Effects of Cholesterol and Cholesterol Derivatives on Hydrocarbon Chain Mobility in Lipids

by

E. Oldfield and D. Chapman
Department of Chemistry,
Sheffield University,
Sheffield S3 7HF.
England.

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SUMMARY

Cholesterol inhibits the chain motion of egg yolk lecithin when it is in the liquid crystalline phase. It also removes the sharp transition from gel to liquid crystalline phase normally observed with a saturated lipid such as dipalmitoyl lecithin. A similar lipid state is produced, partially characterised by a spin probe correlation time $\tau_c \sim 2 \times 10^{-8}$ s.
Small modifications to the cholesterol structure cause marked alterations in the solubilisation properties of the resultant steroid in lecithin bilayer systems.

Cholesterol is an important component of many membrane systems. The use of n.m.r. spectroscopy with lecithin-cholesterol systems has indicated[1] that cholesterol can inhibit the molecular motion of the lipid chains when they are in a liquid crystalline phase. It has also been shown[2] that e.s.r. spin probes are convenient tools for confirming this idea. At the temperature at which a lecithin is normally in a gel phase the effect of cholesterol is to remove the sharp transition which usually exists between the gel and liquid crystalline phases[3]. We have used the e.s.r. spin-probe method to study these effects further.

METHOD

A Varian E-3-X band spectrometer was used for e.s.r. measurements. The structure of the e.s.r. spin probe 12NS is shown in Fig. 1. 1,2-dipalmitoyl-Lα-lecithin was purchased from Messrs. Fluka, Buchs, and was purified on Mallinckrodt SilicAR CC-7 (70-100 mesh), eluting with chloroform-methanol (2:1). Egg yolk lecithin was extracted from Gallus domesticus eggs, and purified on Woelm alumina, according to Dawson[4].

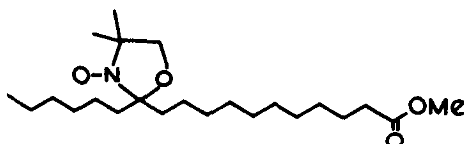

Fig. 1: Methyl 12-(N-oxyl-4',4'-dimethyl oxazolidine) stearate.

This crude fraction was then also repurified on SilicAR. Oxbrain sphingomyelin was purchased from Koch-Light Laboratories, Colnbrook, England, and was used without further purification. Cholesterol, 5α-cholestan-3-one and 3β-chlorocholest-5-ene were purchased from BDH, England. 3β-Methoxycholest-5-ene was prepared by methylating cholesterol with MeI/Ag_2O.

The N-oxyl-4',4'-dimethyloxazolidine derivatives of methyl 4 and 12-keto stearic acids (Fig. 1) were made according to Waggoner[2]. Samples for e.s.r. were prepared by dissolving 1:1 mole ratios of steroid-lipid in $CHCl_3$-MeOH (1:1) or $CHCl_3$, evaporating most solvent with a stream of nitrogen, then evacuating the sample for 3 hours to remove any last traces. 7 wt% lipid dispersions in pH 7 145mM phosphate buffer were homogenised above

their respective lipid, gel → l.c. transition temperature to ensure thorough mixing of all components.

RESULTS

At 20°C the dipalmitoyl lecithin is in a gel phase, and the 12-NS spin-probe spectrum (Fig. 2a) is typical of a highly immobilised nitroxide ($\tau_c \sim 8 \times 10^{-8}$s)[5]. This is consistent with the hydrocarbon chains of the lecithin being in a crystalline condition, and the e.s.r. spectrum approaches that of a rigid glass. The rapid tumbling of the spin-probe in egg yolk lecithin (Fig. 2c) is consistent with the quite fluid condition of the chains of this lipid.

