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MOLECULAR MOTION IN PHOSPHOLIPID BILAYERS IN THE GEL PHASE: SPIN LABEL SATURATION TRANSFER ESR STUDIES*

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SUMMARY. Saturation transfer electron spin resonance has been used to study the molecular motions of a C5-labelled phospholipid spin-probe in gel-phase bilayers of phosphatidylcholines, phospha -tidylethanolamines and phosphatidylglycerols, and of mixtures with cholesterol. The spectra are qualitatively similar to the standard calibration spectra for isotropic motion but show differ -ential quantitative effects in the diagnostic lineheight ratios which are indicative of anisotropic motion. A rather similar motional state is observed at low temperatures for all phospho -lipids, but rapid axial rotation can be triggered by pretransitions, pH and cholesterol.

It is known that several membrane functions such as enzymatic activities and specific transport processes often show enhanced activity when the lipid chains are in a fluid state characteristic of phospholipid bilayers above their gel-to-liquid crystal phase transition (1). However, it is by no means certain that this is a general result, and in addition the fluid lipid is not necessarily directly involved in the enzymatic or transport steps since the timescale of lipid motions is much faster than the enzyme or transport turnover rates. In view of this and the known hetero -geneity of natural membranes, together with the finding that some biological membranes exist in a state in which fluid and gel-phase phospholipids co-exist, it is of considerable interest to investigate the molecular motion in gel-phase lipid bilayers. We have used the recently developed method of saturation transfer electron spin resonance (2-4), which is sensitive to molecular motion in the correlation time range 10^{-7}-10^{-3} sec, to study the motion of a spin

*Abbreviations used: STESR, saturation transfer electron spin resonance; DMPC, DPPC and DSPC, dimyristoyl, dipalmitoyl and distearoyl phosphatidylcholine; DLPE, DMPE and DPPE, dilauryl, dimyristoyl and dipalmitoyl phosphatidylethanolamine; DMPG and DPPG, dimyristoyl and dipalmitoyl phosphatidylglycerol; 5-PCSL, β-[5-(4',4'-dimethyloxazolidinyl-N-oxy)stearoyl]-γ-acyl-α-phos -phatidylcholine.

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-labelled phospholipid in the gel phase of various phospholipids and their mixtures with cholesterol. It is found that well-defined saturation transfer spectra are obtained from these systems, which show a clear sensitivity to motional rates and unambiguous evidence for anisotropic motion.

MATERIALS AND METHODS.

DMPC, DPPC, DSPC, DLPE, DMPE and DPPE were obtained from Fluka, Buchs, Switzerland and the purity was checked by thin layer chroma -tography. Cholesterol was from Merck, Darmstadt (F.R.G.). DMPG and DPPG were synthesized and characterized as previously described (5,6). Phosphatidylcholine spin-labelled on the C-5 atom of the ß-chain (5-PCSL) was synthesized essentially according to the methods in (7,8). Phospholipids and the desired amount of cholesterol, together with 1-2 mole% spin-label, were mixed in chloroform solution and the chloroform evapourated off by nitrogen, followed by vacuum dessication for at least 3 hrs. The dry lipids were then dispersed at a concentration of 80 mM in 0.1 M KCl, 0.01 M Tris, pH 8.0, by vortex mixing at a temperature above the lipid bilayer phase transition.

STESR spectra were recorded on Varian Century Line 9 GHz spectrometers. The sample was thermostatted by nitrogen gas flow and was contained in a 1 mm diameter glass capillary within a 4 mm quartz tube containing silicon oil. Sample temperature was measured by a thermocouple immediately above the microwave cavity. STESR spectra were obtained in the second harmonic, 90^0- out-of-phase, absorption mode, at a modulation frequency of 50 kHz, modulation amplitude of 5 G and microwave power of 63 mW (H_1 field $\sim$ 0.25 G, calibrated by Fremy's salt). Out-of-phase nulls at 1 mW were $\leq$ 1% of the in-phase signal in all cases - see (9) for further details.

RESULTS AND DISCUSSION.

