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Sterol Orientations in Phosphatidylcholine Liposomes As Determined by Deuterium NMR†

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Received June 26, 1985

ABSTRACT: Deuterium magnetic resonance spectra (55.26 MHz) of cholesterol-3α-d_1 and epicholesterol-3β-d_1 in dipalmitoylglycerophosphocholine (DPPC) liposomes were measured as a function of sterol-to-phospholipid ratio below (24 °C) and above (60 °C) the phase transition temperature of DPPC. From the quadrupolar splittings $\Delta\nu_q$, the molecular order parameters S describing the motions of the sterols in the bilayer were calculated, and the most probable angle of tilt α_0 of the molecular axis of the sterols relative to the bilayer normal was determined. We observed that the molecular axis of cholesterol in DPPC liposomes at both 24 and 60 °C is tilted at an angle of 16–19° with the 3β-hydroxyl group projecting parallel to the bilayer normal into the aqueous interface. In contrast, at 24 °C, epicholesterol is aligned parallel (0°) to the bilayer normal, placing the 3α-hydroxyl group essentially perpendicular to the bilayer normal along the aqueous interface. At 60 °C, the average angle of epicholesterol (16–18°) is similar to that of cholesterol, which can project the 3α-hydroxyl group into the hydrophobic bilayer region. On the basis of the observed tilt angles of the two isomeric sterols in DPPC liposomes, a *model* is proposed that can rationalize the differential effects of cholesterol and epicholesterol on membrane properties. The near-parallel alignment, at both 24 and 60 °C, of cholesterol relative to the fatty acyl chains in phospholipid bilayers (16–25°), with the sterol 3β-hydroxyl group projecting into the aqueous interface, would promote strong lipophilic interactions as well as hydration of the hydroxyl function. The nonparallel arrangement, at 24 °C, of epicholesterol and the fatty acyl chains, again assuming an acyl tilt of 16–25°, would reduce lipophilic interactions as compared to those observed for cholesterol, while the hydroxyl function would remain relatively accessible to hydration. At 60 °C, even though epicholesterol would be oriented nearly parallel to the acyl chains, effective lipophilic interactions as well as hydration would be hindered by the 3α-hydroxyl group rotating into the hydrophobic bilayer region. The differential sterol effects of cholesterol and epicholesterol are discussed in light of the model proposed.

Considerable research has focused on the effect of cholesterol on structural and dynamic membrane properties. However, there is still no satisfactory explanation for the unique role of cholesterol in modulating natural and model membranes [for a review, see Presti et al. (1982)]. By comparison, the α-hydroxyl isomeric epicholesterol exerts diminished effects on phospholipid membranes, reflecting greatly reduced sterol–phospholipid interactions (Demel et al., 1972a,b; Bittman & Blau, 1972; Silbert, 1975; Clejan et al., 1979).

Chart I

1 CHOLESTEROL-3α-d_1, R_1 = OH, R_2 = ^{2}H

2 EPICHOLESTEROL-3β-d_1, R_1 = ^{2}H, R_2 = OH

Attempts to rationalize the differential effects of the two isomeric sterols on the basis of stereospecific interactions between the sterol hydroxy function and specific phospholipid sites have not held up to experimental scrutiny. Thus, earlier studies had suggested (Darke et al., 1972; Verma & Wallach, 1973) that the β-hydroxyl group of cholesterol may specifically interact with the phosphatidylcholine (PC)[1] phosphate function in PC–cholesterol bilayers; yet, subsequent NMR studies have clearly ruled out this possibility (Keough et al., 1973; Stockton

† This study was supported in part by U.S. Public Health Service Research Grant HL 08214 from the Program Projects Branch, Extramural Programs, National Heart, Lung and Blood Institute, by NIH Training Grant HL 07311, by Research Grants CA 12150 and NS 14304, and by The Hormel Foundation. High-field ^{2}H NMR measurements were done at the NSF Midwest Regional Instrumentation Facility, University of Illinois, Urbana (E. Oldfield, Director), supported by Grant CHE 79-16100. Preliminary results of this study were presented at the 23rd Experimental NMR Conference, Madison, WI, April 1982.
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[1] Abbreviations: PC, phosphatidylcholine; DMPC, dimyristoylglycerophosphocholine; DPPC, dipalmitoylglycerophosphocholine; NMR, nuclear magnetic resonance; Me_4Si, tetramethylsilane; TLC, thin-layer chromatography; IR, infrared.

