and the nitroxide $2p\pi$ orbital axis is given as $\alpha=\arccos\sqrt{[2S+1)/3]}$ (ref. 15). The isotropic hyperfine splitting constant a which can serve under specified conditions as a measure of the polarity of the environment of the label, was computed using the Eqn $a=1/3(TJ+2TJ)$ (ref. 15).

TABLE I

MOTION PARAMETERS OF THE I(12.3), I(5.10) and I(1.14) LABELS INCORPORATED IN LAMELLAR AND Ca^{2+}-COMPLEXED CARDIOLIPIN STRUCTURES AT 20 °C

Label	$CaCl_2$ (*M*)	$T_{\parallel}$ (*G*)	$T_{\perp}$ (*G*)	*a* (*G*)	T_{xx} (*G*)	T_{zz} (*G*)	*S*	α
I(12.3)	—	24.75	9.13	14.33	5.88	31.25	0.610	30° 38′
	0.05	29.63	8.25	15.38	6.30	33.52	0.779	22° 36′
I(5.10)	—	18.38	10.69	13.25	5.54	28.89	0.325	42° 08′
	0.05	20.69	9.94	13.52	5.54	29.48	0.445	37° 28′
		ΔB_0 (*G*)	h_0/h_{-1}	τ_c *ns*				
I(1.14)	—	2.45	2.64	0.918				
	0.05	2.50	3.07	1.22				

The change in the angular deviation a, with respect to the different positions of the nitrogen oxides relative to the carboxyl groups of the fatty acids, leads to the conclusion that in the presence of Ca^{2+} the restriction of the fatty acids near the polar region ($\Delta a \cong 8°$), for the I(12.3) label, is greater than that near the apolar one ($\Delta a \cong 5°$), for the I(5.10) label. If S is $\leqslant 0.3$, a can no longer be calculated from the ESR spectra, because the motion of the label becomes almost isotropic. For this type of spectra the rotational correlation time τ_c can be calculated using the Eqn:

$$\tau_c = 6.5 \cdot 10^{-10} \Delta B(\sqrt{h_0/h_{-1}} - 1)\text{s/G (refs 17, 18)}$$

This equation holds when τ_c is of the order of $1 \cdot 10^{-9}$ s. From Table I it can be seen that in Ca^{2+}-complexed cardiolipin at the apolar end of the lipid structures, a restriction is still detectable. Fig. 2 shows, in the case of the I(12.3) label, a pro-

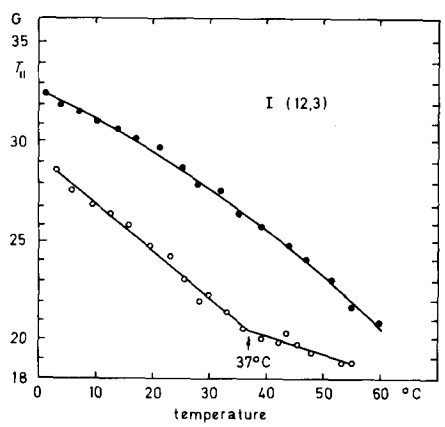

Fig. 2. Temperature dependence of the hyperfine splitting $T_{\parallel}$ of the I(12.3) label in cardiolipin lamellar membranes (○—○) and in the presence of 50 mM $CaCl_2$, pH 7.0 (●—●).

nounced dependence of the hyperfine splitting T_{II} which is measurable over the whole temperature range (5–55 °C). An abrupt change of the fluidity of the lamellar phase of cardiolipin at a temperature of 37 °C is reflected by the label, but in the presence of Ca^{2+} this transition does not occur. If the nitroxide group of the stearic acid is shifted from the polar head to the apolar end of the fatty acid chain, a further change in the membrane's fluidity occurs as shown with the I(5.10) label in Fig. 3a. The two points of phase change are located at 14 and 36 °C, respectively. In the temperature range at which the motion of the label is no longer rapidly anisotropic but, however, not yet rapidly isotropic, the analysis of the spectra in terms of α is inaccurate. For a qualitative analysis of these spectra, several workers therefore prefer an analysis in terms of τ_c instead of α allowing τ_c values up to $5 \cdot 10^{-9}$ s (ref. 8).

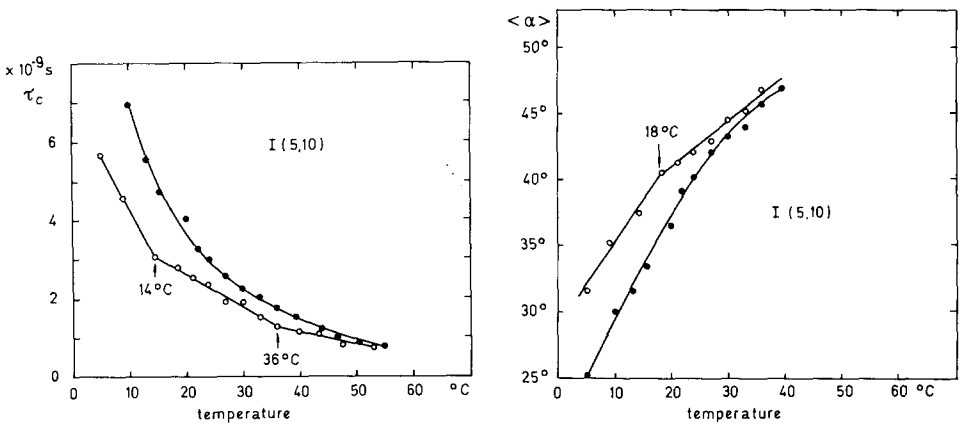

Fig. 3. The motion parameter τ_c is plotted against increasing temperature (a) and the angular deviation α (b), ○—○, lamellar structures, ●—●, $CaCl_2$ (50 mM) precipitated structures of cardiolipin.

