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SATURATION TRANSFER ESR STUDIES OF MOLECULAR MOTION IN PHOSPHATIDYLGLYCEROL BILAYERS IN THE GEL PHASE

EFFECTS OF PRETRANSITIONS AND pH TITRATION

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Summary

The molecular motions of a phosphatidylglycerol spin label have been studied in dimyristoyl and dipalmitoyl phosphatidylglycerol bilayers below their ordered-fluid phase transition, both in the charged state at pH 8.0 and in the protonated state at pH 1.5. The saturation transfer ESR spectra, which are sensitive to motions in the correlation time range 10^{-7}—10^{-3} s, show clear distinctions in the molecular motion both between the charged and the protonated states and between the states above and below the pretransition in the charged bilayers. At low temperatures, below the pH 8.0 pretransition, the saturation transfer ESR spectra indicate rather similar motion at pH 8.0 and 1.5, with the rates being approx. 2-times faster at pH 8.0. The effective rotational correlation times are approx. $0.5—1 \cdot 10^{-4}$ and approx. $1—2 \cdot 10^{-4}$ s, deduced from the outer lineheight ratios which are sensitive only to motion of the long molecular axis, and approx. $0.2—1 \cdot 10^{-4}$ and $0.2—0.5 \cdot 10^{-4}$ s, deduced from the central lineheight ratio which is sensitive also to rotation around the long molecular axis, where the first and second values refer to dimyristoyl and dipalmitoyl phosphatidylglycerol, respectively. As the temperature is increased the outer lineheight ratios at both pH values remain constant until the main transition, indicating little or no increase in motion of the long axis. In contrast, the central lineheight ratio at pH 8.0 shows a sharp decrease before the main transition, corresponding to cooperative onset of rapid rotation around the long molecular axis, at or immediately below the pre-

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transition. The effective correlation time of this long axis rotation is approx. 10^{-7} s, two orders of magnitude faster than that below the pretransition. The bilayers at pH 1.5, for which no pretransition is detected, show only a slow, non-cooperative decrease in the central lineheight ratio with increasing temperature, rapid long axis rotation in dimyristoyl phosphatidylglycerol occurring only at the main transition. The onset of long axis rotation at pH 8.0 is also detected in the conventional spectra of a steroid spin label, which begins to rotate around its long molecular axis with a correlation time of approx. 10^{-9} s whilst the bilayers are still in the gel phase. These observations further strengthen the homology between phosphatidylglycerols in the charged state and the corresponding phosphatidylcholines, and provide the dynamic counterpart of the pH-induced structural change previously observed in phosphatidylglycerol bilayers in the gel phase (Watts, A., Harlos, K., Maschke, W. and Marsh, D. (1978) Biochim. Biophys. Acta 510, 63—74).

Introduction

Phosphatidylglycerols are negatively charged phospholipids found in great abundance both in the plasma membrane of micro-organisms and in the chloroplast membranes of green plants. In previous studies with disaturated phosphatidylglycerols [1], we have observed a strong homology between the behaviour of phosphatidylglycerol bilayers in their fully charged state at pH 8.0, and bilayers of the corresponding phosphatidylcholines at about neutral pH. The transition behaviour, including both the absolute values of the transition temperatures and the occurrence of a well defined pretransition, is very similar in the two systems. The behaviour in freeze-fracture electron microscopy is also very similar, giving a defect, a rippled and a jumbled appearance for samples quenched from below the pretransition, between the pretransition and main transition, and above the main transition, respectively, in both systems. Additionally, a pH-induced structural transition was found in phosphatidylglycerol bilayers in the gel phase, below the ordered-fluid main transition. This was detected by freeze-fracture electron microscopy and it was suggested that it corresponded to a transition from a tilted molecular configuration in the charged phosphatidylglycerol bilayers at pH 8.0 to a non-tilted configuration in the protonated state at pH 1.5 in which the molecules are oriented along the bilayer normal. This suggestion has subsequently been confirmed by X-ray diffraction (Watts, A., Harlos, K. and Marsh, D., unpublished data).

