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INTERACTIONS OF THE CHOLESTEROL SIDE-CHAIN WITH EGG LECITHIN

A SPIN LABEL STUDY

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SUMMARY

The effect in egg lecithin liposomes of cholesterol and cholesterol analogues with side-chains of reduced length on the order parameters of two steroid spin labels has been studied. Analogues with side-chains shorter than cholesterol by more than three carbons cause significantly less ordering than cholesterol. Liposomes containing a cholesterol analogue in which the side-chain is absent cause very little increase in the ordering of a new sterol spin label in which the nitroxide is incorporated into the side-chain. The results suggest that the sterol side-chain exerts a great influence on membrane rigidity within its immediate environment.

INTRODUCTION

The side chain of cholesterol has been shown to be important in many steroid-phospholipid interactions. For example, cholesterol reduces the permeability of egg lecithin liposomes to glycerol and glucose whereas the cholesterol analogue androstan-3β-ol, which has no side chain shows no effect [1]. Enzymes which hydroxylate cholesterol show a contrasting dependence on the steroid side chain. We have shown that the bovine adrenal cortical mitochondrial cholesterol side chain cleavage system shows no side-chain specificity in contrast to the rat liver microsomal cholesterol 7α-hydroxylase, which has a specific requirement for sterols with a side-chain closely similar to that of cholesterol [2, 3]. In the liver system removal of only one carbon from the cholesterol side-chain is sufficient to cause a large decrease in the extent of 7α-hydroxylation.

This specificity is presumably due to a side-chain binding site of high selectivity in the microsomal enzyme, but it would be interesting to know how much of the sterol side-chain is required for maximum interaction with phospholipid. Butler et al. [4] have shown that androst-5-en-3β-ol shows a smaller condensing effect than cholesterol

Abbreviations: 3NC, 3-spiro(2′-(*N*-oxyl-4′,4′-dimethyloxazolidine))cholestane; 25NC, 3β-hydroxy-26-nor-25[2′-(*N*-oxyl-4′,4′-dimethyloxazolidine)]cholestane.

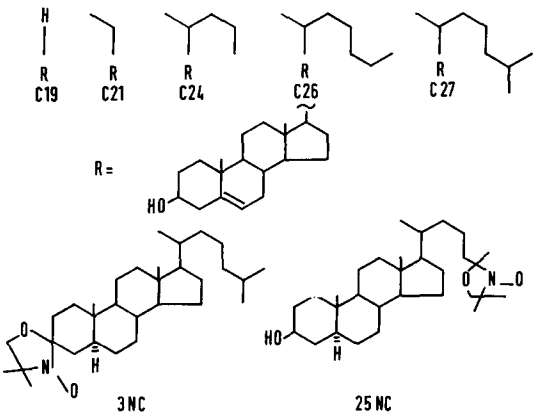

Fig. 1. Structures of sterols and spin labels.

in oriented multibilayers of beef brain lipids. They used the steroid spin label 3-spiro-(2′-(*N*-oxyl-4′,4′-dimethyloxazolidine))cholestane (3NC) to monitor the condensing effect. We report here the effect of cholesterol analogues which retain the cholest-5-en-3β-ol-ring system, but have side-chains of varying length, on the mobility of two spin labels in egg lecithin liposomes. The spin labels used were 3NC, in which the nitroxide is rigidly attached to the steroid ring system, and a new compound, 3β-hydroxy-26-nor-25[2′-(*N*-oxyl-4′,4′-dimethyloxazolidine)]cholestane (25NC), in which the nitroxide is incorporated into the side-chain. The structures of the sterols and spin labels used are given in Fig. 1.

