PHASE TRANSITIONS IN SPHINGOMYELIN THIN FILMS

A SPIN LABEL STUDY

R.A. Long, F.E. Hruska and H.D. Gesser

Department of Chemistry, University of Manitoba, Fort Garry, Manitoba

J.C. Hsia

Department of Pharmacology, University of Toronto, Toronto, Canada

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Summary

3-Spiro-(2'-(N-oxyl-4',4'-dimethyloxazolidine)) - cholestane, (I) and 12-spiro-(2'-(N-oxyl-4',4'-dimethyloxazolidine))-stearic acid (II) have been used as molecular probes to study the interaction of sphingomyelin and cholesterol in both dry and hydrated oriented films at different temperatures. The presence of 50 mole percent cholesterol causes a gel to liquid crystalline phase transition of bovine brain sphingomyelin at 20°C. A temperature induced phase transition involving the phospholipid polar groups has been detected. The mean transition temperature from a rigid to a fluid bilayer lattice structure is 32°C ±0.5°C in hydrated equimolar sphingomyelin - cholesterol films.

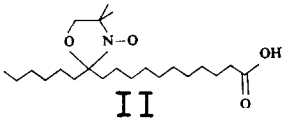
The structure and function of biological membranes are now being actively examined by a variety of physicochemical techniques. Recent x-ray data point out the existence of a lipid-bilayer structure in natural and model membrane systems[1-3] It has been demonstrated that valuable information concerning membrane structure can be obtained from electron spin resonance (ESR) spin label studies [4-10]. Sphingomyelin is an important component of many membrane systems. We report here a spin label study of the organization of bovine brain sphingomyelin thin films. The experiments demonstrate that thin films prepared from sphingomyelin are not oriented multibilayers in either the dry or hydrated state. In the presence of 50 mole percent cholesterol the lipids assume a stacked bilayer structure. The fluidi of the sphingolipids is markedly enhanced with increasing temperature.

EXPERIMENTAL

The spin label 3-spiro-(2'-(N-oxyl-4',4'-dimethyloxazolidine))-cholestane (I), was prepared by the method of Keana et al.[11] The stearic acid spin label (II)

was prepared according to Waggoner et al.[4] Bovine brain sphingomyelin was purchased from Mann Research Lab. and shown by thin layer chromatography to be pure. Cholesterol was recrystallized twice from methanol. Thin lipid films were prepared on a quartz cell by the method described elsewhere.[5] The films contained a fixed sphingomyelin-to-spin label ratio of 150:1. The resonance spectra of the label in the film were recorded with the plane of the film-supporting surface parallel and perpendicular to the direction of the laboratory magnetic field, referred to as the parallel and perpendicular orientations respectively. Hydration of the film was brought about by introducing an aqueous solution of 0.15 M NaCl into the cell. Excess aqueous phase was drained to minimize dielectric loss. All spectra were recorded with a Varian E-3 X-band spectrometer. The magnetic field was calibrated with Fremy's salt. A one cycle goniometer was used to determine the angle between the laboratory magnetic field and the plane of the quartz cell. The temperature in the cavity was determined with a chromel-alumel thermocouple.

I



II

RESULTS

We were able to obtain only angular independent powder spectra of I ($a_{//} \simeq a_{\perp} \simeq 32$ gauss) in dry or hydrated films of sphingomyelin at 20°C. This indicates the absence of any orientation of the spin label under these conditions. This lack of orientation may be a consequence of the technique employed for film preparation, or of the nature of the lipid films. At an elevated temperature, (37°C), angular independent spectra are still obtained but the observed hyperfine splittings $a_{//} \simeq a_{\perp} \simeq 16.8$ gauss indicate a rapid motion of the label suggesting an enhanced fluidity in the film.

