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Infrared Spectroscopic Studies on the Phosphatidylserine Bilayer Interacting with Calcium Ion: Effect of Cholesterol†

Sunhee Choi,* William Ware, Jr., Stephen R. Lauterbach, and Wanda M. Phillips
Department of Chemistry and Biochemistry, Middlebury College, Middlebury, Vermont 05753
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ABSTRACT: Fourier transform infrared (IR) spectroscopic studies of phosphatidylserine/cholesterol/Ca^{2+} complexes are reported using the synthetic phosphatidylserines (PS) 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (DOPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS), and 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (DMPS). IR spectra reveal that cholesterol does not significantly alter the binding nature of Ca^{2+} to PS molecules; Ca^{2+} binds to the phosphate ester group of PS in the presence of cholesterol up to 50 mol % as in the case of pure PS bilayers. However, the IR data indicate that the presence of cholesterol induces disorder of the acyl chain packing, increases the degree of immobilization of the interfacial and polar regions, and increases the degree of dehydration of the PS/Ca^{2+} complexes.

The phospholipid bilayer is a major component of cellular membranes. It acts as a permeability barrier and a matrix where membrane proteins are embedded. Ca^{2+} is involved in the regulation of numerous cellular functions such as stimulus-contraction coupling, protoplasmic motility, intercellular interaction, and stimulus secretion coupling via the interaction with proteins which are embedded in the lipid bilayer (Siegel et al., 1980; Langer, 1987). The lateral distribution and functions of intrinsic membrane proteins are closely influenced by the physical and chemical nature of the lipid bilayer. The structure, phase behavior, and fusion of phospholipid membranes are affected by divalent cations, especially Ca^{2+}. Phosphatidylserine (PS)[1] is a major acidic phospholipid in mammalian plasma membrane (Rothman & Lenard, 1977; Devaux & Seigneuret, 1985). Due to its anionic character at physiological pH, it strongly binds with cations (Poste & Allison, 1973). It has been suggested that the interaction of this lipid with ions in the cytosol is central to a variety of physiological processes. In particular, the raised cytoplasmic Ca^{2+} levels predicted during exocytosis (Douglas, 1968) have been proposed to trigger the membrane fusion reaction by pathways involving ion association with PS (Gingell & Ginsberg, 1978).

The importance of the biological role of the Ca^{2+}/PS interaction has stimulated many structural studies on model membrane systems using various techniques (Seimiya & Ohki, 1973). The calcium ion induces crystallization of the acyl chains of PS, leading to an isothermal phase transition from the liquid-crystalline to the gel state (Papahadjopoulos et al., 1977). Infrared study (Dluhy et al., 1983) has revealed that Ca^{2+} binds to the phosphate ester (PO_2^-) group of PS and causes it to dehydrate. Ca^{2+} does not bind to the carboxylate (CO_2^-) group of PS nor does it dehydrate it. Instead, it immobilizes the CO_2^- group as well as the acyl chain group.

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[1] Abbreviations: FTIR, Fourier transform infrared; DOPS, 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine; POPS, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine; DMPS, 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DPPS, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-L-serine; *N*-methyl-DPPS, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*N*-methyl-L-serine; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; Chol, cholesterol; Tris, tris(hydroxymethyl)aminomethane hydrochloride; ESR, electron spin resonance; NMR, nuclear magnetic resonance.

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There was also indication of trapped water in the interfacial carbonyl group.