Addition of cholesterol at 1:1 mole ratio, to the egg yolk lecithin (Fig. 2d) causes a spectral change consistent with a considerable decrease in mobility of the

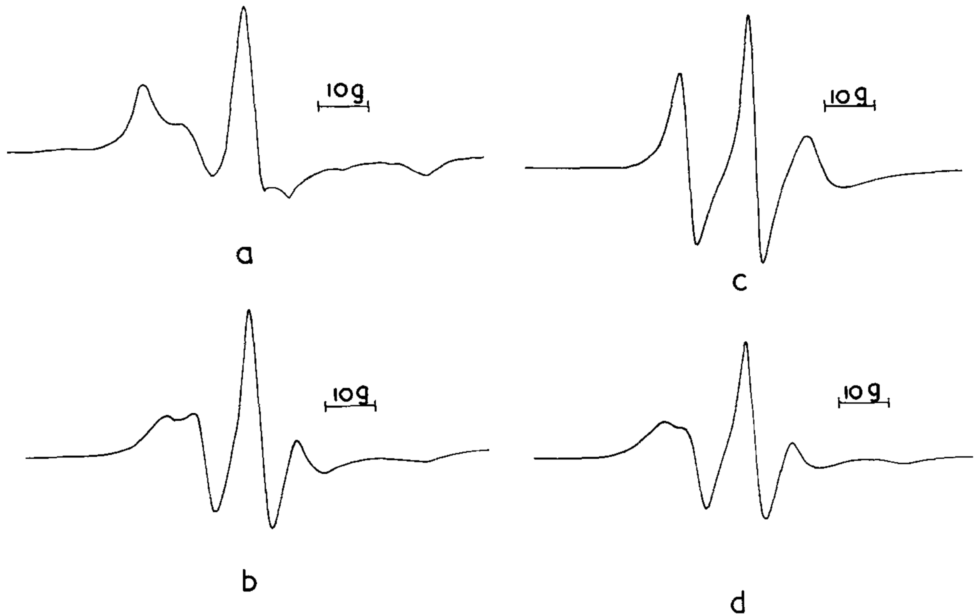

Fig. 2: (a) DPL (b) DPL-cholesterol (1:1) 20°C 12-NS
(c) EYL (d) EYL-cholesterol (1:1) 20°C 12-NS

hydrocarbon chains. The actual increase in correlation time of the spin probe is directly proportional to the amount of cholesterol solubilised by the lecithin.

Modifications to the OH group of cholesterol cause a decrease in solubilisation of the steroid by lecithin. We have observed, using polarised light microscopy, that cholesteryl chloride, methyl ether and cholestanone, are not incorporated to any great extent into this bilayer structure.

Addition of cholesterol to the dipalmitoyl lecithin system, at 1:1 mole ratio, causes a considerable increase in fluidity of the hydrocarbon chains, (Fig. 2b). The system is thus going to an intermediate fluidity, which is very close to that of the unsaturated egg yolk lecithin-cholesterol system, at the same temperature.

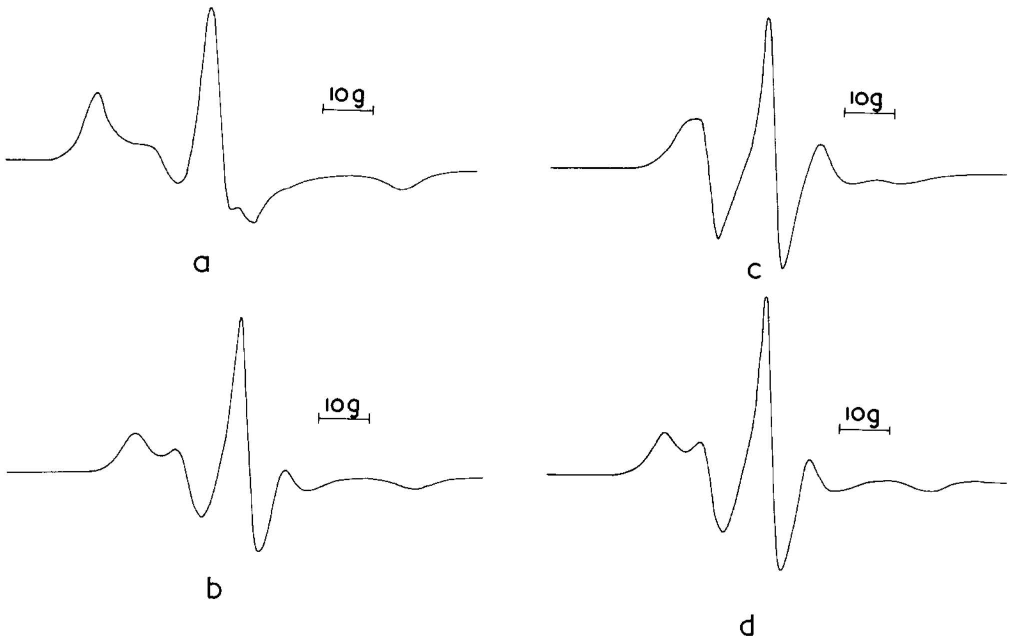

Fig. 3: (a) DPL (b) DPL-cholesterol(1:1)20°C 4-NS
(c) EYL (d) EYL-cholesterol(1:1)20°C 4-NS

