THE STUDY OF HYDROCARBON CHAIN MOBILITY IN MEMBRANE SYSTEMS USING SPIN-LABEL PROBES

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1. Introduction

The fluid nature of certain cell membranes and their relationship to growth temperature [1, 2] is of particular interest. Highly fluid regions in cell membranes have now been demonstrated by both NMR [3] and ESR spectroscopy [4, 5]. The latter technique makes use of spin labels which are incorporated in various ways into the membrane system.

In a recent discussion, Crick [6] raised two points which could be important for the interpretation of spin label spectra of membrane systems. The first concerned the change in the magnitude of mobility of a spin label when a marked change occurs in the lipid system, e.g., the solidification of the hydrocarbon chains. The second concerned the hypothetical situation when two different phases exist in a membrane, would this heterogeneity be indicated by the ESR spectra?

Our recent deuteron resonance experiments with *Acholeplasma laidlawii* B membranes [7] have indicated the heterogeneous nature of this membrane, rigid lipid domains coexisting with more fluid crystalline regions, and this has led us to consider these same points.

2. Experimental

1, 2-Dipalmitoyl-L-lecithin was purchased from Fluka, Buchs., and was purified by chromatography on SilicAR CC-7 (200–350 mesh) using $CHCl_3$–MeOH gradient elution. It was pure by TLC on Merck Kieselgel F and Aluminium Oxide F plates (in $CHCl_3$–MeOH–7 M NH_4OH, 230 : 90 : 15). 1, 2-Dioleyl-L-lecithin was the very generous gift of Unilever Ltd., Colworth Welwyn. The spin label 12NS (methyl 12-spiro-(2′-[*N*-oxyl-4′, 4′-dimethyl oxazoladine])-stearate) was the kind gift of Professor A.D. Keith, University of California, Berkeley.

ESR spectra were recorded on a Varian E-3 (X-band) spectrometer, using a dewared insert and copper–constantan thermocouple regulated N_2 gas flow (± 0.5°K).

Lipid samples were mixed in $CHCl_3$, which was then removed by a N_2 stream, and the residual solvent removed *in vacuo*.

3. Results and discussion

With regard to the detection of phase transitions, early studies using a spin label in dipalmitoyl lecithin (DPL) showed that lipid phase changes, such as gel to liquid crystal, could be detected [8]. However, difficulties can occur and Hubbell and McConnell, using the *N*-oxyl-4′, 4′-dimethyl oxazolidine derivative of 5-keto-palmitic acid, have observed that the detection of the phase transition of DPL is dependent on the concentration of label [9] used.

The difficulties encountered in some cases of accurately monitoring phase changes are illustrated by the spectra of the spin probe 12NS in a dipalmitoyl lecithin–water system. At about 20°C characteristic immobile components are apparent in the spectra (fig. 1a). Some 11C° below the thermal transition (41°C) [10] the motion of the spin label is rapid and isotropic, giving the ESR spectrum of fig. 1b. Varying

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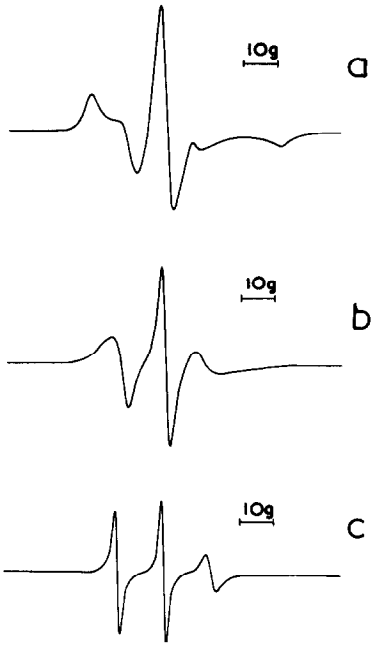

Fig. 1. a) 12 NS in DPL–H_2O, 20°C; b) 12NS in DPL–H_2O, 30°C; c) 12NS in DPL–H_2O, 40°C.

the mole ratio of spin label:lipid from 1:100 to 1:800 caused no significant change in this spectrum At 40°C the motion of the label is extremely rapid (fig. 1c). This indicates that there must be a great deal of motion of the chains near the transition point. Nevertheless at a spin label:lipid ratio of 1:400 (the concentration used for the spectra presented) the thermal phase transition at 41°C is clearly observed using the differential scanning calorimeter (DSC) technique.

The position of the nitroxide group in the probe molecule can also considerably affect the detection of a phase change of this type [11]. Difficulties in monitoring phase changes in lipid systems have also been indicated in studies of the solid to liquid transition of stearic acid, and have been discussed in terms of the presence of local impurity pools [12].