Cholesterol is another essential component of biomembranes, and its importance in many biological functions has been extensively studied [for a review, see Yeagle (1985)]. It plays an important structural role in determining the fluidity of biomembranes; it gives some fluidity to the gel phase by disturbing the gel packing while it gives some rigidity to the liquid-crystalline phase by inducing order in the bilayer. In the presence of Ca^{2+}, cholesterol alters the lateral phase behavior of particular phospholipids, destabilizing the lamellar phase and promoting hexagonal II phase structure (Tilcock, 1984). In the Ca^{2+}-involved cellular processes, cholesterol modulates Ca^{2+}-induced membrane fusion (Bental et al., 1987; Duzgunes, 1988; Chavnin et al., 1988; Cheetham et al., 1990) and Ca^{2+} transport in erythrocytes (Rosier et al., 1986). Therefore, the interaction of phospholipids/cholesterol/Ca^{2+} should be an important factor in determining Ca^{2+}-regulated cellular processes. However, this three-component system is poorly understood in spite of the numerous studies on the interaction of phospholipid with Ca^{2+} (Hope & Cullis, 1980; Dluhy et al., 1983; Altenbach & Seelig, 1984; Casal et al., 1987; Macdonald & Seelig, 1987; Seelig, 1990) and the interaction of phospholipids with cholesterol (Asher & Levin, 1977; Quinn & Chapman, 1980; Seelig & Seelig, 1980; Bush et al., 1980; Umemura et al., 1980; Stubbs, 1983; Yeagle, 1985; Green et al., 1987). Only one reference in the literature which reports the direct study on this subject was found. Surface radio counting (Gregory et al., 1984) has shown that cholesterol alters the Ca^{2+} binding mode of PS monolayers. The molecular basis of this influence of cholesterol is not well understood.

We have studied PS/cholesterol/Ca^{2+} complexes by FTIR spectroscopy to obtain information on the structural influence of cholesterol on the PS/Ca^{2+} assemblies, hoping that the information will give insight as to how cholesterol alters the Ca^{2+} binding modes of PS molecules and the phase behavior of phospholipids. 1,2-Dioleoyl-*sn*-glycero-3-phospho-L-serine (DOPS) has been used as a major synthetic PS lipid, and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS) and 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (DMPS) have been used for comparison.

Experimental Procedures

Materials. 1,2-Dioleoyl-*sn*-glycero-3-phospho-L-serine (DOPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS), and 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (DMPS) were purchased from Avanti Polar Lipids (Birmingham, AL). Cholesterol (99+%), D_2O (99.9 atom %), tris(hydroxymethyl)aminomethane hydrochloride (Tris), and $CaCl_2 \cdot 2H_2O$ were purchased from Sigma Chemical Co. (St. Louis, MO). Chloroform (99.9+%) was obtained from Aldrich Chemical Co. (Milwaukee, WI). The samples were used without further purification.

Sample Preparation. A mixture of phospholipid and cholesterol at the desired molar ratio was dissolved in chloroform. The solvent was evaporated under a stream of nitrogen gas to form a thin film in a test tube. It was dried overnight under vacuum to remove any traces of the organic solvent. The lipid multilayers were formed by hydrating the mixtures with Tris buffer solution (10 mM Tris/100 mM NaCl, pH 7.4) and vortexing. To ensure complete dispersion, the lipid multilayers were heated above the phase transition and cooled at least 3 times. To the hydrated multilayers were added mole fraction aliquots of 10 mM calcium ion concentration in Tris buffer (pH 7.4) and allowed to stand at room temperature for 1 h.

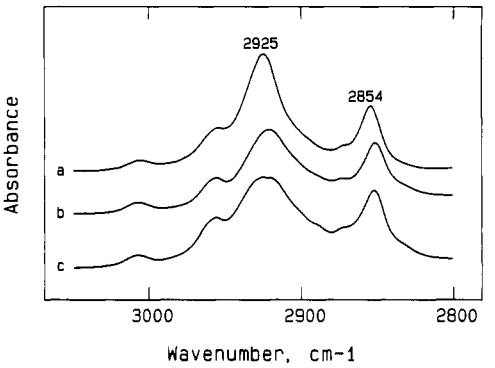

FIGURE 1: Infrared spectra of the C–H stretching (νCH) region for (a) pure DOPS, (b) DOPS/Ca^{2+} (1/1, mol/mol), and (c) DOPS/Chol/Ca^{2+} (1/1/1, mol/mol) in Tris/D_2O buffer (pD 7.4) at 30 °C.

The final concentration of dispersed lipid is in the range of 10 mM. The calcium ion bound sample was then ultracentrifuged at 45 000 rpm for 35 min at 3 °C. The supernatant was removed with a pipet, and the hydrated pellet was spread on a BaF_2 window. It was assembled into a cell of 0.015-mm path length for infrared measurement. Due to the interfering infrared absorption of H_2O, samples were also made up in D_2O buffers. In these cases, the samples were prepared exactly as described above with the exception that the buffers were adjusted to pD 7.4 in D_2O.