In the present work, we have used spin label saturation transfer ESR, a technique which is sensitive to slow motion in the correlation time range 10^{-7}—10^{-3} s [2], to study the molecular motion in phosphatidylglycerol bilayers in the gel phase. In a previous study with phosphatidylcholines [3], the cooperative onset of a rapid, anisotropic long axis rotation of the lipid molecules was detected in the gel phase at a temperature coinciding with or immediately below the phosphatidylcholine pretransition. The results obtained here with phosphatidylglycerols in the gel phase show that in the charged state the spin labels display a cooperative onset of a rapid, anisotropic rotation around the

long axes of the lipid molecules at or immediately below the pretransition. The effective correlation time for this long axis rotation is approx. 100-times faster than that observed well below the pretransition and also 100-times faster than that for the angular motion of the long axis itself, as is also found in phosphatidylcholines. These findings thus extend the structural homology observed between phosphatidylcholines and charged phosphatidylglycerols [1] to their dynamic properties. In addition, it is found that there is also a motional counterpart to the pH-induced structural transition in phosphatidylglycerols in the gel phase. The protonated bilayers at pH 1.5, which do not show a pretransition, do not show a cooperative onset of long axis rotation. The rate of long axis rotation increases only slowly with temperature and, for dimyristoyl phosphatidylglycerol at least, rapid long axis rotation does not take place until the main transition.

Materials and Methods

Dimyristoyl and dipalmitoyl phosphatidylglycerol (DMPG and DPPG, respectively) were synthesized from dimyristoyl and dipalmitoyl phosphatidylcholine (Fluka, Buchs, Switzerland) using headgroup exchange catalysed by phospholipase D (Boehringer-Mannheim, F.R.G.). Synthesis and characterization were as previously described [1,4]. The phosphatidylglycerol spin label, β-5(4′,4′-dimethyloxazolidine-*N*-oxyl)stearoyl-γ-acyl-α-phosphatidylglycerol (5-PGSL), bearing the nitroxide group on the C5 atom of the β-chain, was synthesized from the corresponding phosphatidylcholine spin label by the method used for the unlabelled phosphatidylglycerols. The phosphatidylcholine spin label was prepared essentially according to the methods of Hubbell and McConnell [5] and Boss et al. [6]. The cholestane spin label was obtained from Syva, Palo Alto, U.S.A.

Lipid dispersions were prepared by mixing phosphatidylglycerol with 1—2 mol% spin label in $CHCl_3/CH_3OH$ (2 : 1, v/v), evaporating off the solvent with N_2 and leaving under vacuum overnight. The dry lipid mixture was then dispersed at a concentration 50 mg/ml, in 100 μl of either 0.1 M KCl/10 mM Tris-HCl, (pH 8.0) or 0.1 M KCl/HCl (pH 1.5) buffer, by gently mixing at a temperature above the ordered-fluid phase transition. The pH of the dispersion was checked and adjusted if necessary after preparation. The dispersions were then sealed in 1 mm diameter glass capillaries for ESR measurement.

ESR spectra were recorded with Varian Century Line Series 9 GHz spectrometers, equipped with quartz dewar, N_2 gas-flow, temperature regulation systems. Sample capillaries were contained in standard 4-mm diameter quartz ESR tubes filled with silicon oil for thermal stability. Sample temperature was monitored by a thermocouple situated inside the ESR tube just above the top of the microwave cavity. Saturation transfer ESR spectra were recorded in the second harmonic, 90° out-of-phase, absorption mode (v_2' display) with a modulation frequency of 50 kHz, modulation amplitude of 5 G, and microwave power of 63 mW. (Calibrations indicate this power corresponds to $H_1 \simeq$ 0.25 G for the sample and cavity configuration used). The 90° out-of-phase setting was determined by the self-null method [2] at microwave powers of

1 mW or less; out-of-phase nulls were no greater than 1% of the in-phase signal (see Ref. 7 for further details).

Results

Representative ESR spectra of the 5-PGSL label in DMPG bilayers at pH 8.0 and 1.5 in the gel phase are given in Fig. 1. The spectra are qualitatively similar to the isotropic reference spectra of spin-labelled haemoglobin [2], although there are quantitative differences indicative of anisotropic motion [3,8]. The spectra of the protonated bilayers at pH 1.5 are all very similar, showing only relatively small quantitative differences between −2 and 25°C. The spectrum of the charged bilayers at −2°C is similar to those of the pH 1.5 bilayers; but at higher temperatures, approaching the pretransition, the pH 8.0 spectra become indicative of much more rapid motion [2]. These effects are most marked in the central region of the spectrum, indicating a highly anisotropic motion. The temperature variation of the spectra is summarized in Fig. 2 which gives the diagnostic lineheight ratios (see Fig. 1) derived from the central (C'/C), high-field (H''/H) and low-field (L''/L) regions of the spectra. The outer ratios, H''/H and L''/L, which are sensitive only to motion of the long molecular axis (nitroxide z-axis) [3], remain relatively constant with temperature up to the main transition at both pH 8.0 and 1.5. In contrast, the C'/C ratio, which is sensitive additionally to rotation