0006-2960/86/0425-1062$01.50/0

et al., 1974; Yeagle et al., 1975; Gally et al., 1976; Cullis et al., 1976; Brown & Seelig, 1978). Consideration has also been given to the possibility that the 3β-hydroxyl group of cholesterol may engage in specific hydrogen bonding with the carbonyl oxygen of the phospholipid acyl groups (Brockerhoff, 1974; Yeagle & Martin, 1976; Huang, 1976, 1977; Chatterjie & Brockerhoff, 1978; Presti et al., 1982) and that such hydrogen bonding would be diminished when the sterol hydroxyl is axially oriented with respect to the sterol ring system (Huang, 1976, 1977). Yet, de Kruyff et al. (1973) concluded from calorimetric and monolayer studies that it was unlikely that specific binding between the sterol hydroxyl group and polar phospholipid regions was essential, and this conclusion was consistent with the results of permeability studies on bilayers of cholesterol and phosphatidylcholine, including PC analogues lacking the ester functions, which clearly showed that the acyl carbonyls are not essential for cholesterol–phospholipid interaction (Clejan et al., 1979; Bittman et al., 1981, 1984). Thus, while a 3β-hydroxyl group is undoubtedly necessary for eliciting the typical cholesterol effects on membranes, a rationale for this stereochemical requirement is still lacking.

Deuterium NMR has previously been used to describe the rigid body motions of 3-deuterated cholesterol and to determine the most probable angle of tilt of cholesterol in dimyristoylglycerophosphocholine (DMPC) liposomes (Oldfield et al., 1978). The objective of the present study was to use ^{2}H NMR to determine the orientation of cholesterol-3α-d_1 (**1**) and epicholesterol-3β-d_1 (**2**) (see Chart I) in sterol/dipalmitoylglycerophosphocholine (DPPC) liposomes as a function of sterol-to-DPPC ratio and temperature. We hoped that a model would evolve from these studies that could help in explaining the differential membrane effects of the two stereoisomeric sterols.

Experimental Procedures

Materials and Methods. 1,2-Dipalmitoyl-*rac*-glycerophosphocholine (DPPC) was obtained from United States Biochemical Corp., Cleveland, OH. Mass spectra were recorded on a Dupont DP-102 mass spectrometer; infrared spectra were obtained on a Beckman IR 4240 spectrophotometer. Melting points were determined on a Reichert hot stage and are uncorrected.

Synthesis of Deuterated Sterols. Cholesterol was oxidized with Jones reagent (CrO_3–H_2SO_4 in acetone) to form cholest-5-en-3-one according to the method reported by Djerassi et al. (1956). Cholest-5-en-3-one was recrystallized from acetone and then reduced with sodium borodeuteride (Aldrich Chemical Co., Milwaukee, WI) in absolute ethanol. Cholesterol-3α-d_1 and epicholesterol-3β-d_1 were separated by repeated TLC on layers (0.3 mm) of silica gel H (Merck) with petroleum ether (bp 30–60 °C)–ethyl acetate (85:15 v/v) as developing solvent. For analytical purposes, sterol fractions were made visible by charring after spraying with chromic acid–sulfuric acid reagent; in preparative TLC, fractions were detected after exposure to iodine vapors. The pure sterols were characterized by their R_f values (TLC) and melting points and by IR, mass, and ^{13}C NMR spectra. Cholesterol-3α-d_1: R_f 0.17; mp 146–148 °C; IR (KBr) 3450 (br), 1465 (m) cm^{-1}; mass spectrum m/z 387:386 (92:8); ^{13}C NMR δ 141, 122, 56.8, 56.3, 50.2, 42.3, 39.9, 39.6, 37.3, 36.6, 36.3, 35.8, 32.0, 31.6, 28.3, 28.1, 24.3, 23.9, 22.8, 22.6, 21.1, 19.4, 18.8, and 11.9; the C(3) signal observed at 71.7 ppm for cholesterol was suppressed for deuteriocholesterol. Epicholesterol-3β-d_1: R_f 0.23; mp 139–141 °C; IR (KBr) 3390 (br), 1465 (m) cm^{-1}; mass spectrum m/z 387:386 (94:6); ^{13}C NMR δ 139, 124, 56.8, 56.2, 50.5, 42.4, 39.8, 37.4, 36.3, 35.8, 33.3, 32.0, 28.8, 28.2, 24.3, 23.9, 22.8, 22.6, 20.9, 18.7, and 11.9; the C(3) signal observed at 67.1 ppm for epicholesterol was suppressed for deuterioepicholesterol.