Spectra. For temperature regulation, the cell was placed in a cell mount thermostated by a flow of ethylene glycol/water from a constant-temperature (±1 °C) bath. The temperature was monitored by a copper-constant thermocouple located against the cell window. Spectra at 2 cm^{-1} resolution were recorded with a Mattson Cygnus 100 Fourier transform infrared spectrometer; for each spectrum, 600 interferograms were averaged.

Results

Acyl Chain Region: Acyl C–H Stretching Modes. Figure 1 shows infrared spectra of the C–H stretching region of DOPS, DOPS/Ca^{2+}, and DOPS/Chol/Ca^{2+} bilayers at 30 °C. The spectra of DOPS and DOPS/Ca^{2+} are basically the same as the ones in the literature (Dluhy et al., 1983; Casal, 1987). Both the asymmetric and symmetric C–H stretching modes of the acyl chain methylene groups at 2925 and 2854 cm^{-1}, respectively, have been shifted down by 3–4 cm^{-1} upon Ca^{2+} binding. This low frequency is due to the highly ordered all-trans hydrocarbon chains, showing Ca^{2+}-induced isothermal crystallization of the lipid. In the cholesterol-added complex, DOPS/Chol/Ca^{2+}, these bands have been shifted up by 1 cm^{-1} relative to DOPS/Ca^{2+}, and the asymmetric C–H stretching band is noticeably broadened. This indicates that the acyl chain packing of DOPS/Ca^{2+} is disturbed by cholesterol.

Interfacial Region: Carbonyl Stretching Vibrations. Figure 2 displays Fourier self-deconvoluted (Kauppinen et al., 1981) infrared spectra of the carbonyl stretching region of DOPS, DOPS/Ca^{2+}, and DOPS/Chol/Ca^{2+}. In the spectrum of pure DOPS, there are two asymmetric carbonyl stretching bands at 1742 and 1727 cm^{-1} arising from hydrogen-bonded carbonyl groups of *sn*-1 and *sn*-2 chains, and addition of Ca^{2+} splits these two bands to four bands, which have been assigned to carbonyl groups with different degrees of hydration (Blume et al., 1988). The lowest band occurring at 1704 cm^{-1} has been assigned to one of the C=O groups forming hydrogen bonds with two molecules of water. This band position does not change upon addition of cholesterol. The other three bands have been narrowed in the sample with cholesterol. This sharpening effect is more noticeable in the spectra of samples

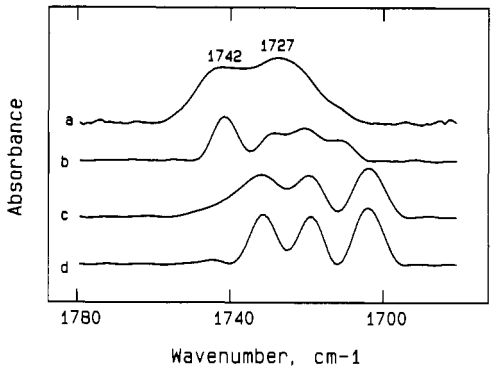

FIGURE 2: Infrared spectra of the C=O stretching (νCO) region for (a) pure DOPS, (b) DOPS/Ca^{2+} (1/0.2, mol/mol), (c) DOPS/Ca^{2+} (1/1, mol/mol), and (d) DOPS/Chol/Ca^{2+} (1/1/1, mol/mol) in Tris/D_2O buffer at 24 °C. Spectra have been Fourier self-deconvoluted with a 10 cm^{-1} half-width Lorentzian line and smoothed to K = 1.8 with a Bessel function (Kauppinen et al., 1981).