MATERIALS AND METHODS

Egg lecithin was obtained from Lipid Products (Nutfield, Surrey, England) and was stored below 0 °C under N_2. It was sufficiently pure to use without further treatment. Cholesterol analogues were prepared as outlined previously [3, 5] and were purified by thin-layer chromatography and recrystallisation. All the sterols used were checked for purity by gas-liquid chromatography. 3NC was purchased from Synvar Associates (Palo Alto, Calif.). 25NC was prepared from 3β-hydroxy-26-nor-25-cholestanone by the method of Keana et al. [6]. It was purified by thin-layer chromatography on silica gel H (Merck). The pure compound had an R_F of 0.45 on silica gel G plates eluted with chloroform/ethyl acetate (85 : 15, v/v). Liposomes were prepared by dispersion on a Vortex mixer in 0.154 M KCl of a film prepared from fresh chloroform solutions of egg lecithin and sterol [1]. They contained the required proportions of sterol and lecithin with 1 mol % spin label. ESR spectra were recorded within 2 h of preparation of the liposomes at 30 °C on a Varian E4 spectrometer with the sample contained in capillary tubes sealed at one end. Order parameters were calculated following Hubbell and McConnell [7]. A value of 25 G was taken for $A_{zz}-A_{xx}$ for 3NC [8] and for 25NC $A_{zz}-A_{xx}$ was estimated to be 25 G from its spectrum in chloroform solution at −172 °C [9].

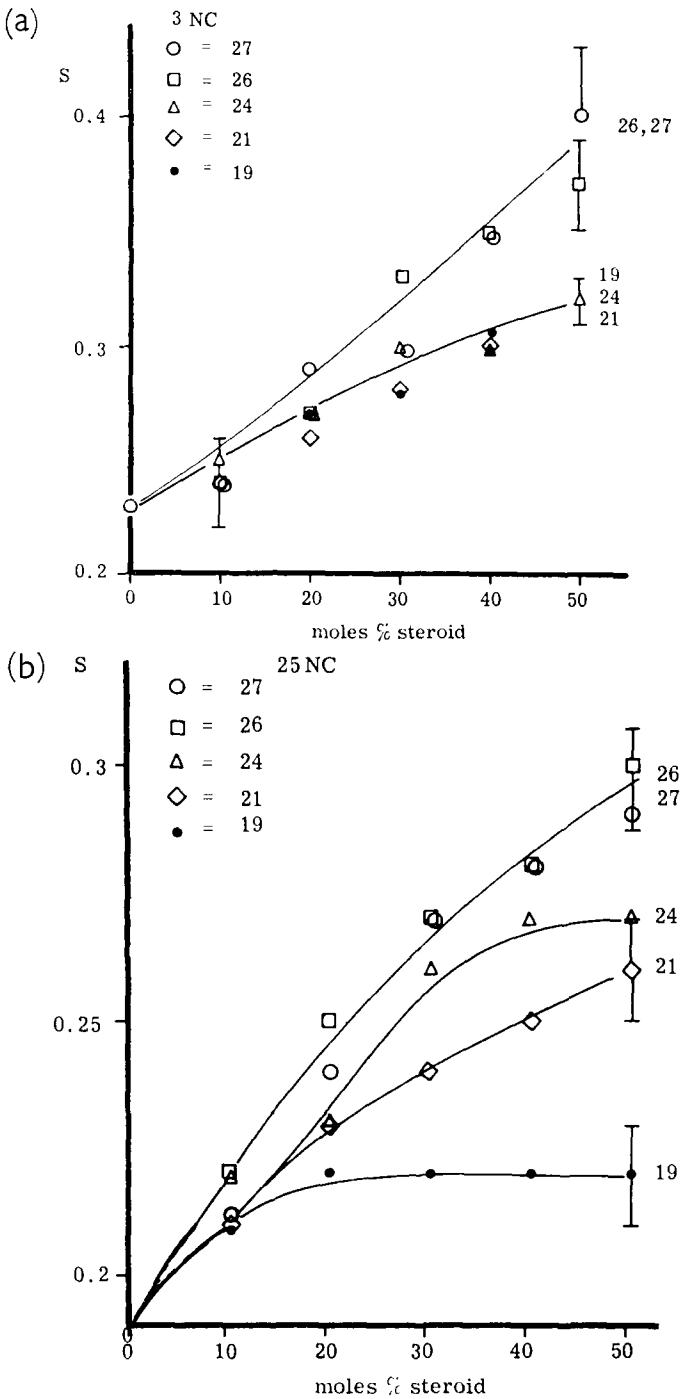

Fig. 2. Effect of sterols on the order parameter (S) of spin labels in egg lecithin liposomes. (a) 3NC. (b) 25NC. Bars indicate errors over 2–4 independent determinations. Order parameters are calculated from the expression $S = A_{\perp} - A_{\parallel}/\Delta A$ for 3NC and $S = A_{\parallel} - A_{\perp}/\Delta A$ for 25NC, where $\Delta A = A_{zz} - A_{xx}$ defined in Materials and Methods. $A_{\parallel}$ and $A_{\perp}$ are the hyperfine splittings parallel and perpendicular, respectively, to the steroid long axis.

