Membrane Condensing Effect of Cholesterol and The Role of Its Hydroxyl Group

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Summary

Cholesterol affects the motional freedom and orientation of the N-oxyl - 4', 4' - dimethyl - oxazolidine derivative of 12-keto stearic acid (I) in dry and hydrated egg lecithin multibilayers. However, cholesterol derivatives with methoxy, carbonyl oxygen or chloro substituents at the 3 position of the cholestene nucleus drastically reduce the condensing effect on the multibilayer structure.

The spin labelled stearic acid was found to orientate in dry egg lecithin films with its long axis preferentially perpendicular to the plane of lipid films with considerable wobbling motion along the axis. Hydration abolishes this orientation, presumably due to an increase in membrane fluidity, therefore permitting the random motion of the label. Cholesterol decreases the wobbling motion of the label in dry multibilayers and increases orientation of the label in the hydrated bilayer structure.

Introduction

The precise role of cholesterol in biological membrane structure and function is yet unknown[1]. Recently, with the use of ESR spin labelling[2] and X - ray diffraction[3] techniques, cholesterol has been shown to improve the order of orientated phosphatidyl choline multibilayer structure. The two objectives of the present work are: one, the study of the cholesterol effect on the hydrophobic interior of oriented multibilayer membrane structure by using a relatively flexible N-oxyl -4', 4' -dimethyl oxazolidine derivative of 12-keto stearic acid.

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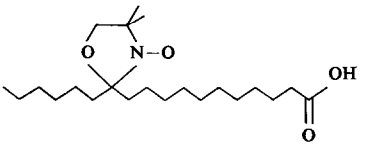

(I)

two, to characterize the contribution of the 3-OH group of cholesterol in the membrane condensing effect.

Experimental

The N-oxyl -4', 4' - dimethyl oxazolidine derivative of 12-keto stearic acid was prepared by the method of Waggoner et al[4]. Egg lecithin was purified by alumina and silicic acid chromatography. Cholesterol was recrystallized twice from methanol M.P. 148.5°. The cholesterol derivatives purchased from Mann Research Lab. were used without further purification. Thin lipid films containing fixed egg lecithin to spin label ratio of 150:1 were prepared by evaporating a chloroform solution of lipids in a standard quartz aqueous flat cell under reduced pressure. After measuring the resonance spectra of the label in this lipid film structure, an aqueous solution of 0.15 M NaCl was introduced into the cell to induce separation of the bilayers.[5]

All spectra were run at room temperature on a Varian E-6 X - band spectrometer. Resonance line position and hyperfine splitting constants were calibrated against Fremy's salt as an external standard. A one cycle goniometer was used to determine the angle between the plane of the flat cell and the laboratory magnetic field, excess aqueous phase was drained to minimize dielectric loss.

Results

The use of the nitroxide spin label in probing membrane structure depends on the fact that the hyperfine splittings of its resonance spectra are sensitive to the label's spacial orientation and motional characteristics.

When the label is interculated in a highly ordered structure such as an orientated membrane, the change in the angular dependence* of the hyper-

*(the angle between the membrane plane and the laboratory magnetic field)

fine splitting constants is a direct measure of the change in order and fluidity of the membrane lattice structure.

In the dry egg lecithin lipid film (<5% H_2O W/W) the resonance spectra of the label are different, depending on whether the supporting flat cell surface is parallel, or perpendicular to the laboratory magnetic field, henceforth refer to as parallel or perpendicular orientation. The difference in the hyperfine splitting constants, Δa, is 3.6 gauss