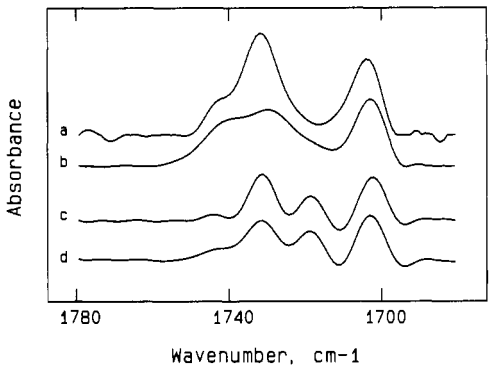

FIGURE 3: Infrared spectra of the C=O stretching (νCO) region for (a) DOPS/Chol/Ca^{2+} (1/1/0.5, mol/mol) and (b) DOPS/Ca^{2+} (1/0.5, mol/mol) at 41 °C and for (c) DOPS/Chol/Ca^{2+} (1/1/0.5, mol/mol) and DOPS/Ca^{2+} (1/0.5, mol/mol) at 24 °C, in Tris/D_2O buffer. Spectra have been Fourier self-deconvoluted the same way as in Figure 2.

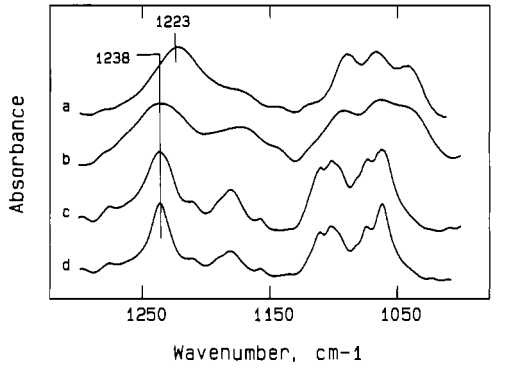

FIGURE 4: Infrared spectra of the PO_2^- stretching (νPO_2^-) region for (a) fully hydrated pure DOPS in Tris buffer, (b) dried pure DOPS, (c) DOPS/Ca^{2+} (1/1, mol/mol), and (d) DOPS/Chol/Ca^{2+} (1/1/1, mol/mol) in Tris buffer (pH 7.4) at 30 °C.

at 41 °C (Figure 3). The spectral features at 41 °C are different from those at 24 °C, which has been attributed to the solid–solid transition around 30 °C (Dluhy et al., 1983).

Polar Group Region: Phosphate Group Vibrations. Figure 4 presents infrared spectra of the PO_2^- stretching bands. The band at 1223 cm^{-1} in fully hydrated DOPS has been assigned to an asymmetric PO_2^- stretching mode ($\nu_{as}PO_2^-$), while the three peaks at 1090, 1067, and 1043 cm^{-1} have been related with symmetric PO_2^- stretching modes ($\nu_sPO_2^-$) (Dluhy et al., 1983). Both the dried pure sample and the fully hydrated DOPS/Ca^{2+} show the $\nu_{as}PO_2^-$ band at 1238 cm^{-1}, which indicates that Ca^{2+} binds with the phosphate ester group and

Table I: Full Width at Half-Maximum Peak Height of the Asymmetric PO_2^- Stretching ($\Delta\nu_{1/2}$) Band for DOPS/Ca^{2+} (1/0.3, mol/mol) with Added Cholesterol in Tris Buffer at Various Temperatures

temp (°C)	cholesterol (mol %)	$\Delta\nu_{1/2}$ (cm^{-1})
41	0	29
	50	22
30	0	24
	50	20
24	0	22
	33	17
	50	13
-13	0	19
	50	11

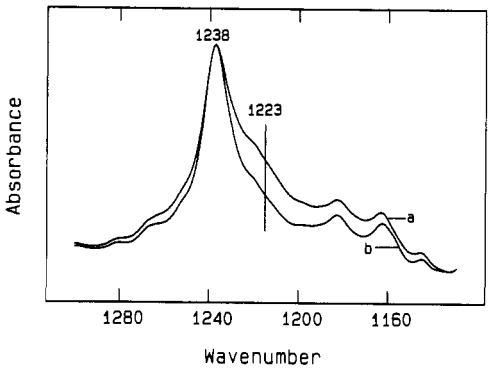

FIGURE 5: Infrared spectra of the $\nu_{as}PO_2^-$ band for the DOPS/Ca^{2+} (1/0.25, mol/mol) complex (a) without and (b) with cholesterol (33 mol % of DOPS) in Tris buffer at 24 °C.