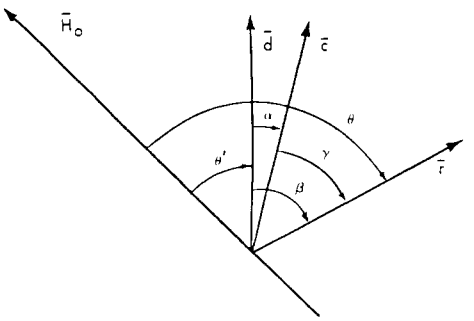

FIGURE 1: Vector and angle designations according to Petersen and Chan (1977). In this study, $\bar{\mathbf{H}}_0$ is the direction of the applied magnetic field, $\bar{\mathbf{r}}$ is the principal component of the electric field gradient, $\bar{\mathbf{d}}$ is the normal to the bilayer surface, and $\bar{\mathbf{c}}$ is the molecular axis of the sterol.

Preparation of Liposomes. A chloroform solution of the sterol and DPPC was dried in a stream of nitrogen, and then in vacuo for 24 h. Liposomes were prepared by vortexing in deuterium-depleted water (0.7 mL; Aldrich) at 25 °C until the preparation appeared homogeneous.

NMR Spectroscopy. Proton-decoupled ^{13}C NMR spectra were recorded at 20 MHz on a Varian FT-80A pulse Fourier transform instrument in $CDCl_3$ at ambient probe temperature (37 ± 1 °C) with Me_4Si serving as internal standard. ^{2}H quadrupole splittings ($\Delta\nu_q$) were measured on a Nicolet NT-360 (8.45 T) spectrometer at 55.26 MHz using the quadrupole echo pulse sequence.

Theory

In motionally restricted systems, deuterium exhibits a "powder pattern" and gives rise to quadrupolar splitting ($\Delta\nu_q$) of the NMR absorption line. The separation of the peaks is given by

$$\Delta\nu_q = \frac{3}{2}\frac{e^2Qq}{h}\frac{3\cos^2\theta - 1}{2} \quad (1)$$

where e^2Qq/h is the deuterium quadrupole coupling constant and θ is the angle between the magnetic field $\bar{\mathbf{H}}_0$ and the principal component of the electric field gradient tensor of the deuterium nucleus (Abragam, 1961). The extent of order in a motionally restricted system can be described with respect to a laboratory set of axes. If there is an effective axis of symmetry within the system, then the motions can be referred to this axis and expressed in terms of a single order parameter (Petersen & Chan, 1977). Thus, the motions of a deuteriosterol can be described by order parameters relative to the bilayer normal, which is the effective axis of symmetry in lipid bilayers (Oldfield et al., 1978). Figure 1 defines the relative disposition of the vectors used in the calculation of the order parameters. Vector $\bar{\mathbf{r}}$, the principal component of the electric field gradient, makes an angle θ relative to the applied magnetic field $\bar{\mathbf{H}}_0$, an angle β with the bilayer normal $\bar{\mathbf{d}}$, and an angle γ with the molecular axis $\bar{\mathbf{c}}$. The molecular axis $\bar{\mathbf{c}}$ is oriented at an angle α relative to $\bar{\mathbf{d}}$, and θ' is the angle between the magnetic field and $\bar{\mathbf{d}}$. Thus, from a measured quadrupole splitting, it is possible to calculate S_β, the order parameter of vector $\bar{\mathbf{r}}$ relative to director $\bar{\mathbf{d}}$, according to eq 1 and

$$\overline{(3\cos^2\theta - 1)} = \tfrac{1}{2}\overline{(3\cos^2\beta - 1)}(3\cos^2\theta' - 1) \quad (2)$$

$$\tfrac{1}{2}\overline{(3\cos^2\beta - 1)} = S_\beta \quad (3)$$

S_β can in turn be separated into a composite of the molecular order parameter S_α and the intramolecular order parameter S_γ:

$$S_\beta = \frac{\overline{(3\cos^2\beta - 1)}}{2} = \frac{\overline{(3\cos^2\alpha - 1)}}{2}\frac{\overline{(3\cos^2\gamma - 1)}}{2} = S_\alpha S_\gamma \quad (4)$$