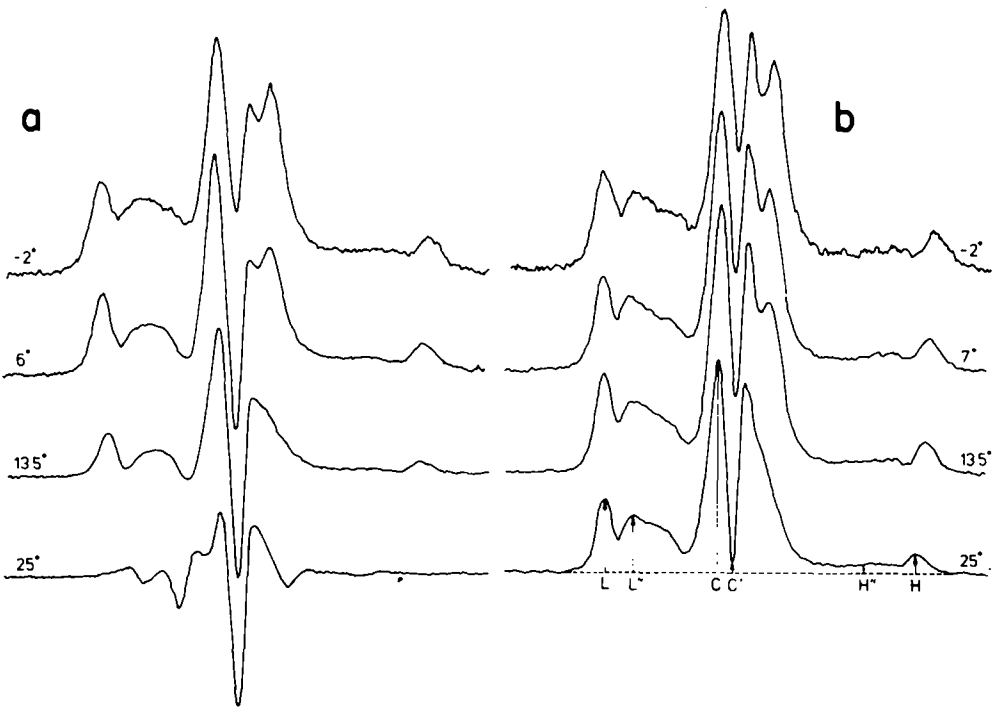


Fig. 1. Second harmonic, 90° out-of-phase, absorption saturation transfer ESR spectra (v_2' display) of the 5-PGSL spin label in multibilayer dispersions of dimyristoyl phosphatidylglycerol. (a) At pH 8.0, (b) at pH 1.5, as a function of temperature.