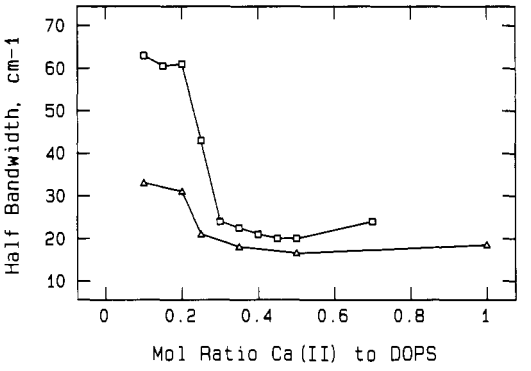

FIGURE 6: Plots of the full width at half-maximum peak height vs mole ratio of the added Ca^{2+} to DOPS without (squares) and with (triangles) cholesterol (50 mol %) at 30 °C.

dehydrates it. The bandwidth of $\nu_{as}PO_2^-$ of the Ca^{2+}-bound phosphate is much narrower than the dried pure DOPS sample, reflecting the immobilization of the phosphate group upon Ca^{2+} binding. The $\nu_sPO_2^-$ bands split into four bands at 1111, 1102, 1074, and 1063 cm^{-1} in the Ca^{2+}-containing sample. The splitting has been attributed to arise from the antiplanar–antiplanar conformation of torsional angles of the two P–O ester bonds (Casal et al., 1987). Addition of cholesterol has sharpened both of the bands. There is a gradual decrease of the $\nu_{as}PO_2^-$ bandwidth as the concentration of cholesterol is increased at all the temperatures studied. The results are tabulated in Table I. Figure 5 compares the $\nu_{as}PO_2^-$ bands of DOPS/Ca^{2+} (1/0.25, mol/mol) and DOPS/Chol/Ca^{2+} (1/1/0.25, mol/mol) at 24 °C. When Ca^{2+} is added to the fully hydrated DOPS, the 1223 cm^{-1} band goes down, and the 1238 cm^{-1} band appears, which is narrower than the dried pure DOPS sample. The presence of cholesterol further reduces the intensity of the 1223 cm^{-1} band and sharpens the 1238 cm^{-1} band. This suggests that cholesterol enhances the dehydration and immobilization of the Ca^{2+}-bound phosphate group. The

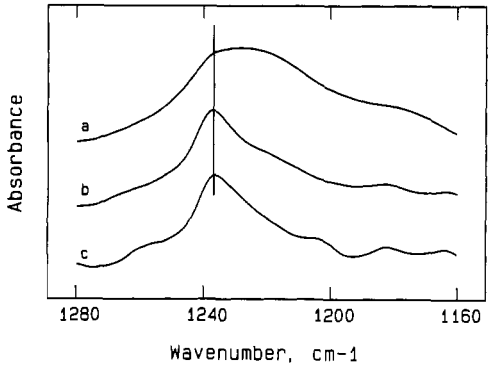

FIGURE 7: Infrared spectra of the asymmetric PO_2^- stretching ($\nu_{as}PO_2^-$) band for Ca^{2+} complexes (0.2 mole ratio of PS) of (a) DOPS, (b) POPS, and (c) DMPS in Tris buffer at 30 °C.

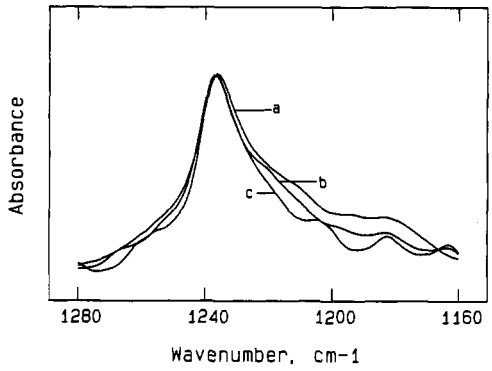

FIGURE 8: Infrared spectra of the asymmetric PO_2^- stretching ($\nu_{as}PO_2^-$) band for cholesterol added (50 mol % of PS) Ca^{2+} complexes (0.2 mole ratio of PS) of (a) DOPS, (b) POPS, and (c) DMPS in Tris buffer at 30 °C.

half-bandwidths of $\nu_{as}PO_2^-$ vs the mole ratio of Ca^{2+} to DOPS with and without cholesterol are plotted in Figure 6. The band narrowing effect of cholesterol is most obvious at low concentrations of Ca^{2+}, but the effect is seen throughout the whole Ca^{2+} concentration range.

