INCORPORATION OF PLANT STEROLS INTO MEMBRANES AND ITS RELATION TO STEROL ABSORPTION

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1. Introduction

Studies of the specificity of structure needed for the incorporation of steroid molecules into cellular membranes and model membranes (liposomes, composed of bilayers of lecithin) have established that the natural sterols can be replaced by closely related compounds [1, 2]. A further investigation of the role of the side chain of the sterol molecule has now been made using the major plant sterols present in the diet, campesterol and sitosterol, which differ from cholesterol only in the possession, respectively, of an extra methyl and ethyl group in the side chain. These, and other plant and fungal sterols, are poorly absorbed in the intestine compared with animal sterols and this specificity of absorption was attributed to the ability of sterols to enter the membranes and soluble lipoproteins of the intestinal cell [3, 4].

The present paper shows that *in vitro* the ability of sterols to enter liposomes and erythrocyte membranes is in the order cholesterol > campesterol > sitosterol and that their behaviour in this system is similar to that seen during intestinal absorption. It also shows that the incorporation of these sterol molecules into erythrocyte membranes can be related to the relative proportions of lecithin and sphingomyelin present.

2. Materials and methods

Egg lecithin preparation, formation of liposomes, lipid extraction and determination of phospholipid and sterols were all carried out as before [5]. The mixture of plant sterols used to prepare the liposomes was a gift from Dr. L.J. Goad and contained 70.7% sitosterol and 29.3% campesterol. Separation of individual sterols was effected by gas liquid chromatography [6]. The liposomes containing cholesterol as well as plant sterols were formed by sonicating 60 mg of lecithin with 35 mg of cholesterol and 130 mg of the plant sterol mixture in 20 ml of water.

2.1. *Exchange of sterols*

Washed erythrocytes containing 1.05–2.53 mg of cholesterol were incubated with liposomes containing 1.16–2.74 mg of plant sterols in 14 ml of 0.15 M phosphate buffer, pH 7.4. Benzylpenicillin (100 units/ml) and streptomycin sulphate (100 μg/ml) were added and the incubation carried out for 16 hr at 37° with shaking. The cells were separated by centrifugation and washed 3 times with buffer before the lipid was extracted and analysed.

3. Results and discussion

The plant sterols were well incorporated into the lipid bilayers of the liposomes since these contained 0.96 ± 0.6 moles of total plant sterol per mole of phospholipid. This is the same molar ratio as is given by cholesterol and is much higher than the values obtained with the other plant sterols stigmasterol and ergosterol [1, 2]. However, campesterol was more readily incorporated than sitosterol into the liposomes since the results given in table 1 show that liposomes

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Table 1
Incorporation of plant sterols (± cholesterol) into liposomes.

Sterols used	Sterol composition (% ± S.E.M.)			
	Starting mixture		Liposomes*	
	(1)	(2)	(1)	(2)
Sitosterol	70.7	55.1	61.6 ± 1.7	41.4 ± 2.9
Campesterol	29.3	23.6	38.4 ± 1.8	29.4 ± 2.6
Cholesterol	–	21.2	–	29.1 ± 3.8

* The values are averages of 4 determinations.

made with these 2 sterols contained on the average a 4:6 mixture instead of the 3:7 mixture of the starting material. Cholesterol is incorporated in preference to either plant sterol since when lecithin was sonicated with a mixture containing roughly 4 times as much plant sterol as cholesterol, the resultant liposomes contained less than 2.5 times as much.

These findings indicate that the insertion of an extra methyl group into the cholesterol side chain reduces its ability to pack with phospholipids and the insertion of an ethyl group reduces it still further. However, comparison with earlier results [1, 2] shows that there is a much more marked reduction in this ability to pack with phospholipids if a double bond is present in the side chain as in ergosterol and stigmasterol. Presumably, freedom of motion of the side chain is necessary for it to adopt the conformation giving the best fit to the spaces between adjacent phospholipid molcules and the rigidity imparted by the double bond prevents this.

The ability of the plant sterols to exchange with erythrocyte membrane cholesterol is shown in table 2. Both sterols enter the membrane and replace endogenous cholesterol but complete exchange of sterols does not occur otherwise about 50% of the membrane cholesterol should have been replaced by plant sterols [7]. As with the liposomes, the plant sterols do not enter the erythrocyte membrane as easily as cholesterol and again campesterol is better incorporated than sitosterol since the proportion of campesterol in the membranes is always higher than in the original liposomes.

There are also species differences in the extent and nature of sterol incorporation. The plant sterols are more readily incorporated into the rat and human erythrocyte membranes than into those of the ox and the proportions of sitosterol and campesterol vary with the total incorporation. Thus in liposomes, where incorporation is greatest, sitosterol makes up 61% of the plant sterols present, in the rat membranes this falls to 51%, in the human membranes to 45% and in the ox membranes, where incorporation is least, to 38%. These differences correlate with the differences in phospholipid composition. The liposomes contain 100% lecithin, the rat membranes, 48% lecithin and 13% sphingomyelin, the human membranes, 29% lecithin and 27% sphingomyelin and the ox membranes, no lecithin and 46% sphingomyelin [8]. It would seem that either membrane cholesterol is associated more firmly with sphingomyelin than with lecithin and does not readily exchange [9] or else the bulkier plant sterols cannot be accommodated so easily between

Table 2
Exchange of plant sterols with the cholesterol of red cell membranes.

Erythrocytes used	No. of experiments	Erythrocyte cholesterol replaced (% ± S.E.M.)	Campesterol:sitosterol in membrane
Rat	4	40.3 ± 8.5	49:51
Human	4	24.3 ± 6.1	55:45
Ox	5	17.7 ± 3.4	62:38

sphingomyelin molecules as between lecithin molecules. Red cell sphingomyelins contain mainly saturated fatty acids [8] and this is consistent with the second hypothesis.

These results also explain the findings that plant sterols are not well absorbed from the intestine although they are as well dispersed in the intestinal lumen as cholesterol [10, 11]. In the diet and in the micellar phase in the intestinal lumen, the percentage of sitosterol is much greater than that of campesterol, whereas in the intestinal wall and in the subsequent stages of absorption in several animal species, the proportion of campesterol is much increased [11–13]. This is what would be predicted on the basis of the above results if absorption were mediated via incorporation into and exchange amongst the membranes and soluble lipoproteins of the mucosal cell [3]. Since cholesterol is incorporated into and exchanges amongst membranes more easily than either of the plant sterols, the plant sterols will be discriminated against more and more the deeper they penetrate into the mucosal cell and the more exchanges they undergo [14]. The progressive discrimination against sitosterol in favour of campesterol is also seen in the above experiments where for every mole of sitosterol in the starting mixture there are 0.4 moles of campesterol. On incorporation into liposomes, this rises to 0.6 and on transfer to membranes to 1.0–1.6. As only the plasma membrane of the intestinal mucosal cell is continually exposed to a high concentration of plant sterols it can be predicted that most of the campesterol and sitosterol of the cell will be in this membrane and as they are transferred inside the cell more slowly than cholesterol, they will tend to block cholesterol uptake at this point.

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