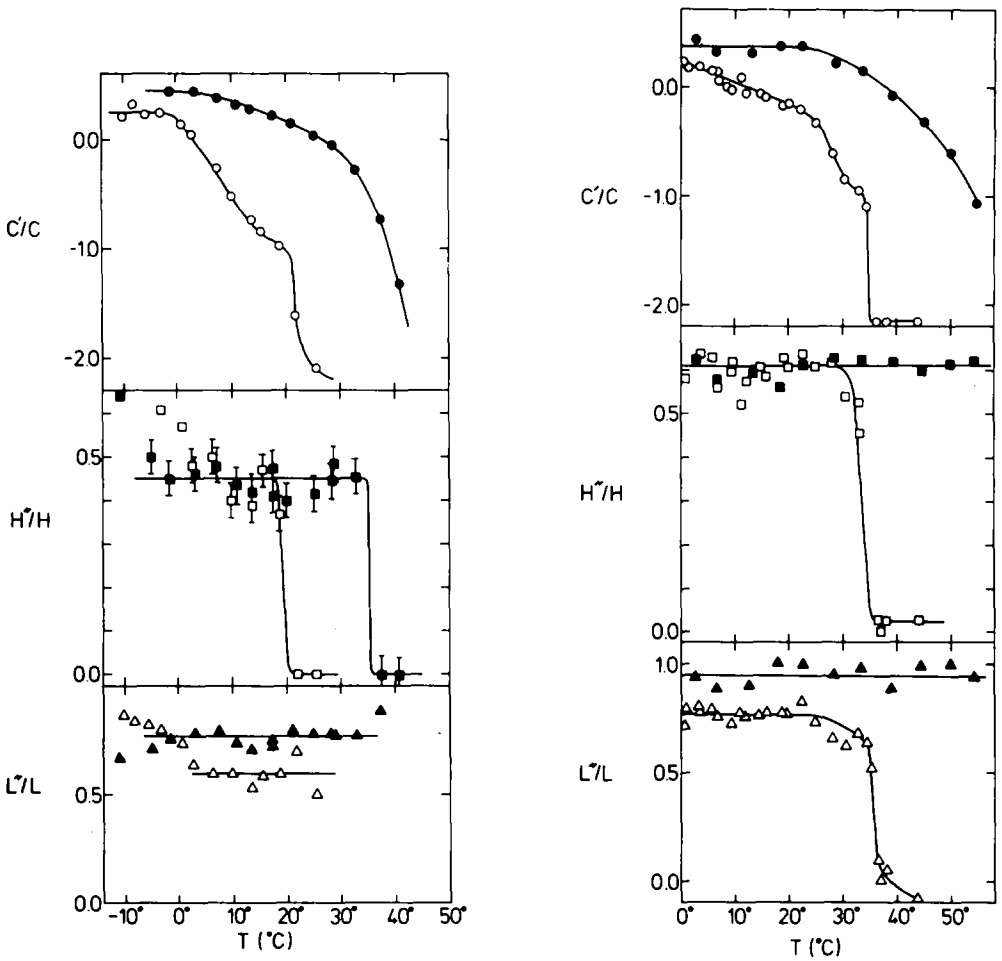


Fig. 2. Central (C'/C), high-field (H''/H) and low-field (L''/L) lineheight ratios (see Fig. 1) in the saturation transfer ESR spectra of DMPG, at pH 8.0 (○, □, △) and pH 1.5 (●, ■, ▲), as a function of temperature.

Fig. 3. C'/C, H''/H and L''/L diagnostic lineheight ratios in the saturation transfer ESR spectra of DPPG bilayers, at pH 8.0 (○, □, △) and pH 1.5 (●, ■, ▲), as a function of temperature.

around the long molecular axis (nitroxide x,y-axis modulation) [3], shows a rapid decrease with increasing temperature, for bilayers at pH 8.0, at a temperature close to or immediately below the pretransition (approx. 8—13°C), whilst still in the gel phase. The protonated bilayers at pH 1.5, which have no pretransition, show only a slow decrease in C'/C with temperature, an abrupt change occurring only at the main transition (approx. 42°C). As in phosphatidylcholines [3], these changes are interpreted as as the onset of a rapid long axis rotation of the spin label at a temperature close to the pretransition in charged DMPG bilayers, and the absence of such a semi-cooperative change in the gel phase of protonated bilayers at pH 1.5.

The highly anisotropic nature of the long axis rotation in the charged DMPG bilayers is seen when the effective correlation times for anisotropic motion are

TABLE I

EFFECTIVE ROTATIONAL CORRELATION TIMES OF THE 5-PGSL SPIN LABEL IN GEL PHASE BILAYERS OF DMPG AT pH 8.0 AND 1.5, DEDUCED FROM CALIBRATION LINEHEIGHT RATIOS OF HAEMOGLOBIN REFERENCE SATURATION TRANSFER ESR SPECTRA

The haemoglobin reference spectra were taken from Ref. 2. Values of the rotational correlation times (τ_2) are given in s.

	Temperature (°C)				
	0	5	10	18	25
pH 8.0					
C'/C	$2 \cdot 10^{-5}$	$5 \cdot 10^{-6}$	$1 \cdot 10^{-6}$	$1 \cdot 10^{-7}$	$\approx 10^{-9}$
L''/L	$9 \cdot 10^{-5}$	$4 \cdot 10^{-5}$	$3 \cdot 10^{-5}$	$3 \cdot 10^{-5}$	$\approx 10^{-9}$
H''/H	$1 \cdot 10^{-4}$	$9 \cdot 10^{-5}$	$5 \cdot 10^{-5}$	$5 \cdot 10^{-5}$	$\approx 10^{-9}$
pH 1.5					
C'/C	$>10^{-4}$	$>10^{-4}$	$4 \cdot 10^{-5}$	$2 \cdot 10^{-5}$	$0.8 \cdot 10^{-5}$
L''/L	$1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$
H''/H	$7 \cdot 10^{-5}$	$8 \cdot 10^{-5}$	$7 \cdot 10^{-5}$	$7 \cdot 10^{-5}$	$6 \cdot 10^{-5}$

