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Lanosterol and cholesterol have different effects on phospholipid acyl chain ordering

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^{2}H nuclear magnetic resonance (^{2}H-NMR) spectra of dioleoylphosphatidylcholine labelled at positions 9 and 10 in the acyl chains of the phospholipid were obtained in the presence of cholesterol and lanosterol. The spectra show in all cases three quadrupole splittings. One is due to the deuterium on position 10 of the *sn*-1 chain and another to the deuterium on position 10 of the *sn*-2 chain. The third deuterium quadrupole splitting arises from the deuterium at position 9 of both chains. Cholesterol, at increasing concentration, produces an increase in the quadrupole splitting from position 9, corresponding to an increase in order of that C-D bond segment arising from the inclusion of cholesterol in the membrane. Little effect is noted on the quadrupole splittings arising from position 10 of either chain. Lanosterol appears to have no effect on the quadrupole splittings from position 9. Lanosterol, likewise, has no effects on the quadrupole splittings from position 10 of both chains. These data therefore suggest little disorganization of the membrane structure due to the 14-methyl group. However, the 14-methyl group prevents lanosterol from causing the increase in motional order of the phospholipid hydrocarbon chains characteristic of cholesterol.

Cholesterol is an important constituent of mammalian cells. In general, it is found in high concentration in plasma membranes of cells, but in markedly lower concentration in intracellular membranes in which it is synthesized. Cholesterol has been shown to exert a variety of physical effects on a phospholipid bilayer, including permeability effects [1], effects on the phase transition of saturated phospholipids [2,3], and effects on the ordering of phospholipid acyl chains [4]. However, the biological significance of these and other cholesterol-related phenomena have yet to be fully elucidated, as is the role (or roles) of cholesterol in cholesterol-requiring cells.

One approach to the question of the biological role of cholesterol is to examine the structural specificity of the cholesterol effects for which assays exist. For example, the phenomenon of cholesterol influence on glucose permeability has been found to be fairly specific for particular sterol structures [1]. Potentially, another aspect of structural specificity may be found in the biosynthetic pathway for cholesterol synthesis. One well-known intermediate in cholesterol biosynthesis is lanosterol, which is notable for three additional methyl groups not found in cholesterol [5]. These methyl groups are located at positions 4 and 14 on the steroid ring system. The methyl group at position 14, in particular, projects into what is referred to as the alpha face of the steroid [6]. In cholesterol, this face is essentially planar. It has been suggested that a planar alpha face is important to cholesterol-like behaviour [7].

It might therefore be expected that the 14-methyl group would have a significant effect on the behavior of the sterol in a membrane. This was substantiated by a comparison of cholesterol and lanosterol in phospholipid vesicles [8]. One might

also expect that such a structural change could have a significant effect on the behavior of the phospholipid acyl chains in a membrane. This was noted in a recent study using fatty acid spin labels in phosphatidylcholine vesicles containing the two sterols. According to these studies, the greatest perturbation on the lipid was in the middle of the acyl chain, or in the region of the membrane in which the 14-methyl group resides [9]. Such results, however, were obtained with labelled fatty acids. It is important to verify these results using as nonperturbing a technique as possible, to get a more clear view of the nature of the structural perturbation of the 14-methyl group.

The method of choice in such a study is deuterium nuclear magnetic resonance (^{2}H-NMR). This technique provides both orientation and orientational order information from the spectra. By using phospholipids specifically deuterated in known positions, the precise region of the membrane being probed can be determined. This has proven a powerful approach to membrane studies [10] but has not been used to assess the effect of sterols, other than cholesterol, on the phospholipid acyl chain orientational order.

In the present study, ^{2}H-NMR of dioleoylphosphatidylcholine deuterated at positions 9 and 10 is used to study the differences between the effects of lanosterol and cholesterol on phosphatidylcholine acyl chains. The deuterium labels are located in just that region of the membrane that is expected to be most perturbed by the 14-methyl group of lanosterol. The results show that this 14-methyl group does not affect the average orientation of the double bond segment of the acyl chain of the phospholipid, when compared to incorporation of a similar amount of cholesterol.