In our preparations, the motional freedom of the I(5.10) label gradually becomes anisotropic at temperatures below 30 °C. In the range from 5–30 °C therefore, the angular deviation α was also computed (Fig. 3b). The difference between the temperature of the phase change calculated from τ_c and that calculated from α may be due to the inaccuracy mentioned above. In all instances, however, addition of Ca^{2+} removes the phase changes. Very similar results were obtained in the case of the I(1.14) label. The critical temperatures, *i.e.* the temperatures at which abrupt changes in the fluidity took place, are 10 and 38 °C, respectively. This holds for unbuffered and Tris-buffered systems. Because of the change of the molecular packing of the acid phospholipids and a dehydration of the liquid crystal caused by Ca^{2+}, a change of the coupling constant a could be expected. In the temperature range ($\geqslant$40 °C) at which the motion of the I(1.14) label is rapid enough to assume that the coupling constant a is related to the polarity of the environment[19], the value of a is 14.0 G, for both preparations, with or without $CaCl_2$. This indicates a polarity between that of *n*-decane and decanol[15].

DISCUSSION

The present observations show that under our experimental conditions in spin-labeled cardiolipin membranes, thermotropic phase changes which could be suppressed by Ca^{2+} are detectable. In the case of cardiolipin structures without Ca^{2+}, there is a marked difference between the results obtained from the I(12.3) label, compared with those from the I(5.10) and I(1.14) labels (Figs 2–4).

Cardiolipin from beef heart contains to a great extent, diglycerides consisting exclusively of linoleic acid as alkyl constituents, however, diglycerides containing saturated fatty acids of various chain length also exist[20]. The difference between the transition point reflected by the I(12.3) label and those ones reflected by the I(1.14) and I(5.10) labels, could therefore be explained by a different environment of the spin label located in the bilayer. As shown in the case of other artificial membrane systems, both, the position of the unsaturated bonds and the degree of unsaturation determines the order–disorder transition temperature[21]. The nitrogen oxide of the I(1.14) and I(5.10) labels is located deep within the membrane where *cis*-carbon–carbon double bonds occur. In such a region, kinks enhance the membrane fluidity and influence the temperature-dependent internal rotation barrier around adjacent single bonds[22].

The difference between the transition points mentioned above could therefore also be due to the position of the *N*-oxide in the core of the membrane. As has been established by experiments performed using different methods, a reaction of Ca^{2+} and acid lipids results in a dehydration and a condensation of the fatty-acid chains[9,23]. The restriction of the Ca^{2+}–cardiolipin complex decreases from the polar to the apolar part of the lipid structures (Table I). This restriction is marked at low temperatures for all the labels and remains nearly constant for the I(12.3) label at all temperatures. The I(5.10) and I(1.14) labels, however, do not show this effect at temperatures above 40 °C (Figs 3 and 4). Although the interaction of Ca^{2+} and cardiolipin took place only in the region of the polar head groups, the Van de Waals attraction between the fatty acid chains must become so strong, that the temperature-dependent phase changes (near the hydrophobic as well as within the apolar core of the fatty acid chains) are no longer detectable within the accuracy of our measurements.

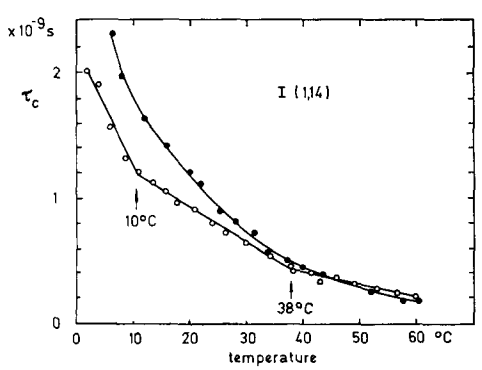

Fig. 4. Temperature dependence of the rotational correlation time τ_c, I(1.14) label: ○—○, lamellar phase, ●—●, cardiolipin precipitated within 50 mM $CaCl_2$, pH 7.0.

The relevance of a possible effect of Ca^{2+} on cardiolipin structures, with respect to the function of the inner mitochondrial membrane, was discussed in detail by Rand and Sengupta[23]. The results presented here suggest that the local Ca^{2+} concentration within a cardiolipin-containing membrane could have a marked temperature-dependent effect on the fluidity of fatty acid chains. Investigations on the possible biological relevance of our results to isolated inner mitochondrial membranes are under study.

ACKNOWLEDGEMENT

This research was supported by the Deutsche Forschungsgemeinschaft.

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