Spectra of I in a dry sphingomyelin film containing 50 mole percent cholesterol are shown in Fig. 1A. In the perpendicular orientation a triplet is observed with $a_{\perp} = 6.4$ gauss whereas in the parallel orientation a powder spectrum was obtained with $a_{//} \simeq 33$ gauss. The long axis of the spin label must therefore be aligned essentially perpendicular to the plane of the film surface with a rotational frequency about this axis <<75MHz. We propose these results

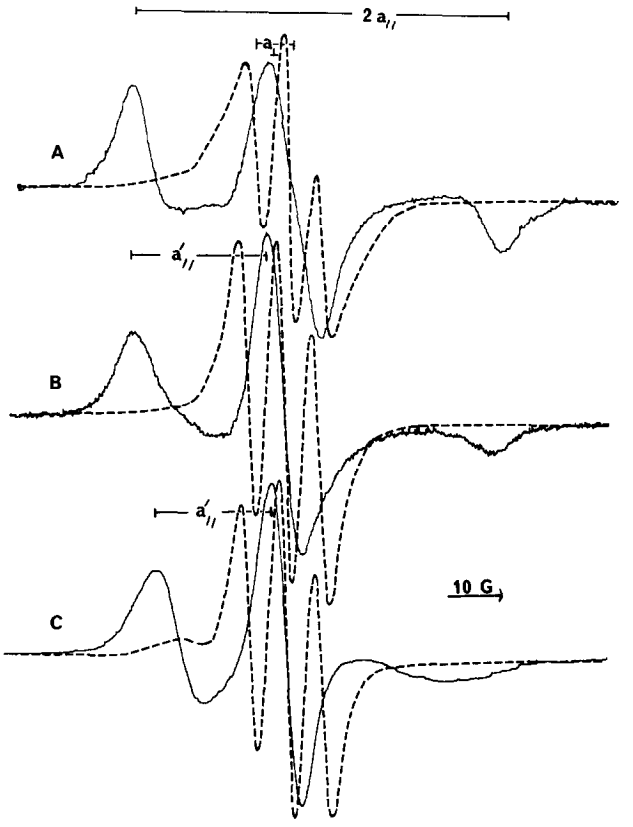


Figure 1. ESR spectra of 3-spiro-(2'-(N-oxyl-4',4'-dimethyloxazolidine))-cholestane in an equimolar sphingomyelin-cholesterol film. The solid line spectra (————) were recorded when the plane of the film was parallel to the direction of the magnetic field. The dotted line spectra (--------) were recorded when the plane of the film was perpendicular to the direction of the magnetic field. (A) dry film at 20°C, (B) hydrated film at 20°C, (C) hydrated film at 37°C. $a_{//}$ is one half the separation, in gauss, between extreme peaks for the parallel orientation in the dry state. $a_{\perp}$ is the hyperfine splitting constant, in gauss, measured between the mid-point of the low field peak and center peak for the perpendicular orientation in both dry and hydrated states. a'// is the peak to peak separation, in gauss, of the low field peak and the center peak for the parallel orientation in the hydrated state.

as evidence that in the presence of cholesterol sphingomyelin readily forms a multibilayer structure.

Hydration of the film at 20°C results in a sharpening of the triplet ($a_{\perp}$ = 6.3 gauss) observed in the perpendicular orientation but a powder spectrum is still found for the parallel mode ($a_{//} \simeq$ 32 gauss). Thus even in the hydrated film, rotation of the label about its long axis is still highly hindered. This is in contrast to previous experiments which demonstrated that hydration of cholesterol-containing egg-and dipalmitoyl-phosphatidylcholine films allowed rapid rotational motion of the label[5,10]. Changes in temperature have a pronounced effect on the spectra, (Compare Fig. 1B and 1C). At 37°C a sharp triplet ($a_{\perp}$ = 6.4 gauss) is still observed for the perpendicular orientation. However, in the parallel orientation $a_{//}$ has decreased from 32 gauss to 19.7 gauss. Therefore, under these experimental conditions the label is still aligned with its long axis perpendicular to the plane of the membrane but its axial rotational frequency must be greater than 75 MHz Thus the label must be less rigidly held in the lattice at this higher temperature. We have taken the separation of the low field and center peaks of the spectra in the parallel orientation ($a'_{//}$) as shown in Fig. 1B and 1C, as an approximate measure of the change in the rotational freedom of the label in the sphingomyelin-cholesterol lattice structure. A plot of $a'_{//}$ as a function of temperature for a 1:1 cholesterol-sphingomyelin film is shown in Fig. 2. The rigid lattice structure ($a'_{//}$ = 24.1 gauss) was maintained from 20°C to 25°C. A linear decrease in $a'_{//}$ indicating increasing fluidity occurs over the range 26°C-38°C. The minimum value of $a'_{//}$ is reached at approximately 38°C. The mean transition temperature from the rigid to fluid bilayer structure is estimated from Fig. 2 to be 32°C±0.5°C. The fact that the change in $a_{\perp}$ over the temperature range 20°C to 42°C is less than 0.2 gauss (Fig. 2) indicates that the lattice rigidity can be altered without a significant change in the average lattice angle of the bimolecular leaflet. We found these temperature effects to be reversible.