RESULTS AND DISCUSSION

Order parameters for 3NC

Each spin label was incorporated into liposome preparations in which the sterol composition varied from 0 to 50 mol %. Experiments were performed using cholesterol and four side-chain analogues (C_{19}, C_{21}, C_{24} and C_{26}, Fig. 1). Order parameters as a function of sterol composition are plotted in Fig. 2a for 3NC and the spectra obtained illustrated in Fig. 3a.

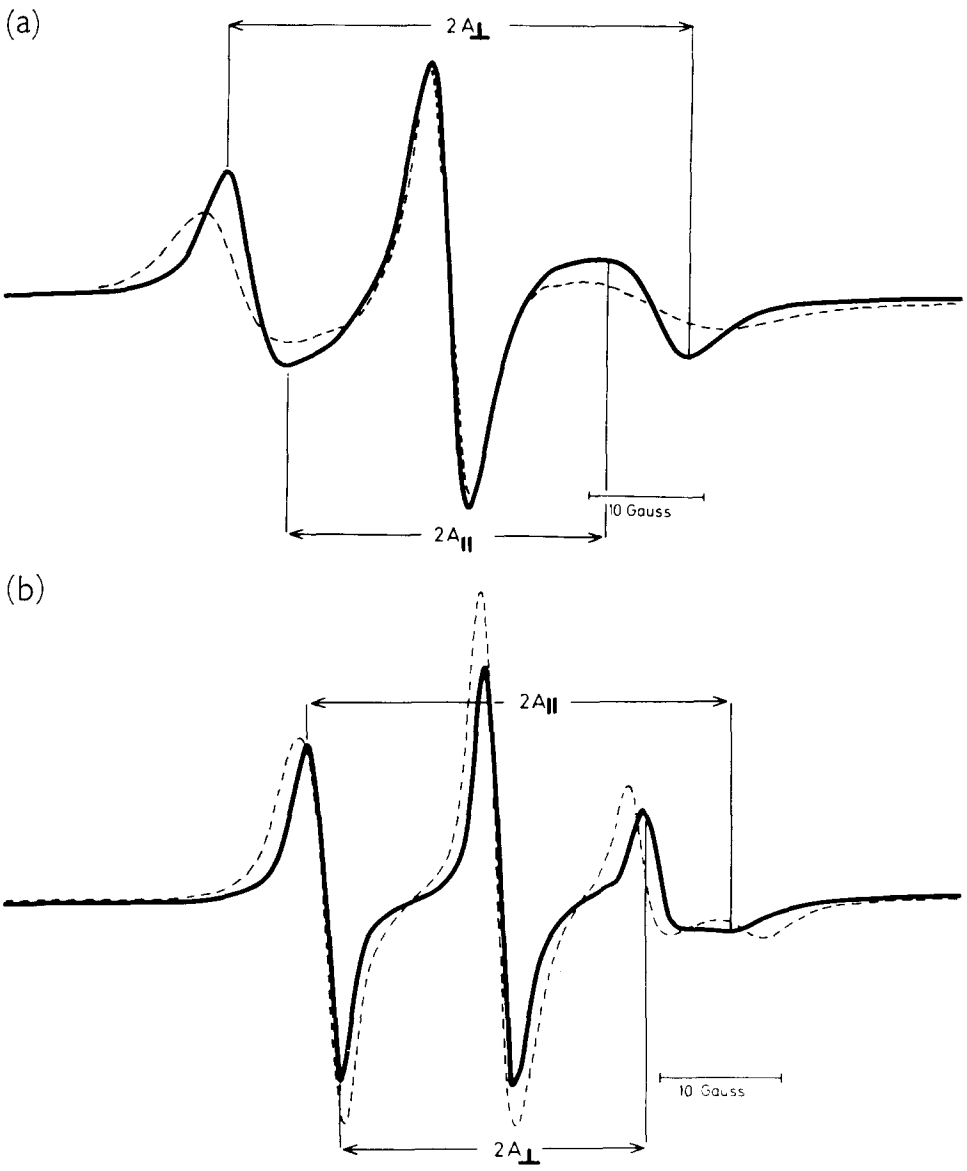

Fig. 3. Spectra of spin labels in egg lecithin liposomes. Continuous line: lecithin alone. Broken line: lecithin/cholesterol (60–40 mol %). (a) 3NC. (b) 25NC.