Materials and methods

Dioleoylphosphatidylcholine specifically deuterated at positions 9 and 10 was obtained from Cambridge Isotope Laboratories. Deuterium-depleted water was obtained from Aldrich.

Multilamellar liposomes were formed by first mixing the lipid components in chloroform, and then drying the lipid, containing 25 mg of the deuterium-labelled phospholipid and the appropriate amount of sterol, out of chloroform into a film onto a glass flask. Deuterium-depleted water was then added and the material vortexed to suspend the lipid. Cloudy suspensions were formed.

^{2}H-NMR spectra were obtained at 41.4 MHz in 10-mm tubes with a JEOL FX270 multinuclear Fourier transform nuclear magnetic resonance spectrometer. Spectra, 50 kHz wide, were obtained using the quadrupole echo sequence and 100-ms repetition rates. 2048 data points were collected and normally 30000 scans were obtained.

Results and Discussion

The ^{2}H-NMR spectrum of pure dioleoylphosphatidylcholine, specifically deuterated at positions 9 and 10, in a multilamellar dispersion appears in Fig. 1. This spectrum is the same as that presented previously for this deuterated phospholipid [11]. Three distinguishable splittings are observed. The outer is for the deuterium at position 9. The middle splitting arises from the deuterium at position 10 on the 1 chain. The smallest

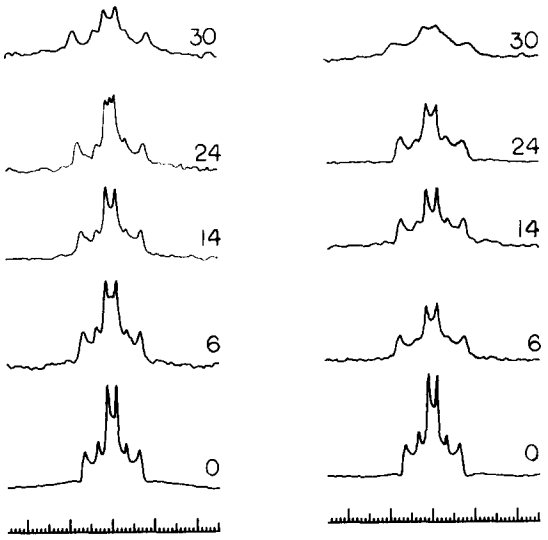

Fig. 1. ^{2}H-NMR spectra at 41.4 MHz of dioleoylphosphatidylcholine labelled at positions 9 and 10 with deuterium on both chains, in deuterium-depleted water. 30000 scans were obtained as described in the text. Spectra on the left represent the effects of added cholesterol in the membrane, at the indicated mole percent. Spectra on the right represent the effects of added lanosterol in the membrane, at the indicated mole percent. Spectra were obtained at 20°C. The smallest division on the scale is 1 kHz.

splitting arises from the deuterium at position 10 on the 2 chain.

These deuterium labels are located in the center of the phospholipid bilayer in an excellent position to report on any effects of sterols. Therefore, Fig. 1 shows the effects of adding cholesterol and lanosterol to these phospholipid bilayers. The two sterols have a different effect on the ^{2}H-NMR resonance of the labelled phospholipids. Fig. 2 shows the quadrupole splittings corresponding to each deuterium-labelled position plotted as a function of cholesterol concentration. The splitting arising from the deuterium at position 9 shows a steady increase in splitting as cholesterol in the membrane is increased. Neither resonance from the deuteriums at position 10 of either hydrocarbon chain of the phospholipids shows much change in splitting as a function of cholesterol concentration.

In contrast, lanosterol has virtually no effect on any of the quadrupole splittings from position 10 or 9, as shown in Fig. 2. Furthermore, temperature studies of lanosterol-containing membranes show small but uniform changes in splitting with temperature (see Fig. 3). These are very similar to the temperature dependence of the quadrupole splittings for the pure phospholipid in bilayers (data not shown).

