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Influence of stigmastanol and stigmastanyl-phosphorylcholine, two plasma cholesterol lowering substances, on synthetic phospholipid membranes. A ^{2}H- and ^{31}P-NMR study

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Cholesterol, stigmastanol, and stigmastanyl-phosphorylcholine (ST-PC) were incorporated into model membranes composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) or 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC). POPC and ST-PC were deuterated at the lipid headgroup, DOPC at the *cis*-double bonds. The influence of the three sterols on the motion and conformation of the lipid headgroups and the hydrocarbon chains was monitored with ^{2}H- and ^{31}P-NMR. All three sterols were freely miscible with the lipid matrix in concentrations of up to 50 mol% without inducing phase separations or nonbilayer structures. However, the molecules exert quite different effects on the phospholipid bilayer. Cholesterol and stigmastanol are largely buried in the hydrocarbon part of the membrane, distinctly restricting the flexing motions of the fatty acyl chains whereas the conformation of the phospholipid headgroups is little affected. In contrast, ST-PC is anchored with its headgroup in the layer of phospholipid dipoles, preventing an extensive penetration of the sterol ring into the hydrocarbon layer. Hence ST-PC has almost no effect on the hydrocarbon chains but induces a characteristic conformational change of the phospholipid headgroups. The ^{2}H- and ^{31}P-NMR spectra of mixed phospholipid/ST-PC membranes further demonstrate that the PC headgroup of ST-PC has a similar orientation as the surrounding phosphatidylcholine headgroups. For both types of molecules the $^-$P-N$^+$ dipole is essentially parallel to the membrane surface. Addition of ST-PC induces a small rotation of the POPC headgroup towards the water phase.

Introduction

Cholesterol plays an important role in the mammalian organism, be it as a building stone of membranes, or be it via its involvement in different hormonal and other metabolic pathways.

Cholesterol is supplied to the human body either by biosynthesis, mostly taking place in the liver, or by uptake from the gastrointestinal tract. Medical intervention to diminish the blood cholesterol level in the case of hypercholesterolemia can be attempted by inhibition of de novo synthesis as well as by decreased absorption of biliary and dietary cholesterol from the gastrointestinal tract. Although presently they seem to be quite safe, long-term administration of drugs which interfere directly with cholesterol synthesis in the liver were expected to cause problems, and efforts have therefore been directed towards the development of extra-systemic drugs which decrease the blood cholesterol level by inhibiting intestinal cholesterol uptake.

Several plant sterols, e.g. β-sitosterol, were found to inhibit intestinal cholesterol uptake and to lower plasma cholesterol more or less efficiently [1,2]. β-Sitosterol has the disadvantage that relatively high doses must be prescribed and that β-sitosterol itself is absorbed to some extent (3–4%) by the organism [3]. stigmastanol (β-sitostanol), the fully hydrogenated ana-

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Abbreviations: ^{2}H-NMR, deuterium nuclear magnetic resonance; ^{31}P-NMR, phosphorus nuclear magnetic resonance; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; MLV, multilamellar vesicles; ST-PC, stigmastanyl-phosphorylcholine=*O*-[hydroxy(5α-stigmastan-3β-yloxy)-phosphinyl]choline hydroxide inner salt.

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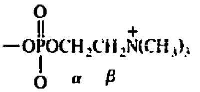
log of sitosterol, seems to be much less absorbed [4], to be excreted more rapidly [5], and to be more effective in lowering plasma cholesterol concentrations [4,6].

Recently the sterol-phospholipid hybrid stigmastanyl-phosphorylcholine (ST-PC; systemic name: *O*-[hydroxy(5α-stigmastan-3β-yloxy)phosphinyl]choline hydroxide inner salt), in which the β-stigmastanol hydroxyl group is esterified with phosphorylcholine [7], was proposed as a cholesterol-lowering drug. In a number of animal species ST-PC could reduce the intestinal cholesterol absorption by up to 90%. The chemical structures of cholesterol, stigmastanol, and stigmastanyl-phosphorylcholine are summarized in Structures below.