With regard to the second point of detecting heterogeneity of packing among the lipids of a membrane, we consider that this is relevant to some biological membranes, especially those membranes whose lipids have a broad thermal phase transition which encompasses the growth temperature of the organism. Two examples of this are the membranes of *Acholeplasma laidlawii* B and *E. coli* grown under normal conditions [13–16].

To examine this we have studied the ESR spectra of 12NS in a simple heterogeneous model membrane system, i.e. mixtures of 1,2-dibehenoyl-L-lecithin (DBL) and 1,2-dioleoyl-L-lecithin (DOL) in water. It has been found that when DOL and DBL are mixed in organic solvent, dried and resuspended in excess water that two thermal transitions occur [17], at T_c (liquid crystal transition temperature) = −22°C and at T_m (temperature of fastest melting) = 67°C. It is apparent from the thermal scans [17] of the mixed system that DOL at 22°C is in a liquid crystalline condition while DBL is in a gel condition and that "clusters" of solid DBL exist in the mixed bilayer. High-angle X-ray diffraction measurements on the DOL–DBL sample show the presence of reflections at Bragg angles consistent with the presence of both gel and liquid crystalline phases.

From the ESR spectra of the individual systems it is apparent that at 22°C the label in the DOL–water system (fig. 2a) is highly mobile, and its mobility is decreased in the BDL–water system (fig. 2b). This is consistent with the unsaturated lipid having fluid hydrocarbon chains (the liquid crystalline state) and the saturated lipid having more tightly packed chains at this temperature. (The DBL system is some 53C° below its transition temperature.) The spectrum of the

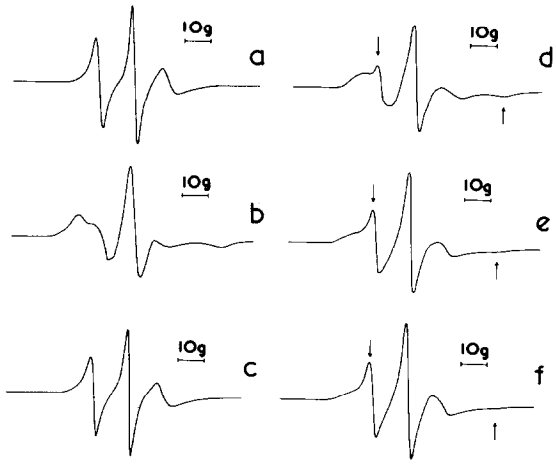

Fig. 2. a) 12NS in DOL–H_2O, 22°C; b) 12NS in DBL–H_2O, 22°C; c) 12NS in DOL–DBL mixed bilayers in H_2O, 22°C; d) 12NS in DBL–H_2O added to DOL–H_2O at 22°C, after 5 min; e) after 20 min; f) after 30 min.

label in the DOL–DBL–water system mixture (fig. 2c) is similar to that shown in fig. 2a (no high field peak is apparent). Thus the label shows only the highly fluid region of this heterogeneous model–membrane system, yet an appreciable portion of this lipid system contains fairly tightly packed chains.

We next considered the question whether a spin label, having been inserted into a lipid in the gel state, could move preferentially from the gel phase to a liquid crystalline phase. Exchange of lipid molecules between individual liposomes of DOL and DBL is known to be slow [17], as is the exchange of molecules between liquid–crystalline lecithin bilayers [18], and between lecithin bilayers and monolayers [19]. When solid DOL and DBL are mixed by homogenization and subsequently dispersed in water, two sharp thermal transitions are observed in the suspension, at −22°C and at 75°C. When spin-labelled DBL liposomes were added at 22°C to DOL liposomes which did not contain any spin label, and the mixture was agitated by hand, the spin label rapidly transferred from the DBL into the liquid crystalline DOL. This can be seen by the changes in the spectra with time shown in figs. 2d–f. (Thermal scans on the sample after the ESR spectra were obtained indicated no detectable mixing of the lipids.) It appears that, even when a spin label is incorporated into a rigid lipid region in a membrane, it may in some circumstances transfer to a more fluid hydrocarbon chain region.

The results of these experiments indicate to us the need for caution in the interpretation of the fluid nature of membrane systems using the spin probe technique. In some systems only the most fluid regions may be being detected by the probe. The possibility of a marked heterogeneity existing among the lipid molecules of some membranes, extending from gel to liquid crystal in packing, needs to be considered.

The problems associated with the detection of phase changes of the lipids adds a further complication to the characterisation of the nature of lipid packing in membrane systems.

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