The interpretation of these data rests upon a previous analysis of the origin of the quadrupole splittings from the region of the 9,10 double bond of oleic acid. Position 10 gives an anomalously small deuterium quadrupole splitting, compared to the quadrupole splittings derived from deuteriums at other positions in the interior of the hydrocarbon chain, removed from the double bond. It

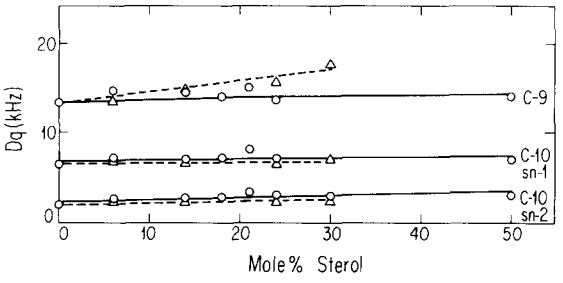

Fig. 2. Plot of the quadrupole splittings, D_q, from Fig. 1 for each labelled position, as a function of mole percent sterol. △, Cholesterol; ○, lanosterol.

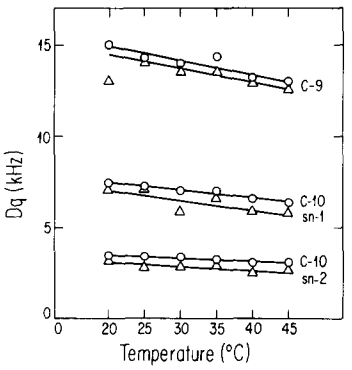

Fig. 3. Plot of the quadrupole splittings, D_q; for 21 mol% lanosterol (○) and for 50 mol% lanosterol (△), as a function of temperature.

has been clearly shown that this is due to a geometric factor. The quadrupole splittings can be scaled by the factor $(3\cos^2\theta - 1)$, where the angle θ is between the C-D vector of interest and the major axis of rotation. Thus, when θ approaches 57°, the scale factor becomes small and the deuterium quadrupole splitting is consequently reduced. This is independent of any analysis of the order parameter pertaining to the C-D bond.

Thus, in the case of the deuterium at position 10 of oleic acid, the small deuterium quadrupole splitting has been shown to be due to this geometric factor, and not to a disordering effect of the double bond [12]. Therefore, the quadrupole splitting of this deuterium primarily reflects the average angle between the C-D bond and the long axis of the chain. Because the orientation is near the magic angle, this quadrupole splitting is very sensitive to the average orientation. The fact that there is virtually no change with lanosterol indicates that the 14-methyl group is not perturbing the phospholipid hydrocarbon chain orientation at the position of the deuterium labelling, even though the labelling is near the putative location of the 14-methyl group of lanosterol in the phospholipid bilayer. In contrast, the quadrupole splittings from position 10 increase a small amount at 50 mol% cholesterol [13]. This indicates a difference between the effects of lanosterol and cholesterol on the phospholipid hydrocarbon chains, a difference which is more evident at position 9.

The resonance from position 9 contains a more important contribution from the order parameter

characterizing the C-D bond segment. These results can be interpreted in terms of an increase in the average order parameter for that position caused by inclusion of cholesterol in the membrane. This has been noted previously for other labelled positions and cholesterol [4]. In addition, it was noted elsewhere that at 50 mol% cholesterol, the splitting from the C-9 position increased to 24.5 kHz [13]. A linear extrapolation of our data agrees well with that result. Lanosterol apparently has no effect on the quadrupole splitting from position 9 even at 50 mol% sterol. This may well result from the 14-methyl, perhaps reflecting the poorer packing of lanosterol in the phospholipid bilayer compared to cholesterol [8].

In general, the observation of differences between the effects of lanosterol and cholesterol on the lipid hydrocarbon chains is in agreement with a recent spin-label study [9]. However, the details differ. In the spin-label study, a large perturbing effect of lanosterol was observed in the region where the 14-methyl is located [9]. This difference may result from steric interactions between the bulky spin label and the 14-methyl group. If there was a strong perturbing effect of the 14-methyl group on phospholipid structure in the membrane, the permeability of the membrane would be expected to increase. However, while lanosterol is less able to reduce the permeability of phospholipid bilayers, compared to cholesterol [8], it does not increase the permeability.

Acknowledgments

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