calculated from the lineheight ratios (see Table I), using the calibrations established from spin-labelled haemoglobin reference spectra by Thomas et al. [2]. It is implicit in this method that if the motion is isotropic then the effective correlation times calculated from all three ratios, L''/L, H''/H and C'/C, should be identical. Anisotropy of the motion is detected by different effective correlation times being obtained from the different ratios [3]. From Table I it is seen that at pH 8.0 the effective correlation times deduced from L''/L and H''/H remain effectively constant and approximately equal at a value of 0.5—1 $\cdot 10^{-4}$ s up to the main transition. On the other hand, the effective correlation time deduced from the C'/C ratio, for bilayers at pH 8.0, starts somewhat lower at a value of approx. $0.2 \cdot 10^{-4}$ s at 0°C, but more importantly, shows a rapid decrease to a value of approx. 10^{-7} s at 18°C. This is indicative of the onset of a much more rapid rotation about the long axis of the label molecules in charged DMPG bilayers in the gel state. The effective rotational correlation time for long axis rotation changes by two orders of magnitude between 0 and 18°C, whereas that for motion of the long axis itself remains essentially constant over this temperature range.

For DMPG bilayers in the protonated state, Table I shows that the effective correlation times for the angular motion of the long axis, given by the L''/L and H''/H ratios, are rather similar to those for the bilayers at pH 8.0 and remain essentially constant with temperature over the range up to the main transition. The L''/L ratio indicates that the motion may be a little slower in the protonated bilayers, having effective correlation times approx. 2-times greater than those deduced for the charged bilayers. In contrast, the effective correlation times deduced from the C'/C ratios differ considerably from those for the charged bilayers. At lower temperatures, the effective correlation times at pH 1.5 are considerably longer (greater than or equal to 10^{-4} s), comparable to those deduced from the L''/L and H''/H ratios, and do not change rapidly with increasing temperature. At 25°C the effective correlation time is still approx. 10^{-5} s, similar to that in the charged bilayers at low temperature.

Thus, although there is some increase with temperature in the rate of rotation about the label long molecular axis in the protonated bilayers, it is much smaller than that for the charged bilayers and is also non-cooperative.

Very similar effects have been observed with DPPG bilayers in the charged and protonated states in the gel phase, the transition temperatures being correspondingly higher than those for DMPG. The temperature variations of the diagnostic lineheight ratios for DPPG bilayers at pH 8.0 and 1.5 are given in Fig. 3. Measurements on DPPG bilayers at pH 1.5 are given only up to 55—60°C, since it was found by thin-layer chromatography that considerable lipid degradation took place during saturation transfer ESR experiments at temperatures higher than this. The L''/L and H''/H ratios again are essentially constant with temperature up to the main transition at both pH values. The C'/C ratio shows a rather cooperative decrease at approx. 25°—30°C for DPPG bilayers at pH 8.0, whereas there is a slower, steady decrease for bilayers at pH 1.5. The effective correlation times deduced from the lineheight ratios of the DPPG saturation transfer spectra are given in Table II and show trends similar to those observed for DMPG. The L''/L and H''/H ratios give essentially constant effective correlation times: approx. $2 \cdot 10^{-4}$ s for bilayers at both pH 8.0 and 1.5. The C'/C ratio shows a change in effective rotational correlation time by approx. 2 orders of magnitude between 5 and 30°C for bilayers at pH 8.0, whereas the corresponding change for bilayers at pH 1.5 is by only a factor of approx. 2.

Further evidence for the onset of a rapid long axis rotation in gel phase phosphatidylglycerol bilayers is given by the conventional ESR spectra of the cholestane spin label. The axes of the hyperfine tensor of this label are oriented such that, if the motion is sufficiently rapid, the conventional spectra will be optimally sensitive to rotation around the long axis of the molecule. The conventional ESR spectra of the cholestane spin label in DPPG bilayers at pH 8.0 are given in Fig. 4. It is seen that the spectra change from a powder-type spectrum at low temperatures to a much narrower, pseudo-three-line

TABLE II

EFFECTIVE ROTATIONAL CORRELATION TIMES OF THE 5-PGSL SPIN LABEL IN GEL PHASE BILAYERS OF DPPG AT pH 8.0 AND 1.5, DEDUCED FROM CALIBRATION LINEHEIGHT RATIOS OF HAEMOGLOBIN REFERENCE SATURATION TRANSFER ESR SPECTRA

The haemoglobin reference spectra were taken from Ref. 2. Values of the rotational correlation times (τ_2) are given in s.

