INTERACTING SPIN LABELS AS PROBES OF MOLECULAR SEPARATION WITHIN PHOSPHOLIPID BILAYERS

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SUMMARY

A steroid spin label probe has been used to measure spin label - spin label distances within the plane of phospholipid bilayer films. Measurements on different phospholipids, and on phospholipids associated with different proportions of cholesterol, have shown the method to be a sensitive probe of molecular organization within the bilayer.

Aqueous phospholipid bilayer systems have been extensively studied using spin labels with a view to clarifying the role of phospholipids in natural membranes.[1-4] Here we report the use of interacting spin labels as a probe of the lateral spatial arrangement of molecules within phospholipid bilayers. Lateral molecular separation is one of the principal factors determining the strength of intermolecular interactions within the bilayer.[5] It thus strongly influences structural and functional characteristics of the bilayer such as the liquid crystal transition point, permeability, and rate of lateral phospholipid diffusion.[6]

The method relies for its success upon resolution of the electron paramagnetic resonance (EPR) spectra from closely separated pairs of spin labels. The spectra of such pairs are

split by the magnetic dipole-dipole interaction between the two spin labels. The magnetic dipolar interaction is inversely proportional to the cube of the spin label separation, and thus the spectral splitting provides a very sensitive probe of spin label-spin label distances.

The spin label used in this investigation was the nitroxide derivative of cholestane, 3-spiro-[2'-(N-oxyl-4',4'-dimethyloxazolidine)]-cholestane. Phospholipid multibilayers were prepared in a flat quartz EPR cell from a chloroform solution of the lipids plus spin label, using the method of Butler *et al.*[7] The concentration of spin label in the original chloroform solution was about ten times larger than that used in normal spin label experiments, and gave a 10 mole % doping in the final multibilayers. EPR spectra were examined at room temperature on a Varian E-9 spectrometer, with the magnetic field parallel and perpendicular to the plane of the multibilayer film.

Multibilayer films of various compositions were examined. Egg lecithin, egg phosphatidylethanolamine, and bovine sphingomyelin were obtained from Lipid Products, Epsom, U.K. Synthetic dipalmitoyl-L-α-lecithin and dioleoyl-L-α-lecithin were obtained from Supelco Inc., Bellefonte, Pa. Lipid fraction 1 was extracted from the white matter of bovine brain.[8] Cholesterol from Steraloids, Pawling, N.Y. was recrystallized from methanol.

A typical spectrum of 10% spin label in multibilayers of lipids extracted from the white matter of bovine brain is given in Fig. 1a). In the perpendicular direction all spin label pairs are oriented homogeneously with respect to the magnetic field and their characteristic spectra are clearly

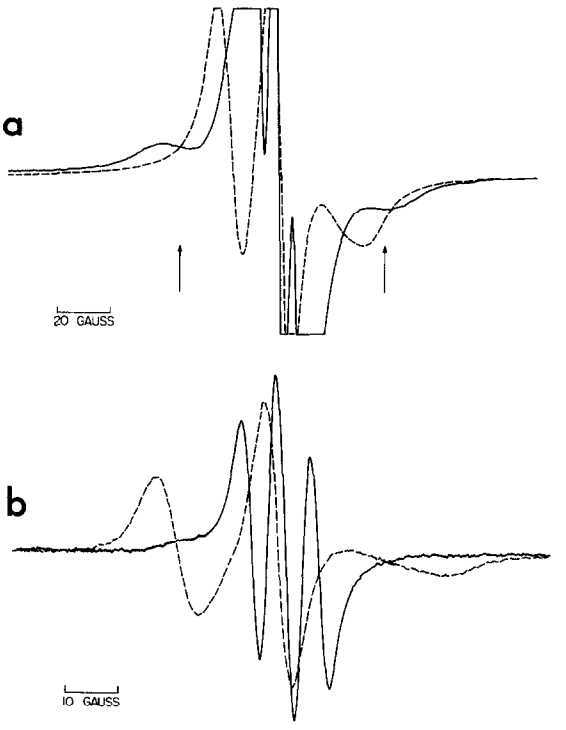

Figure 1. EPR spectra of cholestane spin label in a multibilayer film of lipids from the white matter of bovine brain, hydrated with 0.15M $CaCl_2$: a) 10% spin label doping; b) 1% spin label doping. Solid lines: magnetic field perpendicular to multibilayers. Dotted lines: magnetic field parallel to multibilayers.