The precise mechanism by which stigmastanol and stigmastanyl-phosphorylcholine inhibit cholesterol absorption is as yet unknown but probably involves structural changes in the micelles/vesicles involved in cholesterol absorption. In order to obtain more information, we have studied the interaction of the two compounds with model bilayer systems. We have used multilamellar vesicles (MLV) composed of either 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) or 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC). POPC is the most abundant natural lipid in egg-yolk lecithin (≈ 70 wt% POPC). The gel-to-liquid crystal phase transition temperature is −5°C for POPC and −22°C for DOPC. While the presence of the first *cis*-double bond induces a considerable increase of about 10 $Å^2$ in the surface area, as compared to the fully saturated lipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, only minor effects are noted upon the incorporation of further double bonds [8]. POPC and DOPC have also similar bilayer properties, and the reason of studying both membranes simultaneously was the availability of different selectively deuterated analogues of POPC and DOPC. POPC and ST-PC were selectively deuterated at both methylene segments of the choline moiety and served as probes to monitor the headgroup region of the membrane. DOPC was selectively deuterated at the *cis*-double bonds of both oleic acid chains, and the ^{2}H-NMR spectra consequently provided insight into the hydrocarbon layer of the membrane. For both membrane systems the content of stigmastanol and ST-PC was continuously varied in the range of 0–50 mol%. The data were further compared with those obtained for membranes containing different concentrations of cholesterol.

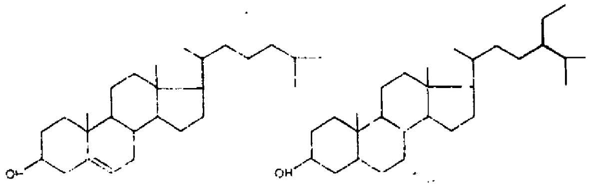
Materials and Methods

To simplify the discussion, the following nomenclature is used for the location of the deuterium labels at the different choline methylene segments:

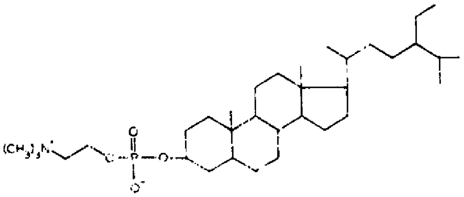


$$\begin{array}{l}\mathrm{O}\\ \| \\ -\mathrm{O\overset{}{P}OCH_2CH_2\overset{+}{N}(CH_3)_3}\\ |\\ \mathrm{O}\quad\alpha\quad\beta\end{array}$$

The α- and β-methylene groups of POPC were selectively deuterated as described by Harbison and Griffin [9]. The C9 and C10 segment (*cis*-double bond) of DOPC (both chains) was deuterated according to Seelig and Waespe-Sarcevic [10]. Cholesterol was purchased from Fluka AG, Switzerland and used without further purification. β-Stigmastanol was prepared by catalytic hydrogenation of stigmasterol (Fluka) and the structure was confirmed by high resolution nmr. ST-PC, unlabeled or deuterated at the α- and β-position was synthesized as follows: 2 g (4.8 mmol) stigmastanol was reacted in 40 ml methylene chloride containing 0.8 ml chinoline with 0.6 ml (6.4 mmol) phosphoroxychloride at 20 C for 3 h. To this solution was added 2 g (8.6 mmol) choline iodide (unlabeled or dideuterated at the



Cholesterol Stigmastanol



Stigmastanyl-phosphorylcholine

Structures

α- or β-position) and 1 ml pyridine. After 5 days, this reaction mixture was reacted with 2 g sodium bicarbonate in 10 ml water, and extracted with a mixture of 60 ml chloroform, 80 ml methanol and 100 ml water. The organic phase was washed with 100 ml 1 M HCl, then with 100 ml water and evaporated to dryness. The residue was dissolved in 60 ml chloroform/methanol (1:1, v/v), and treated with 50 g amberlite MB3 for one day. After filtration, the solution was evaporated and the residue crystallised from a mixture of chloroform-methanol-dioxane, giving 0.32 g (yield 11%) of the target compounds.