Thus, from the values of S_β and S_γ, a value for S_α is obtained, and α, the inclination of the molecular axis $\bar{c}$ to the bilayer normal $\bar{d}$, can then be calculated. Assuming an axially symmetric Gaussian distribution, it is possible to obtain the most probable value of α, i.e., α_0. This average angular deviation of the molecular axis from the bilayer normal (α_0, tilt) can be obtained by numerical integration of the equation (Petersen & Chan, 1977):

$$S_\alpha = \frac{\frac{1}{2}\int_0^\pi \sin\alpha \exp(-\alpha^2/2\alpha_0^2)(3\cos^2\alpha - 1)\,d\alpha}{\int_0^\pi \sin\alpha \exp(-\alpha^2/2\alpha_0^2)\,d\alpha} \quad (5)$$

A computer program written in Basic was used for this integration. As the C–D bond vector in cholesterol cannot reorient due to the rigidity of the sterol ring system, the observed quadrupole splitting is caused by the tilt of the sterol nucleus in the bilayer. Furthermore, as the torsional oscillations of the C(2) and C(4) bonds are of smaller amplitude, the observation is valid over a wide range of temperatures.

Combining eq 1, 2, and 4 results in

$$\Delta\nu_q = \frac{3}{16}\frac{e^2Qq}{h}\overline{(3\cos^2\alpha - 1)}\,\overline{(3\cos^2\gamma - 1)}(3\cos^2\theta' - 1) \quad (6)$$

The peak separation for both cholesterol and epicholesterol can be calculated for the condition $\alpha = 0$, i.e., the molecular axis being aligned with the bilayer normal, by inserting the following values in eq 6: $e^2Qq/h = 170$ kHz (Derbyshire et al., 1969), $\theta' = 90°$, $\gamma = 80°$ for cholesterol (Taylor et al., 1981), and $\gamma = 23°$ for epicholesterol (calculated from the cholesterol value). Thus, for this orientation of the molecular axis, the peak separation would be 56.8 kHz for cholesterol and −98.3 kHz for epicholesterol. Any reduction in the magnitude of $\Delta\nu_q$ observed would then be due to motional averaging of the electric quadrupole interaction through rapid fluctuations in the tilt of the sterol molecule. It is important to note that the sign of the predicted quadrupole splitting for epicholesterol is negative. A calculation of $\Delta\nu_q$ for values of γ ranging from 0 to 90° indicates that the sign of the quadrupolar splitting changes at 54.73°, with higher γ values being positive and lower values being negative. Therefore, in our calculations the sign of the quadrupole splitting for epicholesterol-3β-d_1 is considered negative.[2]

Results

Deuterium-labeled cholesterol (**1**) and epicholesterol (**2**) were used as probes in dipalmitoylglycerophosphocholine (DPPC) liposomes to obtain from the observed ^{2}H quadrupolar splittings the most probable angles of tilt (α_0) of the sterol relative to the bilayer normal. α_0 values of the sterols were determined at various sterol-to-phospholipid ratios at 24 °C and at 60 °C. Because of the rigidity of the sterol ring system, the C–D bond vectors of the 3-deuterated compounds are representative of the orientation of the sterol molecule. The data were analyzed to assess the effect of the configuration of the sterol hydroxyl group, sterol concentration, and temperature on sterol orientation.

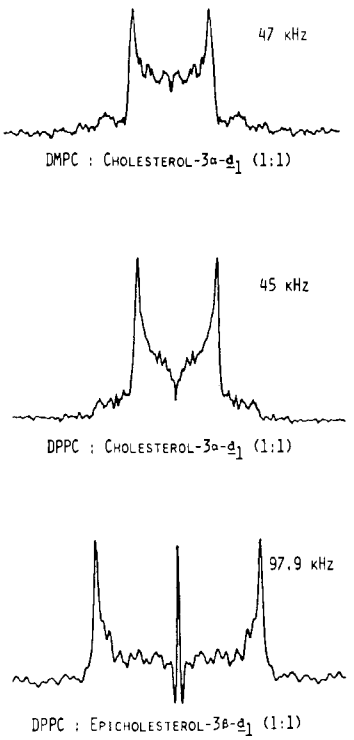

FIGURE 2: 55.26-MHz deuterium NMR spectra of deuterated sterol/phosphatidylcholine (1:1) liposomes measured at 24 °C. Quadrupole splittings ($\Delta\nu_q$) shown are those of DMPC/cholesterol-3α-d_1 (top, 47 kHz), DPPC/cholesterol-3α-d_1 (center, 45 kHz), and DPPC/epicholesterol-3β-d_1 (bottom, 97.9 kHz).