	Temperature (°C)					
	5	15	25	30	40	50
pH 8.0						
C'/C	$1 \cdot 10^{-5}$	$6 \cdot 10^{-6}$	$3 \cdot 10^{-6}$	$2 \cdot 10^{-7}$	$\approx 10^{-9}$	—
L''/L	$1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$7 \cdot 10^{-5}$	$4 \cdot 10^{-5}$	$\approx 10^{-9}$	—
H''/H	$2 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$2 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$\approx 10^{-9}$	—
pH 1.5						
C'/C	$4 \cdot 10^{-5}$	$4 \cdot 10^{-5}$	$3 \cdot 10^{-5}$	$2 \cdot 10^{-5}$	$5 \cdot 10^{-6}$	$6 \cdot 10^{-7}$
L''/L	$2 \cdot 10^{-4}$	$3 \cdot 10^{-4}$	$4 \cdot 10^{-4}$	$4 \cdot 10^{-4}$	$2 \cdot 10^{-4}$	$5 \cdot 10^{-4}$
H''/H	$1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$2 \cdot 10^{-4}$	$2 \cdot 10^{-4}$	$2 \cdot 10^{-4}$	$2 \cdot 10^{-4}$

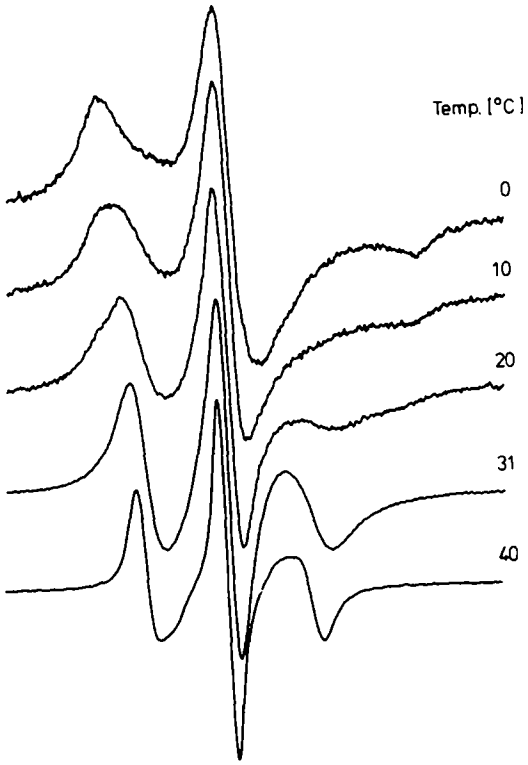
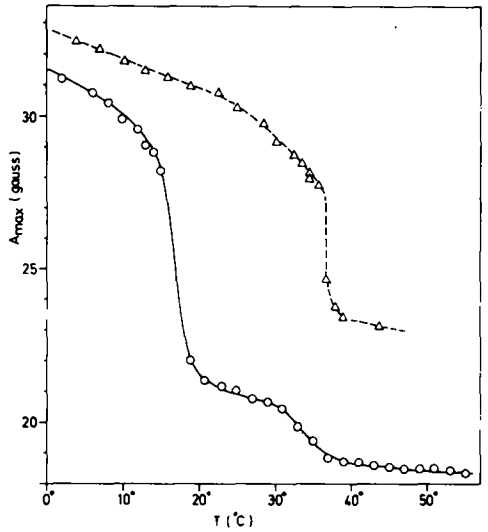


Fig. 4. Conventional ESR spectra of the cholestane steroid spin label in multibilayer dispersions of DPPG at pH 8.0, as a function of temperature.

spectrum at 30°C, whilst still in the gel phase. At the low temperatures the outer hyperfine splitting, $2A_{max}$, has the full anisotropy, $2A_{zz} \simeq 64$ G, whereas at 30°C this is averaged by the long axis rotation to a value $2A_{max} = (A_{zz} + A_{xx}) \simeq 38$ G, producing the pseudo-three-line spectrum. This is clearly seen in Fig. 5 in which the outer hyperfine splitting, A_{max}, of the cholestane spin label is plotted as a function of temperature. There is an apparent cooperative decrease in splitting centred about 15—17°C, below the pretransition, corresponding to the averaging of the A_{zz} and A_{xx} splittings by the long axis rotation, and a smaller decrease at the main transition corresppnding to motion of the long axis itself. In contrast, the outer hyperfine splitting of the 5-PGSL label (Fig. 5), which is sensitive only to the angular motion of the long axis, shows no cooperative decrease in the gel phase; the A_{max} values decrease only slightly until the main phase transition. Similar results are seen for DMPG in Fig. 6. An apparent cooperative decrease is observed in the outer splitting of the cholestane spin label in the gel phase, which merges with the main transition at 22°C. The rate of increase in the long axis rotation of the cholestane spin label in the gel phase bilayers at pH 8.0 is seen from the correlation times calculated from the outer hyperfine splittings and apparent lineheight ratios, using calibrations based on simulations by Polnaszek et al. [9]. Table III shows that the correlation time for long axis rotation decreases