Sample preparation. All measurements were carried out in buffer (10 mM Tris, 100 mM NaCl at pH 7.4). In the case of ^{2}H-NMR measurements, the buffer was prepared with deuterium depleted water in order to minimize the isotropic signal due to natural abundance of ^{2}H in water.

The dry lipids and sterols were dissolved in appropriate amounts of $CHCl_3$/MeOH (65:30, v/v) and mixed. The solvent was removed under a stream of nitrogen and the sample was dried under high vacuum over P_2O_5 for several hours. Buffer was added to yield a final lipid/buffer ratio of 1:2 (wt%:vol%). After sealing the sample tube, the mixture was dispersed by extensive vortexing.

NMR-techniques. All spectra were recorded on a Bruker-Spectrospin MSL 400 spectrometer operating at a frequency of 61.4 MHz for ^{2}H-NMR and 162 MHz for ^{31}P-NMR. For the deuterium NMR measurements the quadrupole echo technique [11] was employed with full phase cycling. The 90°-pulse was in the range of 3.5 to 4 μs, the interpulse delay was $\approx$ 42–45 μs and the recycle delay about 250–400 μs. The spectral width was 50 kHz for headgroup studies and 125 kHz for studies of the hydrocarbon region.

The ^{31}P-spectra were recorded using the Hahn-echo sequence with gated proton decoupling and phase cycling [12]. The 90°-pulse was 2.7 μs, the echo spacing $\approx$ 40 to 45 μs, the recycle delay 1 to 5 s, and the spectral width 50 kHz.

The chemical shielding anisotropy was measured between the edges of the spectrum at half height of the low-field shoulder. The error was estimated to be ± 1 ppm.

All measurements were performed at 305 K.

Results

Cholesterol and stigmastanol yield qualitatively and quantitatively similar results. The lipid-sterol hybrid ST-PC behaves differently from the pure sterols and its effect on the hydrocarbon chains and the lipid head groups will thus be presented separately.

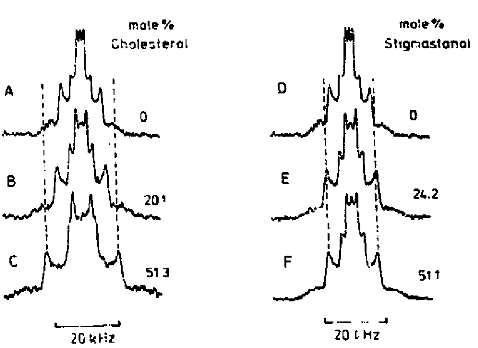


Fig. 1. Effect of cholesterol and stigmastanol on the hydrocarbon chains. ^{2}H-NMR spectra of [9',10'-$^2H_2$]DOPC. Pure DOPC membranes: (A) and (D). Mixtures with cholesterol: (B) 20.1 mol% and (C) 51.3 mol%. Mixtures with stigmastanol (E) 24.2 mol% and (F) 51.1 mol%.

Stigmastanol and cholesterol. Hydrocarbon chain region

The behavior of the fatty acid residues of DOPC upon addition of cholesterol or stigmastanol was monitored with ^{2}H-NMR spectroscopy. Both chains of DOPC were selectively deuterated at the C-9 and C-10 positions, i.e. at the *cis*-double bond. The membranes were in the liquid-crystalline bilayer phase at the measuring temperature of 305 K. In pure DOPC membranes three different quadrupole splittings can be distinguished with separations of 12.5 kHz, 6.0 kHz, and 2.1 kHz, respectively (Fig. 1A). The assignment of these resonances is as follows [13]: The two C-9 deuterons give rise to identical splittings with a value of 12.5 kHz, the two smaller splittings stem from the C-10 deuterons, indicating slightly different orientations of the two *cis*-double bonds in the DOPC membrane. Upon incorporation of cholesterol all three splittings increase considerably (cf. Figs. 1B and 1C). At a cholesterol content of about 50 mol%, the three splittings have almost doubled (cf. Table I). This result is in agreement with the well-known stiffening effect of cholesterol on lipid bilayers (cf. Refs. 14 and 30). The presence of the rigid sterol ring decreases the flexing motions of the neighbouring hydrocarbon chains and produces a more extended trans-conformation. At the highest cholesterol concentration measured ($\approx$ 50 mol% cholesterol) the fatty acyl chains adopt an almost rigid all-*trans* conformation [15].