This view is substantiated when the spin probe 4-NS is substituted for 12-NS. 4-NS is more immobilised than 12-NS in equivalent systems, due to its localisation near the polar-apolar interface (Fig. 3). Again, the resemblance of the dipalmitoyl lecithin-cholesterol (Fig. 3b), to the egg yolk lecithin-cholesterol system (Fig. 3d) is striking.

The e.s.r. spectrum of sphingomyelin with the stearate probe 12-NS at room temperature shows quite strong probe immobilisation (Fig. 4a). At this temperature the lipid is in a gel phase. Addition of cholesterol at 1:1 mole ratio produces an effect essentially the same as that observed in the dipalmitoyl lecithin-water system, that is, an increase in probe mobility (Fig. 4b). It is apparent that the effect of cholesterol, at 1:1 mole ratio, with sphingomyelin and dipalmitoyl lecithin - both of which are in a gel phase at room temperature, is to produce a

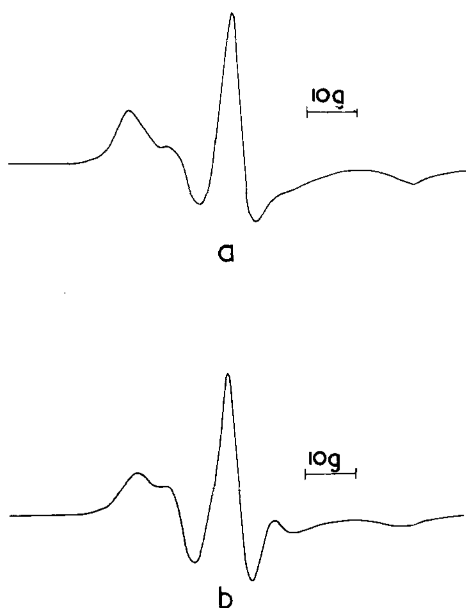

Fig. 4: (a) Sphingomyelin (b) SM-cholesterol(1:1)20°C 12-NS

state of the lipid hydrocarbon chains that is almost the equivalent of that produced by its action in the l.c. phase of egg yolk lecithin.

DISCUSSION

It may be that in numerous membrane systems there are areas of lipid adjacent to areas of protein, in a 'mosaic' organisation. The controlling of the permeability of the lipid regions may to some extent be governed by the fluidity of the lipid hydrocarbon chains. The mechanism whereby this fluidity is controlled must in general be linked to the turnover of the lipid. It seems likely that in myelin, where the turnover is very low, so that long term stability is required, that the relatively saturated lipids are kept in a fluid condition by the presence of cholesterol. The results with ox-brain sphingomyelin and dipalmitoyl lecithin are consistent with this idea.

Where there is a rapid turnover of phospholipids or where long term stability is not required, the role of cholesterol may be to decrease the mobility of unsaturated hydrocarbon chains. This is apparently the case in the egg-yolk lecithin system, and may be a general mechanism in for example, plasma membranes.

It appears that the 3-OH group is necessary for incorporation of steroid into the egg lecithin bilayer in appreciable quantities. Long et al[6], have recently concluded, using egg-yolk lecithin, that "cholesterol derivatives with methoxy, carbonyl oxygen or chloro substituents at the 3 position of the cholestene nucleus

drastically reduce the condensing effect on the multi-bilayer structure". As we have shown these steroids are not completely incorporated in the bilayer structure, at 1:1 mole ratios "condensing effects" if they occur, are expected to be small.

The observation of a common lipid state produced by cholesterol with saturated and unsaturated lipids, partially characterised by a spin-probe correlation time $\tau_c \sim 2 \times 10^{-8}$s may be biologically significant.

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