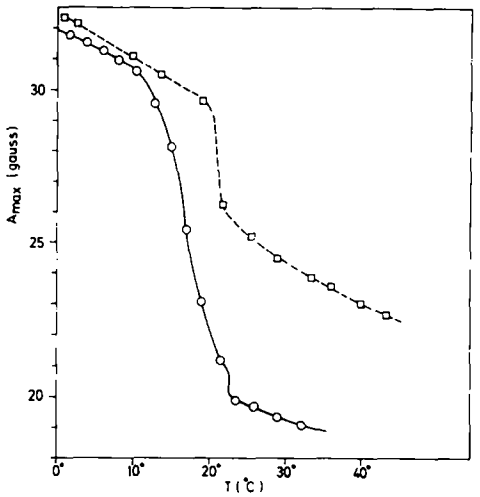


Fig. 5. Maximum outer hyperfine splitting, A_{max}, (see Fig. 4) in the conventional ESR spectra of DPPG bilayers at pH 8.0, as a function of temperature, for the cholestane spin label (○———○) and the 5-PGSL label (△- - - - -△).

Fig. 6. Maximum outer hyperfine splitting, A_{max}, in the conventional ESR spectra of DMPG bilayers at pH 8.0, as a function of temperature for the cholestane spin label (○———○) and the 5-PGSL label (□- - - - -□).

from values greater than 10^{-8} s at low temperatures to values less than 10^{-9} s, whilst still in the gel phase.

Results with the cholestane spin label in protonated phosphatidylglycerol bilayers at pH 1.5 are not so well defined. The spectra exhibit two components

TABLE III

ROTATIONAL CORRELATION TIMES OF THE CHOLESTANE SPIN LABEL IN GEL PHASE BILAYERS OF DPPG AND DMPG AT pH 8.0

Rotational correlation times were deduced from linesplittings and lineheights in the conventional ESR spectra. Calibrations were taken from spectral simulations by Polnaszek et al. [9]; see also Refs. 3 and 7. Values of the rotational correlation times (τ_R') are given in s.

	Temperature (°C)				
	4	15	20	29	40
DPPG					
A_{max}/A_{zz}	$>10^{-8}$	$3.5 \cdot 10^{-9}$	$1.2 \cdot 10^{-9}$	$0.7 \cdot 10^{-9}$	$\approx 10^{-10}$
B'	$>2 \cdot 10^{-9}$	$>2 \cdot 10^{-9}$	$2 \cdot 10^{-9}$	$0.8 \cdot 10^{-9}$	$\approx 10^{-10}$
C'	$>2 \cdot 10^{-9}$	$>2 \cdot 10^{-9}$	$2 \cdot 10^{-9}$	$0.7 \cdot 10^{-9}$	$\approx 10^{-10}$
	Temperature (°C)				
	1	10	15	20	25
DMPG					
A_{max}/A_{zz}	$>10^{-8}$		$3.5 \cdot 10^{-9}$	$1.5 \cdot 10^{-9}$	$\approx 10^{-10}$
B'	$>2 \cdot 10^{-9}$	$>2 \cdot 10^{-9}$	$>2 \cdot 10^{-9}$	$1.6 \cdot 10^{-9}$	$\approx 10^{-10}$
C'	$>2 \cdot 10^{-9}$	$>2 \cdot 10^{-9}$	$>2 \cdot 10^{-9}$	$1.7 \cdot 10^{-9}$	$\approx 10^{-10}$

at higher temperatures, whilst still in the gel phase. One component is immobilized with close to the full hyperfine anisotropy, whereas the other component shows motional averaging due to long axis rotation. The cholesterol-like cholestane spin label presumably disrupts the molecular packing in the gel phase such that the long axis rotation in phosphatidylglycerol bilayers at pH 8.0 is faster than that for the phospholipid spin label 5-PGSL, giving rise to motional averaging on the conventional ESR timescale, and also allows more extensive rotational motion in bilayers at pH 1.5.