Mixtures of stigmastanol with DOPC bilayers show similar effects as all three quadrupole splittings increase with increasing stigmastanol concentration up to 25 mol% (Figs. 1D, E). At higher stigmastanol levels no further increase can be observed (Figs. 1E, F). This is demonstrated more clearly in Fig. 2 for the C-9 deuterons of the DOPC membrane. For cholesterol, the C-9 splitting varies linearly with the cholesterol concentration up to 50 mol% cholesterol. Addition of

TABLE I

Quadrupole splittings Δν (kHz) of $[9',10'\text{-}^2H_2]$DOPC in mixtures with cholesterol, stigmastanol, and ST-PC, respectively, measured in buffer at 305 K

	Sterol concn. (mol%)	Quadrupole splitting (kHz)		
		$\Delta\nu_1$	$\Delta\nu_2$	$\Delta\nu_3$
Pure $[9',10'\text{-}^2H_2]$DOPC	0	2.1	6.0	12.5
Cholesterol	10.4	2.7	6.7	13.9
	20.1	3.5	7.1	15.5
	31.9	4.4	8.2	18.3
	51.3	5.8	8.9	22.8
Stigmastanol	10.1	2.3	6.4	13.6
	17.4	2.9	7.0	14.7
	24.2	3.4	7.4	15.5
	28.5	3.4	7.3	15.5
	42.7	3.4	7.2	15.6
	51.1	3.1	7.0	15.4
ST-PC	8.8	1.8	6.1	12.7
	21.8	1.5	6.0	13.0
	46.3	1.1 *	5.0	13.1
	50.8	1.1 *	5.5	13.1

* Single peak, width at half-height.

stigmastanol induces a slightly smaller variation of cholesterol up to a concentration of 25 mol%. Above this concentration stigmastanol has no further effect on the hydrocarbon chains.

Stigmastanol and cholesterol. Phospholipid headgroup region

^2H- and ^{31}P-NMR studies were performed with POPC deuterated at the α-and β-choline segments, again varying the sterol content of the membrane. Typical ^2H-NMR results obtained with stigmastanol are summarized in Fig. 3. The α-splitting of the choline moiety remains practically constant, the β-splitting decreases linearly with increasing stigmastanol concentration. The measurement of the phosphorus chemical shielding anisotropy reveals a small increase from −49.5 ppm for pure POPC to −48 ppm for POPC with 50% stigmastanol. Taken together, these results demonstrate only a small influence of stigmastanol on the phosphocholine headgroup which is in distinct contrast to its effect on the hydrocarbon chains. As an aside it may be noted that the β-quadrupole splitting exhibits no plateau value at 25% stigmastanol (as observed for the hydrocarbon chains) but decreases continuously up to 50 mol% stigmastanol.

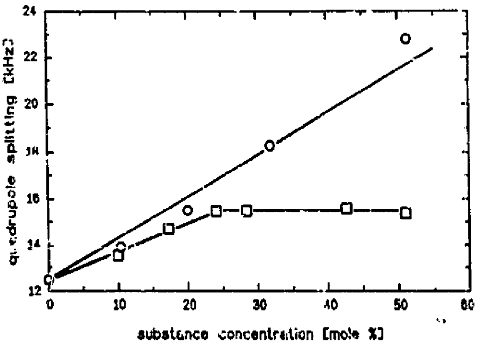


Fig. 2. Effect of cholesterol and stigmastanol on the hydrocarbon chains. Comparison of the outer quadrupole splittings in membranes composed of $[9',10'\text{-}^2H_2]$DOPC/cholesterol and $[9',10'\text{-}^2H]$DOPC/stigmastanol. Above 25 mol% stigmastanol the quadrupole splittings level off. □, Stigmastanol; ○, cholesterol.