Typical STESR spectra of phosphatidylcholines and phosphatidyl-ethanolamines of various chain-lengths, all the bilayers of which are in the gel-phase at 15^oC, are given in Fig. 1. The transition temperatures of DSPC and DPPE are 55^oC and 63^oC, of DPPC and DMPE are 42^oC and 48^oC, and of DMPC and DLPE are 23^oC and 29^oC, respectively. Thus each of these pairs of lipids should be at approximately similar states within their gel phase, on a reduced temperature scale. With the exception of DMPC, which is already in its pre-transition region, the spectra of the different gel phase bilayers are all qualitatively rather similar and also bear a fairly close resemblence to the spectra of spin-labelled haemoglobin (2) undergoing isotropic rotation with correlation times in the range 10^{-4}- 10^{-3}sec. This similarity between the different bilayers is borne out in Table 1 in which the diagnostic line-height ratios L"/L, H"/H and C'/C (see Fig. 1) are compared and effective rotational correlation times are calculated by comparison with the

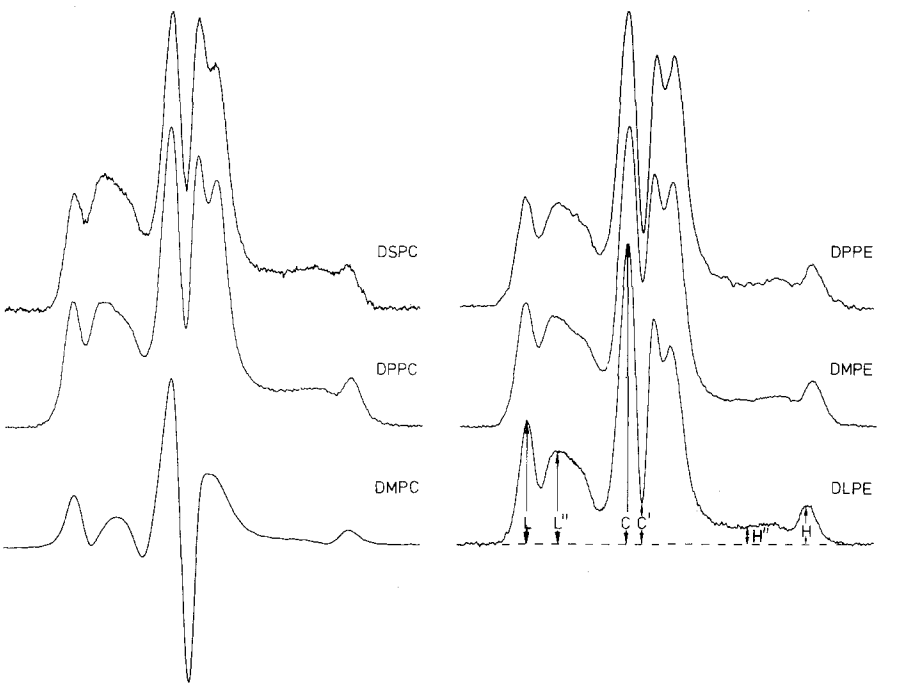

Fig. 1: STESR spectra of the phosphatidylcholine spin label 5-PCSL in gel phase bilayers of various phospholipids, at 15°C.

corresponding ratios from the standard isotropic calibration spectra of reference (2). Since the lipid motion is most likely anisotropic, it is not to be expected that the effective correlation times calculated from the different line-height ratios will be identical. In particular the L"/L and H"/H ratios will only be sensitive to motions in a direction perpendicular to the long axis of the lipid molecule, whereas the C'/C ratio is also sensitive to rotation around the long molecular axis.From Table 1 it is seen that the L"/L and H"/H ratios for DSPC, DPPC, DPPE, DMPE and DLPE all give effective correlation times $\sim 10^{-4}$ sec for motion of the long molecular axis. (For DSPC the spectra are approaching the limit of motional sensitivity of STESR, so perhaps the motion is somewhat slower, and for DLPE the effective correlation time is a little faster than 10^{-4} sec corresponding to incipient transitional effects with this lipid.) For DSPC and DMPE the C'/C ratios yield the same slow effective correlation time as the L"/L and H"/H ratios indicating that rotation around the long molecular axis is not appreciably faster than the angular oscillations of the long axis itself, whereas the C'/C ratio for DPPC yields an effective

Table 1. Diagnostic lineheight ratios, effective rotational correlation times (in brackets) and out-of-phase/in-phase amplitude ratios of spin-labelled PC in gel-phase bilayers of phosphatidylcholines, phosphatidylethanolamines and phosphatidylglycerols, T = 15°C.