Table I: Quadrupole Splittings ($\Delta\nu_q$) and Order Parameters of Cholesterol-3α-d_1/DPPC Liposomes Measured at 55.26 MHz

DPPC:cholesterol ratio	temp (°C)	$\Delta\nu_q$ (kHz)	S_β	S_α	α_0 (deg)
8:2	24	44.0	−0.345	0.759	17
7:3	24	44.6	−0.350	0.769	16
1:1	24	45.0	−0.353	0.776	16
8:2	60	41.5	−0.325	0.714	19
7:3	60	43.8	−0.343	0.754	17
1:1	60	45.0	−0.353	0.776	16

Table II: Quadrupole Splittings ($\Delta\nu_q$) and Order Parameters of Epicholesterol-3β-d_1/DPPC Liposomes Measured at 55.26 MHz

DPPC:epicholesterol ratio	temp (°C)	$\Delta\nu_q$ (kHz)	S_β	S_α	α_0 (deg)
10:1	24	−96.9	0.760	0.986	0
7:3	24	−97.3	0.763	0.990	0
1:1	24	−97.9	0.768	0.996	0
7:3	60	−70.9	0.556	0.721	18
1:1	60	−75.0	0.588	0.762	16

Figure 2 (center and bottom) shows the typical powder pattern of the ^{2}H NMR spectra of cholesterol-3α-d_1 (45 kHz) and epicholesterol-3β-d_1 (97.9 kHz) in DPPC liposomes (1:1) at 24 °C. The $\Delta\nu_q$ value (47 kHz) of cholesterol-3α-d_1 in DMPC liposomes (Figure 2, top) was measured for comparative purposes and was found in good agreement with results (49.5 kHz at 23 °C) obtained by Oldfield et al. (1978) and others (Gally et al., 1976; Dahl, 1981; Taylor et al., 1981, 1982).

[2] The sign of the deuterium quadrupole splitting has generally been considered to be positive (Lucken, 1969). Thus, when dealing with nonspherically symmetrical molecules containing deuterium, caution must be taken in the calculation of order parameters from quadrupole splitting values.

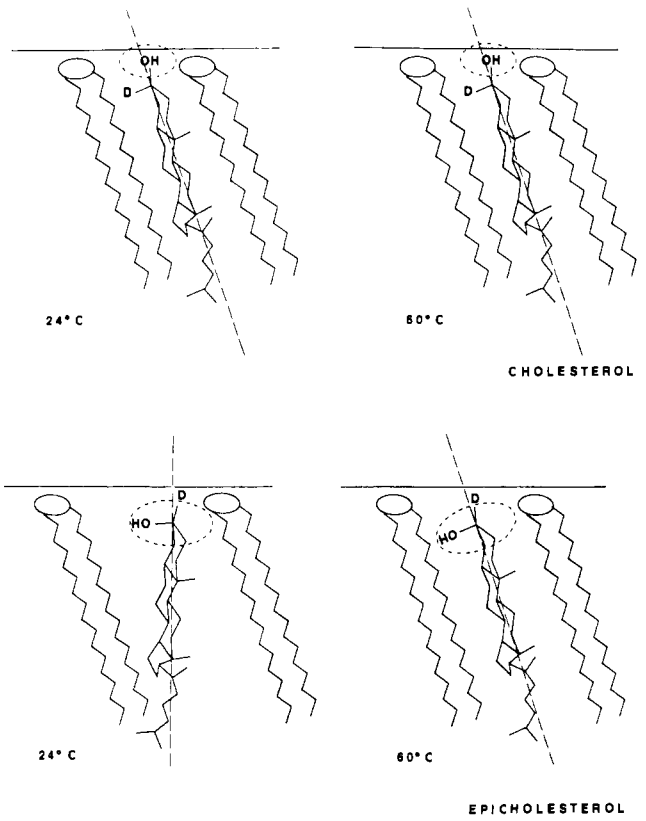

FIGURE 3: Orientation of cholesterol-3α-d_1 (top) and epicholesterol-3β-d_1 (bottom) in DPPC/sterol liposomes at 24 (left) and 60 °C (right). The orientation of the phospholipid fatty acyl chains shown is based on values measured on cholesterol/PC liposomes (Oldfield et al., 1978). The tilts of the sterols are those measured in the present study (Tables I and II).