Discussion

The present saturation transfer ESR studies have shown a clear distinction between the molecular motions in the charged (pH 8.0) and protonated (pH 1.5) states of the gel phase phosphatidylglycerol bilayers, as recorded by the phospholipid spin label. In the charged bilayers there occurs a rapid, more cooperative, increase in the rate of rotation around the label long molecular axis, whereas in the protonated bilayers there is only a slow, gradual increase, the main part of the increase (for DMPG bilayers, at least) taking place at the main transition. In this respect, the motional behaviour in the charged phosphatidylglycerol bilayers resembles that in phosphatidylcholines in the gel phase [3], the occurrence of the rapid long axis rotation correlating with the existence of a calorimetric pretransition in both these lipids. This further extends the structural similarity noted previously between these two lipids [1] to dynamic considerations also. Quantitatively, it is found that the overall motion is somewhat greater in the charged phosphatidylglycerols than in the phosphatidylcholines (effective correlation times are approx. 2—3-times faster), and that the increases in slope in the temperature dependence of the C'/C parameter are not so abrupt. This possibly indicates looser molecular packing in the charged phosphatidylglycerols than in the phosphatidylcholines in the gel phase. However, it is to be noted that the saturation transfer ESR spectra are sensitive to sample shape, size and orientation [11] and possibly also to vesicle size. Quantitative differences between phosphatidylglycerols and phosphatidylcholines might thus also be attributable in part to the different degrees of dispersability of these lipids.

Two points are relevant to the discussion of the detection of a highly anisotropic, long axis rotation of the phospholipid probe by saturation transfer ESR. Firstly, simulations have been made of the saturation transfer spectra arising from anisotropic rotation [10]. These have supported the principle of identification of long axis rotation by means of the C'/C ratio, as introduced previously in the analysis of the spectra in gel phase phosphatidylcholines [3]. Secondly, saturation transfer ESR experiments on gel phase dipalmitoyl phosphatidylcholine using other positional isomers of the phosphatidylcholine spin label (Fajer, P., Watts, A. and Marsh, D., unpublished data) have revealed that the onset of rapid long axis rotation is also accompanied by some segmental motion of the lipid spin label chains. Thus, the label molecules appear to display some flexible motions in the gel phase and do not simply rotate as rigid rods. In addition, it should be noted that axial rotation detected by a spin label on the chain C5 atom does not necessarily imply long

axis rotation of the whole molecule. However, the rotation of the rigid cholestane spin label molecule, which has a similar cross-sectional area to a phospholipid molecule, suggests that long axis rotation of the latter is very likely.

The phosphatidylglycerol bilayers in the protonated state at pH 1.5 do not show a pretransition [1], and similarly the saturation transfer spectra do not show an abrupt increase in long axis rotation. In this respect, the protonated bilayers resemble somewhat phosphatidylethanolamines, although in the latter case there is far less rotational motion in the gel phase than for phosphatidylglycerols [3].

Finally, it should be emphasized that the saturation transfer ESR measurements made here refer to the motions of the labelled molecule and thus are likely to be considerably different from those of the unlabelled parent molecules in the absence of spin label. The differences arise both from the perturbing effect of the spin label on the host lipid and from the effects of the nitroxide group on the motion of the labelled molecule. Several lines of evidence, however, lead us to believe that the changes observed in the long axis rotation of the spin label reflect motional changes which occur in bilayers of the parent unlabelled lipid. Firstly, the changes display a certain degree of cooperativity and correlate with the existence of a pretransition in the unlabelled bilayers. Secondly, the changes in long axis rotation are observed with two rather different spin labelled molecules, namely a phospholipid and a steroid. Thirdly, the spin label motion has a well defined anisotropy consistent with the symmetry of the unperturbed bilayers.

A rather important conclusion from this work is that the rotational motion detected by saturation transfer ESR in phosphatidylglycerols can be triggered isothermally simply by varying the pH.

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References

1 Watts, A., Harlos, K., Maschke, W. and Marsh, D. (1978) Biochim. Biophys. Acta 510, 63—74
2 Thomas, D.D., Dalton, L.R. and Hyde, J.S. (1976) J. Chem. Phys. 65, 3006—3024
3 Marsh, D. (1980) Biochemistry 19, 1632—1637
4 Marsh, D. and Watts, A. (1978) FEBS Lett. 85, 124—126
5 Hubbell, W.L. and McConnell, H.M. (1971) J. Am. Chem. Soc. 93, 314—326
6 Boss, W.F., Kelley, C.J. and Landsberger, F.R. (1975) Anal. Biochem. 64, 289—292
7 Marsh, D. (1981) in Membrane Spectroscopy (Grell, E., ed.), pp. 51—142, Springer-Verlag, Berlin
8 Marsh, D. and Watts, A. (1980) Biochem. Biophys. Res. Commun. 94, 130—137
9 Polnaszek, C.F., Marsh, D. and Smith, I.C.P. (1981) J. Magn. Resonance, in the press
10 Robinson, B.H. and Dalton, L.R. (1980) J. Chem. Phys. 72, 1312—1324
11 Beth, A.H., Wilder, R., Wilkerson, L.S., Perkins, R.C., Meriwether, B.P., Dalton, L.R., Park, C.R. and Park, J.H. (1979) J. Chem. Phys. 71, 2074—2082