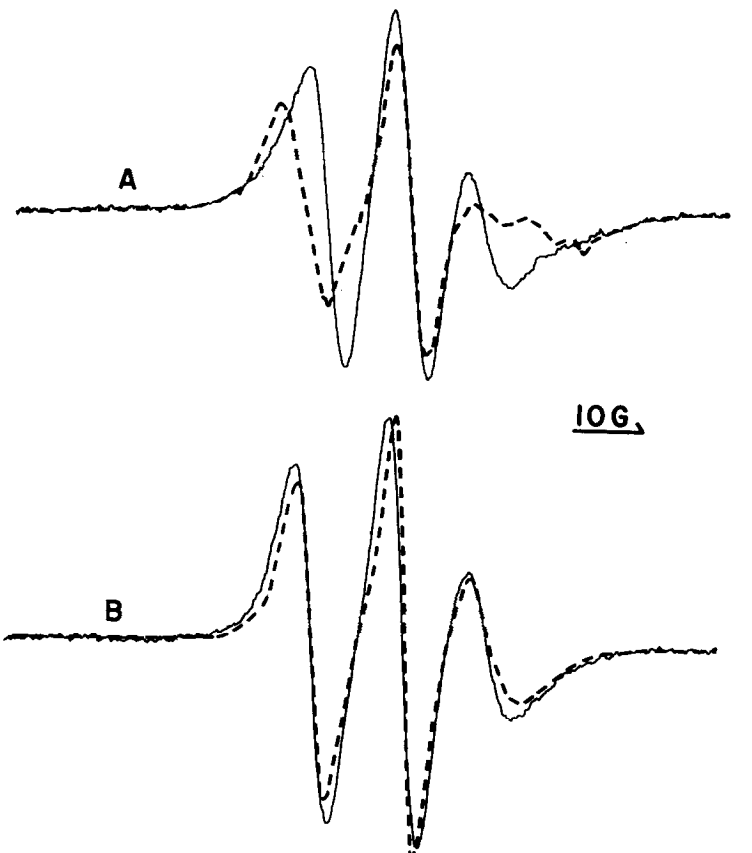

Figure 1. ESR spectra of N-oxyl - 4', 4' - dimethyl derivative of 12-keto stearic acid in orientated (A) Dry egg lecithin film, solid line spectrum (———), when supporting flat quartz cell surface is parallel to the laboratory magnetic field (parallel orientation) dotted line spectrum (------) when the film is perpendicular to the magentic field (perpendicular orientation.)
(B) Same sample as above after addition of 0.15 M NaCl solid line spectrum (———) parallel orientation; dotted line spectrum (------) perpendicular orientation.

(Figure 1A) indicating wobbling motion along the long axis of the stearic acid molecule, and poor orientation of this axis with respect to the membrane surface, assuming this axis is preferentially perpendicular to the plane of the lipid film. However, upon introduction of the salt solution, the resonance spectra are now almost orientation independent, $\blacktriangle a \simeq 0.5$ gauss, (Fig. 1B) indicating the label is going through chaotic random motion due to an increase in fluidity of the hydrocarbon interior of the bilayer unit membrane structure.

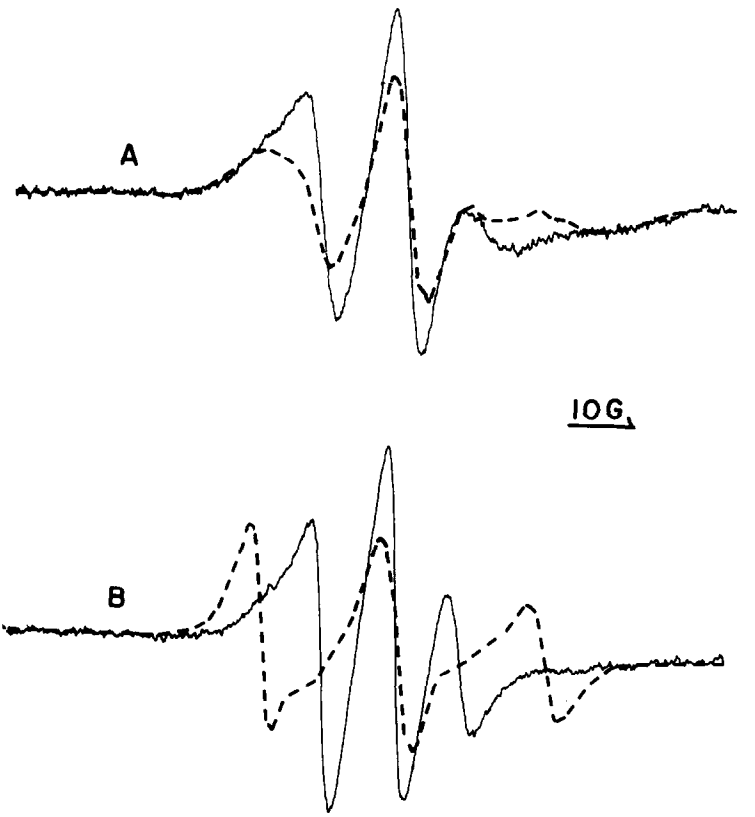

Figure 2. ESR spectra of N-oxyl -4', 4' - dimethyl derivative of 12-keto stearic acid in orientated egg lecithin - cholesterol samples in the presence and absence of aqueous phase.
(A) In dry equalmolar egg lecithin - cholesterol lipid film. Solid line (———) spectrum taken at parallel orientation dotted line spectrum (------) obtained at perpendicular orientation.
(B) Above sample in the presence of 0.15 M NaCl aqueous phase. Solid line spectrum (———) represents parallel orientation dotted line spectrum (------) represents perpendicular orientation.