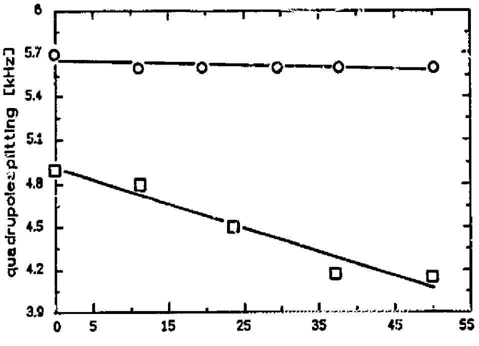


Fig. 3. Influence of stigmastanol on the α- and β-deuterated methylene segment of the phosphocholine headgroup of POPC membranes. Variation of the ^2H-NMR quadrupole splittings of the choline headgroup segments with sterol concentration. □, β-segment; ○, α-segment.

The influence of cholesterol on the POPC headgroup has been studied before [16,17] and is qualitatively similar to that of stigmastanol, i.e. the presence of cholesterol has only a small effect on the motion and the orientation of the phospholipid headgroup.

Stigmastanyl-phosphorylcholine. Hydrocarbon chain region

ST-PC exhibits quite different properties in mixture with POPC and DOPC bilayers. For the hydrocarbon chain region this is most obvious by inspection of Table I which demonstrates that ST-PC has only a minor effect on the fatty acyl chains. The C-9 quadrupole splitting of the DOPC bilayer increases by at most 400 Hz, those of the two C-10 deuterons even decrease slightly. A comparison of ST-PC with the other sterols is given in Fig. 4 demonstrating that the hydrocarbon chain stiffening effect decreases in the order cholesterol > stigmastanol ≫ ST-PC.

Stigmastanyl phosphorylcholine. Headgroup region

ST-PC has a distinct effect on the orientation of the $^-P\text{-}N^+$ dipole in POPC membranes as is demonstrated

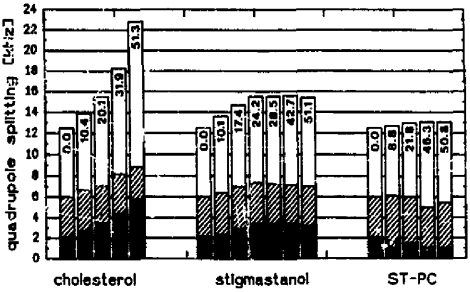


Fig. 4. Variation of the three quadrupole splittings of [9′,10′-$^2H_2$]DOPC when mixed with cholesterol, stigmastanol, and ST-PC, respectively. The mole fractions of the sterols are given in the figure.

by the ^{2}H-NMR spectra of Fig. 5. Addition of about 50 mol% ST-PC decreases the quadrupole splitting of the α-choline segment of POPC from 5.9 kHz to 3.9 kHz (−34% change) and increases the β-splitting from 4.9 kHz to 7.9 kHz (+61% change).

The quadrupole splittings vary approximately linear with the ST-PC concentration (Fig. 6) indicating a strictly correlated, counterdirectional variation of the two quadrupole splittings. Linear regression analysis of the data of Fig. 6 yields:

$$\Delta\nu_\alpha = 6.07 - 4.03 X_b \text{ (kHz)}$$

$$\Delta\nu_\beta = 4.7 + 6.01 X_b \text{ (kHz)}$$

where X_b (mole/mole) denotes the mole fraction of ST-PC in the membrane. Elimination of X_b leads to a linear relationship between $\Delta\nu_\alpha$ and $\Delta\nu_\beta$