	L"/L	H"/H	C'/C	out/in
DSPC	1.16 ($\geq 10^{-3}$ s.)	0.95 ($\sim 10^{-3}$ s.)	0.41 ($\geq 10^{-4}$ s.)	8%
DPPC	0.99 (4.10^{-4} s.)	0.73 (3.10^{-4} s.)	0.33 (3.10^{-5} s.)	12%
DMPC	0.57 (3.10^{-5} s.)	0.44 (6.10^{-5} s.)	-0.83 (2.10^{-7} s.)	9%
DPPE	0.96 (4.10^{-4} s.)	0.60 (1.10^{-4} s.)	0.24 (2.10^{-5} s.)	4%
DMPE	0.89 (2.10^{-4} s.)	0.58 (1.10^{-4} s.)	0.48 ($\geq 10^{-4}$ s.)	15%
DLPE	0.75 (7.10^{-5} s.)	0.45 (7.10^{-5} s.)	0.14 (1.10^{-5} s.)	13%
DPPG, pH 8.0	0.75 (9.10^{-5} s.)	0.60 (1.10^{-4} s.)	-0.07 (6.10^{-6} s.)	9%
DPPG, pH 1.5	0.95 (3.10^{-4} s.)	0.57 (1.10^{-4} s.)	0.31 (3.10^{-5} s.)	15%
DMPG, pH 8.0	0.60 (3.10^{-5} s.)	0.43 (6.10^{-5} s.)	-0.82 (2.10^{-7} s.)	11%
DMPG, pH 1.5	0.73 (7.10^{-5} s.)	0.40 (6.10^{-5} s.)	0.26 (2.10^{-5} s.)	12%

correlation time 10 times faster than that deduced from L"/L and H"/H indicating the onset of a more rapid rotation around the long molecular axis which is associated with incipient pretransitional effects in DPPC. (DPPE is an exception, its conventional spectra show strong spin-spin broadening and for this reason the C'/C ratio is reduced relative to that which would be obtained from motional contributions alone.) DMPC which is already in the pretransition region at 15°C shows the anisotropic rotation effect even more clearly. The L"/L and H"/H ratios are reduced somewhat relative to the other lipids, giving an effective correlation time of $\sim 5.10^{-5}$ sec, but the C'/C ratio gives an effective correlation time 100 times smaller than this indicating a much more rapid preferential long axis rotation in this state. DLPE has a C'/C ratio which gives an effective correlation time less than 10 times faster than that from L"/L and H"/H. This somewhat faster long axis rotation rate is attributed to incipient main transition effects.

A detailed study of the temperature dependence shows that these results can be summarized as follows. The phosphatidylcholines,

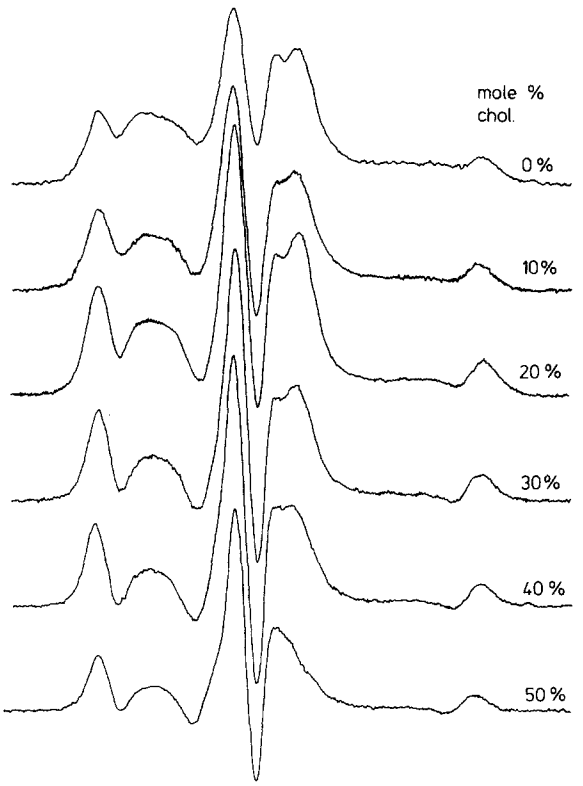

Fig. 2: STESR spectra of the 5-PCSL spin label in gel phase bilayers of DPPC with different cholesterol contents.

which show a calorimetric pretransition, display a rapid rotation around the long molecular axis with an effective correlation time $\sim 10^{-6} - 10^{-7}$ sec in the gel phase above the pretransition, whereas the phosphatidylethanolamines, which show no calorimetric pretransition, do not display this rapid long axis rotation until the main gel-to-liquid crystal phase transition. The data for DMPG and DPPG at two different pH's (Table 1) provide an interesting extension of this motional distinction. In the charged state, at pH 8.0, DMPG and DPPG display a pretransition and behavior in freeze-fracture electron microscopy very similar to DMPC and DPPC, whereas in the protonated state at pH 1.5 they display no pretransition and featureless freeze fracture faces similar to phosphatidylethanolamines (6). Table 1 shows that a similar distinction is observed in the STESR spectra. A more rapid, anisotropic rotation around the long axis is observed at pH 8.0 than at pH 1.5 (C'/C ratios), whereas the rate of motion of the long axis itself is similar at

