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OPTIMUM INTERACTION OF STEROL SIDE CHAINS WITH PHOSPHATIDYLCHOLINE

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Summary

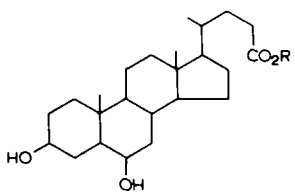
The specificity of the interaction between the cholesterol side chain and egg phosphatidylcholine was precisely defined by examining the effect of three new analogues of cholesterol with modified side chains on the ordering of two steroid spin labels in liposomes. The complete side chain of cholesterol was shown to be required for maximum ordering. Sterols with side chains shorter or longer than cholesterol caused significantly less ordering.

The role of cholesterol in membrane structure has been examined by a variety of physical techniques including NMR and ESR (for example, see refs. 1,2). The results of these and other studies are consistent with the interpretation that cholesterol causes the membrane phospholipid to possess an intermediate fluidity between a gel and a fluid state. Many different studies have shown that for maximum interaction with a phospholipid the sterol requires a planar ring structure, an equatorial 3β-hydroxyl group and an intact side chain [3].

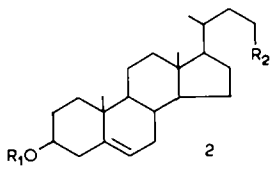
The role played by the side chain in this interaction has been studied in several ways [3—5]. Stevens and Green [4] measured the incorporation of several testosterone esters into egg phosphatidylcholine liposomes. The optimal incorporation was observed when the fatty acyl moiety had eight carbons, corresponding approximately to the length of the side chain of cholesterol. However, testosterone esters are not closely analogous to cholesterol in their molecular shape and polarity. In this laboratory we have examined the effect, by spin labelling, of varying the side chain lengths with sterols containing the cholesterol ring system [5]. These analogues are more closely related to cholesterol, the only difference being the modified side chains. This work suggested that sterols with side chains shorter than cholesterol by more than three carbons caused significantly less ordering of the spin labels in egg phos-

phatidylcholine liposomes than did cholesterol. These results encouraged us to synthesise further analogues of cholesterol to define the requirement for a side chain in the interaction with phospholipid more precisely. In this paper we report on the synthesis of three new analogues of cholesterol (Fig. 1) and their effect in egg phosphatidylcholine liposomes on the ordering of the two steroid spin labels described before, 3-spiro(2′-(*N*-oxyl-4′,4′-dimethyloxazolidine))cholestane (3NC) and 3β-hydroxy-26-nor-25 (2′-(*N*-oxyl-4′,4′-dimethyloxazolidine)cholestane) (25NC) [5].

Reagents used were of standard commercial grade and purified as required. Egg yolk phosphatidylcholine was prepared using the method of Pangborn [6] and was pure by thin-layer chromatography. Liposomes were prepared containing sterols, phospholipid and spin labels and ESR spectra obtained as described previously [5].



1a R = H
1b R = Me



2	R_1	R_2
a	H	$-CO_2H$
b	H	$-CO_2Me$
c	Tetrahydropyranyl	$-CO_2Me$
d	,,	$-CH_2OH$
e	,,	$-CHO$
f	,,	$-CHOHCH_3$
g	,,	$-CHOHCH_2CH(CH_3)_2$
h	,,	$-CHOH(CH_2)_4CH_3$
i	H	$-CH_2CH_3$
j	H	$-(CH_2)_2CH(CH_3)_2$
k	H	$-(CH_2)_5CH_3$

Synthesis of cholesterol analogues

Hyodeoxycholic acid (1a) was esterified with diazomethane and the ester (1b) converted into 3β-hydroxycholenic acid (2a) by the method of Bharuca et al. [7]. After esterification with diazomethane the 3β-hydroxycholenic acid methyl ester (2b) was converted into its tetrahydropyranyl ether (2c) [8]. The ether was reduced to the corresponding alcohol (2d) with lithium aluminium hydride and the alcohol oxidised to the protected aldehyde (2e) with celite-silver oxide [9]. The aldehyde was then reacted with the appropriate Grignard reagent [10] and the resulting secondary alcohol (2f, 2g, 2h) reduced via its tosylate to give a saturated side chain essentially as described by Arthur et al. [11]. Deprotection was effected by treatment of the steroid with acid [8] and the product was purified by chromatography and recrystallisation. All intermediates were characterised by infrared and NMR

spectroscopy and mass spectrometry and were shown to be pure by thin-layer chromatography. The product sterols were shown to be pure by thin-layer chromatography and by gas liquid chromatography. Their physical characteristics were as follows:

24-methyl-5-cholen-3β-ol, m.p. 133°C, M^+ 358 C_{25} sterol (2i);

26-nor-27,27-dimethyl-5-cholesten-3β-ol, m.p. 128—129°C, M^+ 400 C_{28} sterol (2j);

26-nor-27-*n*-propyl-5-cholesten-3β-ol, m.p. 124—125°C, M^+ 414 C_{29} sterol (2k).

Order parameters for 3NC and 25NC

Experiments were carried out using the C_{25}, C_{28}, and C_{29} analogues and also with cholesterol using egg phosphatidylcholine liposomes containing 0—50 mol% sterol. Order parameters for 3NC and 25NC were calculated as described previously and the spectra and order parameters for cholesterol were essentially the same as those previously described [5]. The values of the order parameters for liposomes containing 40 and 50 mol% sterol are given in Table I. The results for cholesterol are the mean of twelve independent experiments and those for the analogues of at least four independent

TABLE I

ORDER PARAMETERS FOR CHOLESTEROL ANALOGUES IN EGG PHOSPHATIDYLCHOLINE LIPOSOMES

	mol% sterol	Sterol			
		C_{27}	C_{25}	C_{28}	C_{29}
3NC	40	0.34 ±0.02	0.30 ±0.01	0.31 ±0.01	0.31 ±0.01
	50	0.39 ±0.02	0.35 ±0.01	0.36 ±0.01	0.36 ±0.01
25NC	40	0.264±0.01	0.255±0.01	0.257±0.01	0.255±0.01
	50	0.29 ±0.01	0.270±0.01	0.270±0.01	0.270±0.01

experiments. In every case the order parameter was found to increase with increasing sterol concentration. The presence of cholesterol caused significantly more ordering than either the shorter (C_{25}) or the longer (C_{28}, C_{29}) side chain analogues at concentrations greater than 40 mol% with both spin labels. The differences in order parameter at compositions below 40 mol% were too small to be distinguishable by these methods.

These results and those reported previously agree in outline with those of Stevens and Green [4]. They found that a testosterone ester with an eight carbon side chain gave maximum incorporation into egg phosphatidylcholine liposomes. They suggested that since this compound is slightly longer than cholesterol, a cholesterol-like molecule with one extra carbon in the side chain (i.e., our C_{28} analogue) would be incorporated to a greater extent than cholesterol. Although our experimental conditions are not directly comparable, the spin label results indicate that this may not be the case. Cholesterol

itself appears to have the optimal structure for a maximum sterol-phospholipid interaction.

Recent results from X-ray and neutron diffraction studies [12,13] show that cholesterol occupies a position in a phosphatidylcholine bilayer such that its side chain terminates in the region of the ends of the fatty acyl chains of the phospholipid. On this basis, the C_{28} catalogue and more especially the C_{29} analogue, which is much longer, would be likely to penetrate into the terminal region of the adjacent half of the phospholipid bilayer. Such an interaction or the change that might take place in a phospholipid bilayer to relieve the consequences of such an interaction would be likely to result in a decrease in order of the bilayer. This is what is observed in these experiments. The order of the liposomes containing the sterols with longer side chains is reduced to the same level as that observed with sterols with shorter side chains

These results confirm and extend our previous findings. Taken together, the results obtained with all the analogues of cholesterol suggest that the complete side chain has a specific role to play in the interaction with phospholipid and that the cholesterol molecule is the optimum size to fit into an egg phosphatidylcholine bilayer to give the maximum possible interaction with the phospholipid.

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