$$\Delta\nu_\beta = 13.74 - 1.49\Delta\nu_\alpha$$

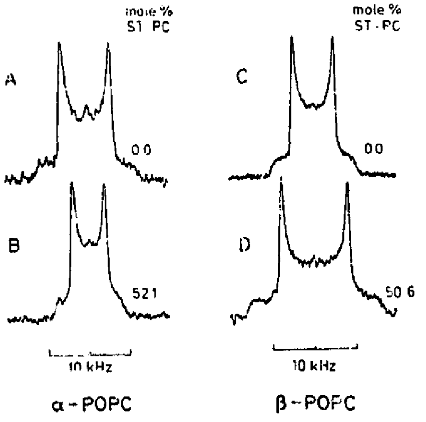


Fig. 5. ^{2}H-NMR spectra of the α- and β-headgroup segment of POPC in mixture with ST-PC. The spectra (A) and (C) represent the pure POPC membrane spectra, (B) and (D) correspond to the membranes with about 50 mol% ST-PC.

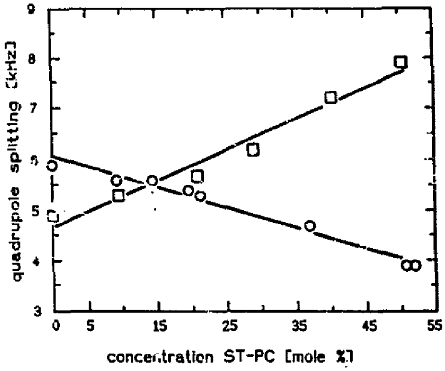


Fig. 6. Variation of the α- and β-splittings of the POPC headgroup with the ST-PC concentration. ○, α-segment; □, β-segment.

^{31}P-NMR spectra of the same POPC samples are displayed in Fig. 7. The pure POPC bilayer is characterized by the typical powder-type pattern with a single chemical shielding anisotropy [18]. As more and more ST-PC is added, the chemical shielding anisotropy increases in magnitude by 10% from −50.5 ppm (pure POPC) to about −55 ppm (50% ST-PC). At the same time a new resonance of reduced width appears, the intensity of which is directly correlated to the amount of ST-PC in the membrane. Since the ^{2}H-NMR spectra indicate only a single ^{2}H-NMR quadrupole splitting, a

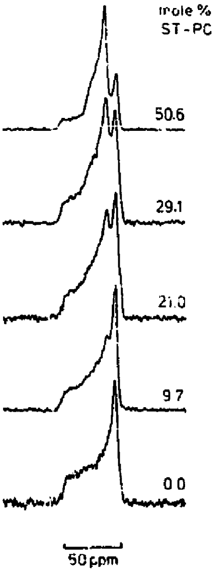


Fig. 7. ^{31}P-NMR spectra of membranes composed of POPC/ST-PC. With increasing ST-PC concentration a second signal emerges which must be ascribed to the ST-PC headgroup.

TABLE II

Quadrupole splittings Δν (kHz) and phosphorous chemical shielding anisotropy Δσ (ppm) of membranes composed of stigmastanyl-phosphorylcholine and POPC

	Sterol concn. (mol%)	$\Delta\nu_\alpha$ or $\Delta\nu_\beta$ [kHz]	Δσ POPC [ppm]	Δσ ST-PC [ppm]
α-POPC/ST-PC	0	5.9	−50.5	−
	9.3	5.6	−	−
	14.4	5.6	−52.5	−
	19.5	5.4	−52.5	−25
	21.1	5.3	−	−
	36.9	4.7	−52.5	−25
	50.8	3.9	−55.0	−22/−27
	52.1	3.9	−	−
β-POPC/ST-PC	0	4.9	−50.0	−
	9.6	5.3	−50.5	−
	21.0	5.7	−51.5	−23
	29.1	6.2	−53.0	−26
	40.3	7.2	−54.5	−25
	50.6	7.9	−54.0	−24
POPC/α-ST-PC	9.9	9.5; 10.9		
	20.1	9.5; 10.9		
	29.9	9.5; 11.1		
	40.4	9.4; 11.3		
	50.0	9.4; 11.5		
POPC/β-ST-PC	10.1	5.5		
	20.2	5.9		
	29.8	6.4		
	40.2	6.9		
	50.5	7.5		

phase separation can be excluded. We therefore attribute the second phosphorus signal to the PC headgroup of ST-PC. The numerical values of the phosphorus chemical shielding anisotropies are included in Table II.