The $\nu_{as}PO_2^-$ bandwidths of Ca^{2+} complexes of DOPS, POPS, and DMPS are compared in Figure 7. The bandwidth of $\nu_{as}PO_2^-$ decreases in the order DOPS > POPS > DMPS. The presence of cholesterol has reduced the bandwidths of $\nu_{as}PO_2^-$ for all three lipids (Figure 8). The half-bandwidth difference between the sample with and without cholesterol is 30 cm^{-1} for DOPS, 5.5 cm^{-1} for POPS, and 6.5 cm^{-1} for DMPS, at the concentration of 0.2 mol of Ca^{2+}/mol of PS. The symmetric phosphate stretching bands also become sharpened by cholesterol (see the lower frequency part of Figure 4), consistent with the behavior of the asymmetric phosphate stretching mode.

Carboxylate Stretching Mode. The carboxylate stretching band of pure DOPS at 1625 cm^{-1} has been shifted down to 1621 cm^{-1} in the PS/Ca^{2+} complex, which is consistent with the previous work (Dluhy et al., 1983). The interpretation is that the carboxylate group remains hydrated but that the conformation is more rigid in the PS/Ca^{2+} complex. Addition of cholesterol has not affected this band (spectrum not shown), indicating that cholesterol affects neither the hydration nor the mobility of the carboxylate group.

Discussion

Our infrared spectral results have shown that cholesterol affects (a) the acyl chain region, (b) the carbonyl interfacial region, and (c) the phosphate ester group region. The effect of cholesterol on each region will be discussed separately.

Many techniques including IR have shown that cholesterol disturbs highly ordered acyl chain packing in the gel phase of lipid bilayers (Demel & Dekruijff, 1976; Umemura et al., 1980; Presti et al., 1982; Yeagle, 1985). The existence of unsaturated bonds in the acyl chain is also known to lower the order of packing (Seelig & Seelig, 1977; Stubbs et al., 1981; van de Ven & Levine, 1984; Mulders et al., 1986). We observed both of these cases. The asymmetric C–H stretching band ($\nu_{as}CH$) of DOPS/Ca^{2+} (1/1, mol/mol) occurring at 2921 cm^{-1} is shifted up by 1 cm^{-1} and has a broadened band width in DOPS/Chol/Ca^{2+} (1/1/1, mol/mol), indicating that cholesterol disturbs the acyl chain packing. In POPS/Chol/Ca^{2+}, $\nu_{as}CH$ appears at 2921 cm^{-1} (spectrum not shown). The fact that the $\nu_{as}CH$ bands of POPS/Chol/Ca^{2+} and DOPS/Ca^{2+} appear at the same frequency suggests that the degree of disorder in their acyl chains is almost the same. Since POPS has one double bond and DOPS has two double bonds, we may say that the presence of cholesterol at 50 mol % of the lipid gives the same disordering effect on the acyl chain packing as the existence of one additional double bond. This kind of correlation has been observed in the phase behavior of mixed phosphatidylserine (PS) and phosphatidylethanolamine (PE) in the presence of Ca^{2+} (Tilcock et al., 1984). Ca^{2+} can segregate dioleoyl-PS (DOPS) into crystalline cochleate domains in an equimolar mixture of dioleoyl-PE (DOPE) and DOPS, but such effects are not observed for mixtures containing more unsaturated (dilinoleoyl) species of PS or cholesterol containing DOPS/DOPE mixtures.

The infrared spectrum of the interfacial carbonyl stretching region has been sharpened and better resolved in the cholesterol-containing complex without changing the band positions. It is noticed that the lowest band at 1704 cm^{-1} arising from the disolvated carbonyl groups (Blume et al., 1988) does not change upon addition of cholesterol. This can be interpreted in two ways. One is that cholesterol does not change the degree of hydration of the interfacial region, leaving the trapped water intact. The other is that the 3β-OH group of cholesterol is hydrogen-bonded with the carbonyl group. At present, we cannot tell which is the case. The previous Raman and IR studies (Bush, 1980) have shown that in the dehydrated DOPC/cholesterol system, there is no hydrogen bonding between the acyl carbonyl group and cholesterol 3β-OH. The other three bands have been narrowed in the sample with cholesterol. This sharpening effect is more noticeable in the spectra of samples at 41 °C (Figure 3). Cholesterol seems to increase the degree of immobilization of the interfacial region.