The spectrum (Fig. 3a) was similar to previously published spectra (for example ref. 9). All the steroids used caused an increase in the order parameter of 3NC as the concentration of sterol was increased. The increase in ordering caused by the C_{19}, C_{21} and C_{24} analogues was significantly less than that caused by cholesterol or the C_{26} analogue at sterol compositions of greater than 40 mol %.

Order parameters for 25NC.

Order parameters for 25NC incorporated in liposomes of varying sterol composition are shown in Fig. 2b. A typical spectrum is shown in Fig. 3b.

The nitroxide in 25NC would be expected to have more degrees of motional freedom than the more rigidly constrained nitroxide in 3NC. This greater degree of freedom is clearly shown by the spectrum of 25NC (Fig. 3b). The lines are narrower and more well-defined than those observed with 3NC, which is consistent with a more rapid and isotropic motion of the nitroxide [9]. The order parameters are also consistent with this model. At 0% sterol the value is 0.18 for 25NC compared with 0.23 for 3NC.

The effects of the sterols on the order parameter of 25NC show distinct differences when compared with the effects on 3NC. Cholesterol and the C_{26} analogue both cause an increase in the order parameter to about the same extent. The C_{21} and C_{24} analogues also increase the ordering but to a lesser extent than cholesterol. The effect of the C_{19} analogue, which has no side-chain, is more remarkable. This sterol causes an increase in the order parameter up to only 20 mol % sterol. Above this composition of sterol no further increase in the order parameter is observed.

Two high field lines are observed in the spectrum of 25NC in egg lecithin liposomes. The more extreme of the two is sensitive to factors which affect the rigidity of the liposome, e.g. to temperature and sterol content, and this line was used in the present study for the calculation of order parameters. The less extreme high field line is not as sensitive and is little affected by sterol content. These two lines may reflect two general independent environments of the nitroxide in 25NC in the liposomes or they may be a consequence of the isotropic nature of a liposome preparation with respect to the magnetic field. Gaffney and McConnell [11] have reported similar spectra for a phospholipid spin label observed in an isotropic distribution of hydrated bilayers of egg lecithin.

CONCLUSIONS

Cholesterol has been shown to increase the ordering of various spin labels in egg lecithin liposomes and multibilayers [12] and our results are consistent with the reported trends. The results in Fig. 2b suggest that the extreme line in the spectrum of 25NC reflects an environment which is very sensitive to the sterol side-chains which presumably lie towards the centre of the bilayer. The effect of the sterol side-chain on this environment is clearly greater than that observed with 3NC in which the nitroxide is thought to reside in the head-group region of the bilayer [10].

These results are also in accord with the analysis of Rothman and Engelman [13] from model building and with the NMR evidence of Kroon et al. [14] which suggest that cholesterol shows differential mobility of its nucleus and side-chain in phospholipid vesicles.

The experiments reported here suggest that the steroid side-chain exerts a very strong effect on membrane rigidity within its immediate environment. This is particularly clear at sterol compositions of greater than 30 mol % where the condensing effect of the ring system is sufficient for the influence of the side-chain to come into play. A reduction of the side-chain by more than one but no more than three carbons is the minimum for this effect to be observed.

It would be interesting to know if the effects of the cholesterol analogues on the mobility of spin labels in egg lecithin liposomes are parallelled by measurements of sterol-phospholipid interactions by other techniques. Preliminary results indicate that the degree of condensation of an egg lecithin monolayer by the cholesterol analogues decreases as the length of the side-chain is reduced (Suckling, K. E. and Malcolm, B. R., unpublished).

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