Tables I and II list the ^2H quadrupole splittings ($\Delta\nu_q$) for cholesterol-3α-d_1 or epicholesterol-3β-d_1 containing DPPC liposomes, which we measured at 24 °C and at 60 °C at various DPPC-to-sterol ratios. The values of S_β, S_α, and α_0 given were calculated from the observed $\Delta\nu_q$ values as described under Theory by using eq 1–6.

For cholesterol-3α-d_1 (Table I), the quadrupole splittings measured below and above the phase transition temperature of pure DPPC are smaller than the value of 56.8 kHz that can be calculated for the condition $\alpha = 0$. As internal reorientation of the C–D vector with respect to the fused sterol ring system cannot occur, the smaller splitting is likely to be due to the tilt of cholesterol relative to the bilayer normal. At 24 °C, the quadrupole splittings observed for cholesterol-3α-d_1 (44.0–45.0 kHz) are quite independent of the sterol concentration, and so are the calculated angles of tilt (16–17°) of the molecular axis of cholesterol relative to the bilayer normal. At 60 °C, the quadrupole splittings of cholesterol-3α-d_1 (41.5–45.0 kHz) are somewhat concentration-dependent, and the tilt of the molecular axis (16–19°) decreases slightly with increasing cholesterol concentration. The tilt (16–19°) of cholesterol observed at 24 °C or at 60 °C projects the sterol hydroxyl group essentially parallel to the bilayer normal into the aqueous interface (Figure 3, top).

In contrast to the cholesterol/DPPC system, the ^2H quadrupole splittings measured on epicholesterol-3β-d_1/DPPC liposomes at 24 °C and at 60 °C were quite different (Table II). The $\Delta\nu_q$ values of epicholesterol-3β-d_1 at 24 °C were only slightly smaller than −98.3 kHz predicted for the condition $\alpha = 0$. At this temperature, the observed quadrupole splittings (96.9–97.9 kHz) and calculated angles of tilt (α_0) are quite independent of the DPPC-to-epicholesterol ratio. The data show that the molecular axis of epicholesterol in DPPC liposomes at 24 °C is aligned very close to the bilayer normal, resulting in an orientation of the sterol hydroxyl group essentially perpendicular to the bilayer normal along the aqueous interface (Figure 3, lower left). At 60 °C, the quadrupole splittings observed for epicholesterol (70.9–75.0 kHz) are somewhat concentration-dependent. This translates into α_0 values of 16–18°, thus approximating those found for cholesterol (16–19°) at this temperature. However, in contrast to cholesterol, this tilt of epicholesterol can project the polar 3α-hydroxyl group away from the aqueous interface and toward the hydrophobic bilayer region (Figure 3, lower right).

Discussion

Many attempts have been made to understand the relationship between the effect of a sterol on membrane properties and the sterol structure. Cholesterol with its planar steroid nucleus, its long nonpolar side chain at C(17), and its β-oriented hydroxyl group is known to cause reduced membrane permeability, broadened gel to liquid crystalline phase transition, and reduced average molecular surface areas, i.e., a maximum condensing effect (de Bernard, 1958; Demel et al., 1967; Rand & Luzzati, 1968; Joos & Demel, 1969; Demel & de Kruyff, 1976; Lala et al., 1978; Clejan et al., 1979; Suckling et al., 1979). In contrast, 3α-hydroxy sterols, such as epicholesterol, exert diminished effects on average molecular surface areas and on membrane permeability (Demel et al., 1972a,b; Bittman & Blau, 1972; Silbert, 1975; Clejan et al., 1979). Neutron and X-ray diffraction studies (Rand & Luzzati, 1968; Franks, 1976; Worcester & Franks, 1976) had earlier shown that the sterol hydroxyl group in cholesterol/PC bilayers is located near the water interface and close to the phospholipid ester functions. These observations lent support to postulated modes of cholesterol interaction that involved specific interaction of the sterol β-hydroxyls with the phospholipid phosphate groups and with the carbonyl oxygens of the phospholipid acyl functions (hydrogen bonding). However, later studies convincingly proved that the ester phospholipid carboxy functions are not essential sites for cholesterol interaction (Clejan et al., 1979; Bittman et al., 1981) and that a specific interaction between cholesterol and the phospholipid phosphate group is not required to bring about the typical cholesterol effects (Keough et al., 1973; Stockton et al., 1974; Yeagle et al., 1975; Gally et al., 1976; Cullis et al., 1976; Brown & Seelig, 1978). Cholesterol rather seems to act as a spacer molecule to increase the separation between the phosphocholine head groups (Yeagle et al., 1977; Oldfield et al., 1978; Yeagle, 1978) and to rigidify the long aliphatic chains in order to maximize lipophilic interactions without disturbing the bilayer structure (Suckling et al., 1979). Thus, it is quite clear now (i) that the interaction of cholesterol in phospholipid bilayers causes changes in membrane permeability properties, restrictions in motional freedom of the fatty acyl chains, and a condensing effect distinctly different from the effects produced by epicholesterol and (ii) that specific hydrogen bonding between the sterol 3-hydroxyl group and specific phospholipid polar regions is not essential to bring about these effects. However, a rationale for the structural and steric requirements of the cholesterol effect is still lacking.