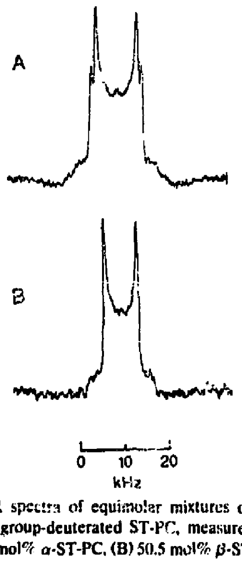


Fig. 8. ^{2}H-NMR spectra of equimolar mixtures of nondeuterated POPC and headgroup-deuterated ST-PC, measured at 305 K. (A) 50.0 mol% α-ST-PC, (B) 50.5 mol% β-ST-PC.

More detailed insight into the ST-PC headgroup conformation was obtained by employing headgroup deuterated ST-PC. Fig. 8 displays characteristic ^{2}H-NMR spectra of α- and β-deuterated ST-PC in equimolar mixture with nondeuterated POPC. For the α-segment of ST-PC two quadrupole splittings of 9.4 kHz and 11.5 kHz were observed, indicating a small motional inequivalence of the two deuterons. For the β-segment this inequivalence was smoothed out and a single splitting of 7.5 kHz separation was detected. The variation of the quadrupole splittings with the ST-PC content of the membrane are summarized in Table II. The α-splitting is only slightly dependent on the composition of the membrane, the β-splitting increases from about 5.5 kHz at 10% ST-PC to 7.5 kHz at about 50% ST-PC. The behavior of the phosphocholine dipole when attached to stigmastanol is thus clearly different from that of the same headgroup in the neighboring phospholipid molecule.

Discussion

Cholesterol, stigmastanol, and stigmastanyl-phosphorylcholine are freely miscible with POPC and DOPC bilayer membranes up to concentrations of at least 50 mol%. This follows from the observation that the ^{2}H-NMR signals change homogeneously over the whole concentration range and provide no evidence for a phase separation. This also holds true for membrane systems containing ST-PC, even though the ^{31}P-NMR spectra of this system show different chemical shielding anisotropies for the phospholipid phosphorus and the ST-PC phosphorus atom.

In spite of their similar membrane miscibility, the three sterols have quite different effects on the membrane matrix: cholesterol and stigmastanol interact preferentially at the level of the hydrocarbon chains with little effect on the phospholipid head group; ST-PC exhibits just the opposite behavior modifying the phospholipid headgroups and leaving the hydrocarbon chains unaltered.

From neutron diffraction studies with selectively deuterated cholesterol it is known that the hydroxyl group of cholesterol is located at the lipid water interface in the vicinity of the lipid glycerol backbone [19]. The rigid sterol frame is thus intercalated between the fatty acyl chains reducing considerably the segmental flexing motion, as has been established by quite a number of different methods (cf. Refs. 14 and 20). Since the cholesterol OH-group is positioned below the layer of $^-$P-N$^+$ dipoles, the absence of any effects on the phosphorus and the α-CH$_2$ choline segment is easily explained.

The similarity of the ^{2}H- and ^{31}P-NMR spectra of stigmastanol and cholesterol suggests a similar average position for stigmastanol in the lipid matrix. Again the sterol ring is buried in the hydrocarbon region of the membrane whereas the hydroxyl group must be anchored near the fatty acid carbonyls. Stigmastanol has a smaller effect on the hydrocarbon chain ordering than cholesterol, a finding which is in agreement with related studies on precursors and derivatives of cholesterol [21–23]. Among all steroids investigated, cholesterol exerts the largest stiffening effect on the membrane fatty acyl chains. Measured in terms of quadrupole splittings, the maximum stiffening effect of stigmastanol is only 50% of that of cholesterol.