We have used the stearic acid spin label II to probe the hydrophobic regions of the sphingomyelin films. In a hydrated sphingomyelin film at 20°C the label

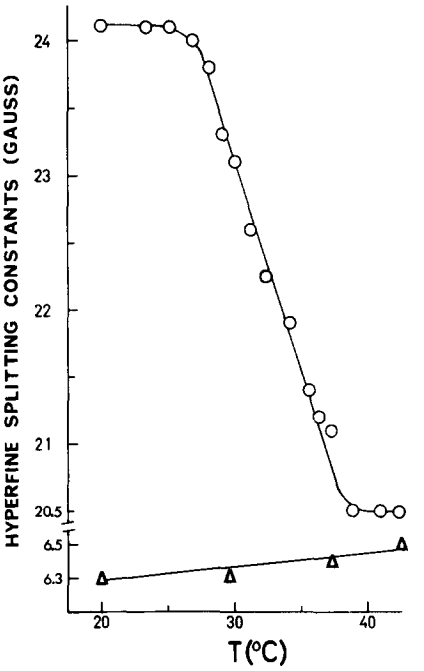


Figure 2. A plot of hyperfine splitting constants of 3-spiro-(2'-(N-oxyl-4',4'-dimethyloxazolidine))-cholestane in a hydrated equimolar sphingomyelin-cholesterol film as a function of temperature. (0) a'//, (Δ) $a_{\perp}$

is strongly immobilized indicative of a very rigid hydrocarbon region. At 20°C the motion of this label in a hydrated 50 mole percent sphingomyelin-cholesterol film is essentially the same as that in a 50 mole percent cholesterol-egg phosphatidylcholine film the data for which we reported in an earlier work.[6] We were able to conclude that there was a preferential alignment of the long axis of the stearic acid molecule perpendicular to the plane of the bilayer, with considerable wobbling motion of the molecule, presumably a consequence of the high degree of fluidity in the hydrocarbon region of the lamellar structure. In view of the above mentioned similarity in the spectra we may reasonably suggest a similar degree of fluidity for the hydrocarbon interior of cholesterol containing sphingomyelin bilayers. This fluidizing effect of cholesterol on the hydrocarbon chains has been observed by Oldfield and Chapman[12] in aqueous dispersions of sphingomyelin.

CONCLUSIONS

The presence of 50 mole percent cholesterol causes a gel-to-liquid crystalline phase transition in hydrated sphingomyelin thin films at 20°C.

At 20°C the spin label I is oriented with its long axis perpendicular to the plane of the hydrated cholesterol containing-sphingomyelin multilayers with little rotational freedom about this axis. Since our results with the stearic acid spin label demonstrated that the hydrophobic interior of the bilayers exist in a highly fluid state at 20°C the restricted rotational motion of I must be a consequence of hindering interactions involving the polar moieties of sphingomyelin. Increasing the temperature presumably relaxes these molecular forces in the bilayer structure, resulting in an increased average area occupied by each lipid molecule at the bilayer-water interface. This loosening of the lattice structure allows increasing rotational motion about the long axis of I.

Shah and Schulman[13] have postulated the existence of an intramolecular ion-dipole association in the sphingomyelin molecule between the hydroxyl group and the ionic oxygen of the phosphate group. Our results indicate that there is a strong intermolecular interaction, presumably hydrogen bonding, between sphingomyelin molecules, resulting in a cross-linked structure at the membrane-water interface.

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