In this study, we have observed a unique difference between the orientation of cholesterol and epicholesterol in phospholipid bilayers, which must have a significant bearing on sterol–phospholipid interactions and membrane properties (see Tables I and II and Figure 3). On the basis of the observed tilt angles of the two isomeric sterols in DPPC liposomes, we propose a *model* that can rationalize the differential membrane effects

of cholesterol and epicholesterol. At temperatures below (24 °C) and above (60 °C) the phase transition temperature of pure DPPC, cholesterol is inclined at an angle of 16–19°, which is close to the reported tilt (16–25°) of the phospholipid fatty acyl chains in bilayer membranes (Oldfield et al., 1978; Pearson & Pascher, 1979; Jähnig et al., 1979). This near-parallel alignment promotes strong lipophilic interactions between the sterol and the aliphatic chains. It also projects the cholesterol hydroxyl group into the aqueous interface, thus facilitating its hydration (Figure 3, top). This view of cholesterol–phospholipid interaction is quite consistent with earlier observations (Stockton et al., 1974; Gally et al., 1976; Oldfield et al., 1978; Dahl, 1981). However, in the case of epicholesterol/DPPC liposomes at 24 °C, the sterol molecule is oriented parallel to the bilayer normal (0°). If one assumes that the phospholipid fatty acyl chains would attempt to preserve their tilt of 16–25° (Figure 3) in the presence of epicholesterol, as they do in the presence of cholesterol (Oldfield et al., 1978), this would place epicholesterol nonparallel to the acyl chains (Figure 3, lower left). Obviously, this nonparallel orientation would reduce lipophilic interactions. At 60 °C, the tilt of epicholesterol (16–18°) would become similar to that of the phospholipid acyl chains (Figure 3, lower right), which would be expected to increase lipophilic interactions. Yet, this change in tilt places the 3α-hydroxyl group deeper into the hydrophobic region. Even though epicholesterol is now oriented nearly parallel to the acyl chains, effective lipophilic interaction is hindered because the sterol 3α-hydroxyl group rotates into the hydrophobic bilayer region. Differences observed in anisotropy and rate of sterol motions between cholesterol and epicholesterol in PC vesicles (Brainard & Cordes, 1981) are consistent with this model.[3]

Conclusions

It appears that sterol orientation in phospholipid bilayers is affected (i) by the accessibility of the sterol hydroxyl function at the aqueous interface and its susceptibility to hydration and (ii) by the degree of lipophilic interaction between the phospholipid acyl chains and the respective sterol. In the case of cholesterol, hydration of the hydroxyl group coupled with maximum lipophilic interactions due to the near parallel alignment of the sterol ring system with the phospholipid acyl chains produces the optimal membrane effects. In contrast, with epicholesterol the lipophilic interactions are decreased at lower temperature (24 °C) due to differences in orientation between the sterol and the phospholipid acyl chains; at higher temperature (60 °C), lipophilic interactions are perturbed because the axially oriented hydroxyl group of epicholesterol protrudes into the hydrophobic bilayer region. This model is consistent with the known differential effects of cholesterol and epicholesterol on bilayer membrane properties.

Acknowledgments

We thank Professor Eric Oldfield for stimulating discussions and for instrument time on the Nicolet NT-360 NMR spectrometer. We are indebted to Dr. Barbara Malewicz for her help in the preparation of the manuscript and to Catherine A. Guy for technical assistance.

Registry No. DPPC, 2797-68-4; cholesterol-3α-d_1, 51467-57-3; epicholesterol-3β-d_1, 98936-76-6; cholesterol, 57-88-5; epicholesterol, 474-77-1; cholest-5-en-3-one, 601-54-7.