seen, indicated by the two arrows in Fig. 1a). In the parallel direction the axes joining the various pairs have different orientations with respect to the magnetic field direction. In this case the spectra from the pairs are spread over a relatively large magnetic field range and their individual intensities are too low to be observable. In both cases the strong central lines arise from non-interacting spin labels, and the characteristic anisotropy in their three-line hyperfine structure shows the multibilayers to be well-ordered.[1-4] It is worth noting that the lines attributed to spin label pairs in the perpendicular direction cannot arise from disordered multibilayers,

because their separation is greater than the maximum hyperfine separation in the parallel direction. One would expect each of the two pair lines in Fig. 1a) to exhibit a five-line hyperfine structure arising from the coupling of the two nitrogen nuclear spins of I = 1. However, the splittings between the individual hyperfine lines are half those of the isolated label (ca. 3 gauss in the perpendicular direction). The hyperfine structure of the pair lines is presumably unresolved due to motional broadening (such as is seen in the parallel direction of the isolated spin label)[3] and dipolar broadening by more distant spin labels.

Comparison of the central lines of the isolated spin label spectra at 1% doping (Fig. 1b) and 10% doping (Fig. 1a) shows considerable dipolar broadening of the isolated spin label spectrum at 10% doping (note the change in magnetic field scale between a) and b)). This broadening is inevitable at the spin label concentrations necessary to obtain observable intensities in the pair lines, and is one of the main factors limiting the applicability of the method. The pair lines can be resolved only if their overlap with the central lines is small. Thus the method is only applicable to relatively well-ordered systems in which neighbouring spin labels are closely spaced and their dipolar interaction is not partially averaged out by rapid motion of large amplitude. For instance, pair lines are not resolved in pure egg lecithin bilayers, a system in which the spin label has been shown to have large amplitude motion.[3]

The various representative phospholipid systems to which the method has been applied are listed in Table I. These include a natural mixture of phospholipids, purified phospho-

TABLE I

LATERAL SEPARATIONS OF CHOLESTANE SPIN LABEL PAIRS IN VARIOUS PHOSPHOLIPID BILAYERS

Phospholipid	Cholesterol (mole %)	r(Å)
Beef brain lipid/0.15M $CaCl_2$	50*	7.19 ± 0.05
Sphingomyelin/0.15M NaCl	0	6.84
Sphingomyelin/0.15M NaCl	50	7.23
Phosphatidyl ethanolamine/ 0.15M NaCl	50	7.35
Egg lecithin/0.15M NaCl	30	7.46
Dipalmitoyl lecithin/0.15M NaCl	30	6.97
Dioleoyl lecithin/0.15M NaCl	30	7.55
Dipalmitoyl lecithin/dry	15	6.59
Dipalmitoyl lecithin/0.15M NaCl	15	6.88

*approximate natural composition.

lipids with naturally-occurring mixture of hydrocarbon chains and synthetic phospholipids with a single type of hydrocarbon chain. Cholesterol composition is expressed as mole % of total phospholipid plus cholesterol. The spin label separations, r, are calculated from the pair line splittings using the customary dipole-dipole Hamiltonian[9] and assuming a g-value[10] of 2.0058 in the perpendicular direction. The spin label separations are seen to be sensitive to phospholipid composition, cholesterol composition, hydrocarbon chain composition and hydration by the aqueous phase. This shows the method to be representative of lateral distances within the bilayer and also to be an effective probe of phospholipid organization and phospholipid-cholesterol interactions.

The effect of molecular composition on spatial separ-

ation within the bilayer is most clearly seen in the case of hydrocarbon chain composition and cholesterol composition. In multibilayers of the saturated phospholipid, dipalmitoyl lecithin, the spin labels are constrained to approach much closer than in the multibilayers of the unsaturated dioleoyl lecithin or the natural egg lecithin. The implied, larger intermolecular interactions in saturated lecithins are in line with their higher transition temperatures. The effect of cholesterol is clearly seen in comparing dipalmitoyl lecithin bilayers with 15% and 30% cholesterol. The increased cholesterol composition appears to cause a forcing apart of the molecules within the bilayer which is in agreement with the observed decrease in transition temperature[11] and other spin probe studies.[12,13] A marked change in spacing is also seen on hydration of dipalmitoyl lecithin - 15% cholesterol film. The increased separation is presumably associated with the change from a quasi-crystalline to a liquid crystalline phase on addition of the aqueous phase. Variation in spacing with phospholipid headgroup is less obvious. Comparison of egg lecithin - 30% cholesterol with beef brain lipids suggests some change with phospholipid composition, possibly associated with the negatively-charged phospholipids in beef brain lipid.

These results show that in favourable circumstances the interacting spin label method is a sensitive probe of lateral molecular arrangement within phospholipid bilayers. In these cases it contrasts favourably with X-ray diffraction which commonly yields a single broad band at high angle, corresponding to a mean spacing of 4.5 Å between the fluid hydrocarbon chains.[11,14]

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