The ^{2}H- and ^{31}P-NMR data obtained for ST-PC suggest a different membrane location for this molecule. The $^{-}$P-N^{+} dipole of the PC headgroup appears to be sufficiently polar to anchor the molecule in the surface layer of the phospholipid $^{-}$P-N^{+} dipoles. The sterol ring of ST-PC is thus hindered in penetrating deeply into the hydrophobic region. This is in agreement with the deuterium NMR spectra of the *cis*-double bonds of DOPC which remain virtually constant upon addition of ST-PC. On the other hand, the $^{-}$P-N^{+} dipole of ST-PC induces a conformational change of the neighboring phospholipid headgroups. The observed variations of the PC headgroup splittings ($\Delta\nu_{\alpha}$ decreases, $\Delta\nu_{\beta}$ increases with increasing ST-PC) are qualitatively similar to those caused by the adsorption of positive electric charges to the membrane surface [24–26]. The molecular model associated with this conformational change is a rotation of the $^{-}$P-N^{+} dipole with its positive end towards the water phase ([25]; Caniparoli and Seelig, in preparation). Based on the analogy with positive electric surface charges we therefore conclude that the addition of ST-PC to a POPC bilayer moves the phospholipid $^{-}$P-N^{+} dipoles out of the plane of the membrane into the waterphase.

Due to the adsorption of water molecules at the membrane surface but also because of contributions of electric dipoles intrinsic to the lipids (headgroups, -C=O, -CH$_3$) even neutral phospholipid membranes are characterized by a distinct dipole potential of 300–500 mV (membrane interior positive, cf. Ref. 27). The rotation of the PC headgroup of ST-PC creates a dipole component with the opposite polarity ($^{+}$N end further in water phase than PO^{-} end). This headgroup dipole potential reduces the intrinsic membrane dipole potential which may have functional consequences.

Two distinct differences between ST-PC and true surface charges should be pointed out. First, the variation of $\Delta\nu_{\alpha}$ and $\Delta\nu_{\beta}$ with the ST-PC concentration amounts to only 10–20% of what is typically observed with hydrophobic ions (cf. Ref. 26). Secondly, the variation at the β-segment is larger than that at the α-segment ($\Delta\nu_{\beta} \cong -1.5\ \Delta\nu_{\alpha}$). For positive electric surface charges, independent of the chemical nature of the ion, the corresponding relation is $\Delta\nu_{\beta} \cong -0.5\ \Delta\nu_{\alpha}$. We have previously studied POPC membranes containing phloretin as a non-charged, but strongly dipolar membrane component [17]. The conformational change of the PC headgroup was small and in the same order of magnitude as reported here for ST-PC but of opposite polarity. The β-splitting was again more affected than the α-splitting, indicating that electric dipoles produce different effects at membrane surfaces than do true electric charges.

Finally, we consider the headgroup of ST-PC itself. The characteristic parameters are $\Delta\sigma = -25$ ppm, $\Delta\nu_{\alpha} \cong 10$ kHz, and $\Delta\nu_{\beta} \cong 6$ kHz. A comparison with the existing literature reveals a close similarity of ST-PC with the phosphoethanolamine headgroup with $\Delta\sigma = -37.5$ ppm, $\Delta\nu_{\alpha} = 10.1$ kHz, and $\Delta\nu_{\beta} = 4.0$ kHz (measured immediately above the liquid crystalline phase transition) [16,28]. Like the PC headgroup the phosphoethanolamine headgroup is also extended parallel to the membrane surface as deduced from the NMR parameters [28] and confirmed by neutron diffraction studies [29]. However, the torsion angles characterizing the rotation around the various single bonds are slightly different for PC and PE. Nevertheless, it can be concluded that the PC headgroup has similar average orientations in both POPC and ST-PC and is oriented essentially parallel to the surface of the membrane.

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