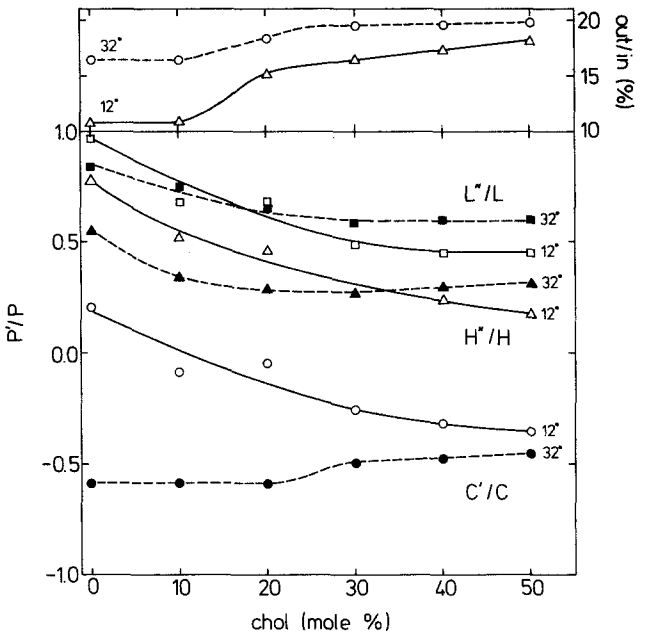

Fig. 3: Diagnostic peak height ratios L"/L (□), C'/C (o), H"/H (Δ) and out-of-phase/in-phase amplitude ratios as a function of cholesterol content, for the 5-PCSL label in gel phase DPPC bilayers at 12°C (———) and 32°C (- - - -).

the two pH's (L"/L and H"/H ratios). In particular all three ratios for DMPG at pH 8.0 are very close to those for DMPC and the ratios at pH 1.5 are very close to those for DLPE. The out-of-phase/in-phase amplitude ratios in Table 1, which are proportional to T_1 (2), are all of the order of 10% (with the exception of DPPE which is spin-spin broadened), indicating that it is motional differences and not T_1 differences which are being observed in the STESR spectra.

Figure 2 shows the effect of cholesterol addition on the STESR spectra of DPPC at 12°C. Again all the spectra show a qualitative similarity to the standard reference spectra of spin-labelled haemoglobin (2), indicating an increasing rate of motion with increasing cholesterol content due to the disruption of the close-packing of the lipid chains by the irregularly-shaped cholesterol molecule. Fig. 3 gives the plots of the diagnostic lineheight ratios as a function of cholesterol content at a temperature above and below the pretransition in the gel phase. It is seen that the changes in lineheight ratios with cholesterol are considerably smaller above the pretransition, where there is already more motion and

Table 2. Effective rotational correlation times of spin-labelled PC in gel-phase bilayers of DPPC as a function of mole% cholesterol content. T = 12°C.

τ(sec)	0%	10%	20%	30%	40%	50%
L"/L	6.10^{-4}	2.10^{-4}	8.10^{-5}	2.10^{-5}	1.10^{-5}	1.10^{-5}
H"/H	5.10^{-4}	1.10^{-4}	9.10^{-5}	2.10^{-5}	2.10^{-5}	1.10^{-5}
C'/C	2.10^{-5}	8.10^{-6}	8.10^{-6}	5.10^{-6}	3.10^{-6}	3.10^{-6}

looser molecular packing, than they are below the pretransition. In fact the higher cholesterol concentrations appear to reduce the temperature dependence of the spectra, particularly the C'/C ratio, and would tend to stabilize the fluidity of the gel phase against perturbations (c.f. previous conventional ESR studies, ref. 10,11). Again the out/in amplitude ratios indicate only small increases in T_1, (presumably due to a decrease in molecular packing density), which are insufficient to account for the changes in the STESR spectra. Table 2 gives the effective rotational correlation times, deduced from the lineheight ratios, as a function of cholesterol. The cholesterol reduces the effective correlation times both from the L"/L and H"/H ratios and from the C'/C ratio, and in fact reduces the anisotropy between them. Thus as a result of the disruption of molecular packing by cholesterol,the rate of motion of the chain segments in a direction perpendicular to the chain axis approaches more closely that of the faster rotation around the long molecular axis.

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