The cholesterol effect observed at equimolar egg lecithin to cholesterol ratio in a dry lipid film appears to be a decrease of the wobbling motion observed in Fig. 1A. The largest hyperfine splitting in the perpendicular orientation is 23 $\pm$ 2 gauss, and 12 $\pm$ 1 gauss at the parallel orientation (Fig. 2A). Upon introducing the aqueous phase wobbling motion along the long axis became apparent. The difference in hyperfine splitting between the parallel and perpendicular orientation is 10 $\pm$ 1 gauss (Fig. 2B) compared with a value of 0.5 gauss for cholesterol free films (Fig. 1B)

The increased anisotropic motion of the spin label is probably the direct result of better orientation and higher rigidity of the membrane lattice structure due to the presence of cholesterol.

The angular dependence of the hyperfine splittings and the resonance

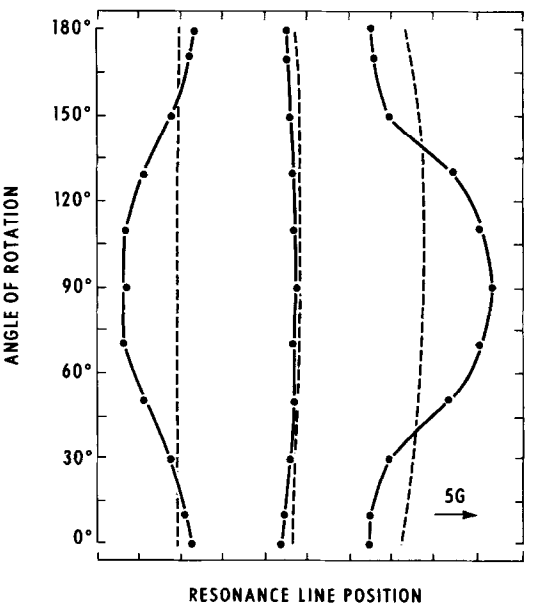

Figure 3. Dependence of the hyperfine splitting and resonance line positions of N-oxyl - 4', 4' - dimethyl derivative of 12-keto stearic acid as a function of the angle between the membrane supporting polished quartz cell surface and the external magnetic field. Field strength and resonance line position calibrated against Fremy's salt, solid line (———), equimolar egg lecithin - cholesterol, dotted line (------) egg lecithin.

line positions, relative to Fremy's salt in the presence and absence of cholesterol, in the hydrated state are shown in Fig. 3.

The g factor anisotropy of the spin label is evident from the angular dependence of the resonance line position of the centre peak. The change in the center line position between the parallel and perpendicular orientation for a pure egg lecithin film is 0.6 gauss, and 1.0 gauss for equimolar cholesterol lecithin films. The shift of the center line position of the nitroxide resonance spectrum with addition of cholesterol presumably results from the increased anisotropic motion of the label.

The plane symmetry of the angular dependence of the hyperfine splittings, i.e. spectra are identical at 0° and 180°, clearly indicates the same symmetry of the membrane preparations. The maximum hyperfine splitting observed at the perpendicular orientation suggests that the long axis of the stearic acid molecule is preferentially aligned perpendicular to the plane of the lipid films[6]. However, the decrease from the theoretical maximum splitting of 32 gauss[7] demonstrates that there is considerable wobbling motion of the label. Discrepancies between the results reported here and that of Libertini et al[6] presumably arise from differences in the extent of membrane hydration.

When the cholesterol derivatives, cholesteryl methyl ether, 5-cholestene -3β- chloride, or 4 - cholestene -3- one, are substituted for cholesterol in the lipid films, theΔa values are $1.5 \pm .5$ gauss in the hydrated state compared to 10 ± 1 gauss (Figure 2B).

Conclusion

Cholesterol increases the rigidity and order of egg lecithin lattice structure in the dry lipid film state as well as in hydrated multibilayers. The 3-OH group on the cholestene nucleus is necessary for this orientation and condensing effect.

The role of the 3-OH group is presumably to anchor the cholesterol molecule at the membrane-water interface thus providing stereospecific

hydrophobic and electrostatic interactions between the cholesterol and the phospholipid molecules. Hydrogen bonding between the 3-OH group and an oxygen atom of the phosphotidic acid or to water molecules is considered to be important for this anchoring effect.

Acknowledgement

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