[3] Recent studies by Dufourc et al. (1984) have also shown that sterol orientation can be a key factor in determining membrane properties. The $\Delta\nu_Q$ value of about 97.5 kHz, which we obtained for epicholesterol-3β-d_1/DPPC at 24 °C, approximates the value (100 kHz) predicted by Dufourc et al. for the "second solution".

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Substrate and Inhibitor Activities of the Screw Sense Isomers of Metal–Nucleotide Complexes in the Formyltetrahydrofolate Synthetase Reaction†

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Received August 13, 1985

ABSTRACT: Phosphorothioate analogues of ATP and isomers of CrATP and CrADP were used to examine the nucleotide stereoselectivity of formyltetrahydrofolate synthetase from procaryotic and eucaryotic sources. Substrate activity of the thio-ATP analogues increased as the site of sulfur substitution was changed from the γ to the α position. Thus, adenine nucleotide analogues substituted with sulfur at an α nonbridging position (ATPαS isomers) were the most active, and ATPγS was inactive. When Mg^{2+} was used as the divalent cation, both enzymes showed a clear preference (higher V/K_m value) for the S_P isomer of ATPβS although the magnitude of the preference was greater with the bacterial enzyme. With Cd^{2+} as the divalent cation the R_P isomer was preferred, but the difference was greater with the yeast enzyme. Both (S_P)-MgATPβS and (R_P)-CdATPβS have the Δ or right-hand screw sense configuration of the metal chelate ring. The reversal of stereoselectivity when the cation was changed indicates that the metal ion is coordinated to the β-phosphate group. No stereoselectivity was observed when ATPαS isomers were used in the presence of Mg^{2+} or Cd^{2+}, suggesting that the metals are not coordinated to the α-phosphate. ATPβS was also found to be a competitive inhibitor of MgATP and CdATP, and the lowest K_i values were obtained with the Λ screw sense isomers. The screw sense isomers of bidentate CrATP exhibited no detectable substrate activity but were competitive inhibitors of MgATP. The left-hand (Λ) screw sense isomer of bidentate CrATP and bidentate CrADP bound with greater affinity (lower K_i values) to both enzymes than did the Δ isomer. Although the Λ metal–ATP isomers bind with equal or better affinity than the Δ isomers, they are not as effective as substrates, indicating that geometric constraints imposed by the enzyme are different for binding and catalysis. The bacterial and yeast enzymes have similar stereochemical requirements for the metal nucleotide substrate but apparently differ in the degree of stereoselectivity.

Stereochemical and structural aspects of metal–nucleotide–protein interactions have been extensively studied for a number of enzymes with the use of phosphorothioate analogues of the nucleotide (Eckstein, 1983; Cohn, 1982) and inert chromium– and cobalt–nucleotide complexes (Dunaway-Mariano & Cleland, 1980b; Cleland, 1982). Substitution of a nonbridging oxygen by a sulfur atom in the α- and β-phosphoryl groups generates chirality at the phosphorus center, giving rise to a pair of diastereomers, R_P and S_P. Absolute configurations have been established for ATPαS[1] (Burgers et al., 1979; Burgers & Eckstein, 1978) and ATPβS (Jaffe & Cohn, 1978b). Coordination of the β- and γ-phosphates in the bidentate complex of CrATP creates a chiral center at the β-phosphorus, and two screw sense isomers[2] exist, Λ, or left-handed, and Δ, or right-handed (Cleland, 1982) (Figure 1). In addition, two isomers each of the Λ and Δ forms exist. These have been postulated to result from strong hydrogen bonding between the H_2O ligands and the pseudoaxial oxygen on the γ-phosphate and another H_2O and either the α- or

† This investigation was supported by National Institutes of Health Research Grant AM 07140 to R.H.H.
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[1] Abbreviations: H_4folate, tetrahydrofolate; 10-CHO-H_4folate, N^{10}-formyltetrahydrofolate; ATPαS, ATPβS, ATPγS, ADPαS, and ADPβS, adenine nucleotide analogues substituted with sulfur on the indicated phosphate in the nonbridging position; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl; PEI, poly(ethylenimine); Tris, tris(hydroxymethyl)aminomethane; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TLC, thin-layer chromatography; HPLC, high-pressure liquid chromatography; EPR, electron paramagnetic resonance.

[2] The screw sense nomenclature is that of Cornelius and Cleland (1978).

0006-2960/86/0425-1067$01.50/0