The most significant effect of cholesterol on the PS/Ca^{2+} complex can be seen at the phosphate ester group. The presence of cholesterol does not change the position of symmetric and asymmetric phosphate stretching modes. This means that the presence of cholesterol alters neither the binding site of the PS lipid nor the Ca^{2+}-induced antiplanar–antiplanar conformers of the two P–O bonds. However, cholesterol reduces the phosphate stretching bandwidth. In the spectrum of a complex with a low concentration of Ca^{2+}, both bound and unbound phosphate stretching bands are detected (Figure 5); a band at 1238 cm^{-1} is due to the bound dehydrated phosphate group while a band on the shoulder at 1223 cm^{-} is due to the unbound hydrated phosphate group. At the same level of low Ca^{2+} concentration, this shoulder at 1223 cm^{-1} disappears, and the bandwidth at 1238 cm^{-1} becomes narrowed in the cholesterol-containing samples. This is interpreted as cholesterol enhancing the dehydration of the phosphate group and further immobilizing it. The $\nu_{as}PO_2^-$ band-narrowing effect by cholesterol was observed at all levels

of Ca^{2+} concentration. The bandwidths vs Ca^{2+} concentrations are plotted in Figure 6. It is interesting to note that the inflection point and the minimum value of $\nu_{as}PO_2^-$ bandwidth occur at the same concentration of Ca^{2+} for both samples with or without cholesterol. This indicates that cholesterol does not change the binding stoichiometry of Ca^{2+} with DOPS which is known to be 1/2, mol/mol (Ekerdt & Papahadjopoulos, 1982). Our result is not the same as the previous surface radiocounting study (Gregory & Ginberg, 1984). It reported that the Ca^{2+}/PS surface ratio was approximately 1/2 in expanded monolayers of pure anionic lipid while an increase in surface-associated Ca^{2+} to a number ratio of 1/1 was observed in PS/cholesterol films. The difference may be due to the different types of assemblies. The previous study is on the monolayer while our study is on the bilayer assembly. In the monolayer, the binding should be within the same layer, and the presence of cholesterol as a spacer prevents one Ca^{2+} ion from associating with two adjacent PS molecules in the same layer. However, in the bilayer assembly, the binding can occur between opposing layers, and the disrupted molecular arrays of bilayers by cholesterol would not affect the binding stoichiometry. This may be the case in the present systems.

It has been generally accepted that the lipid molecular area plays a major role in determining the metal ion binding affinity of PS bilayers (Casal et al., 1987, 1989). The IR and ^{31}P NMR studies have shown that the affinity of PS for Ca^{2+} (Casal et al., 1987) or Mg^{2+} (Casal et al., 1989) increases with decreasing molecular area which is in the order DOPS > POPS > DMPS (Demel et al., 1987). We have compared the $\nu_{as}PO_2^-$ bandwidth of Ca^{2+} complexes of DOPS, POPS, and DMPS in Figure 7. The band of DOPS is broad over the range of 1220–1240 cm^{-1}, indicating that a large amount of lipid is unbound. However, the bands of POPS and DMPS are quite sharp at 1238 cm^{-1} with a weak shoulder at 1223 cm^{-1}, indicating that the amount of the bound lipid is higher than the unbound lipid. This confirms that the bandwidth of $\nu_{as}PO_2^-$ can be directly related with the affinity of lipid toward Ca^{2+}. POPS and DMPS have higher surface charge than DOPS and hence they have higher affinity toward Ca^{2+}, and this is reflected on the bandwidth of $\nu_{as}PO_2^-$.

The presence of cholesterol has reduced the bandwidths of $\nu_{as}PO_2^-$ for all of these three lipids (Figure 8); the half-bandwidth difference between the sample with and without cholesterol is 30 cm^{-1} for DOPS, 5.5 cm^{-1} for POPS, and 6.5 cm^{-1} for DMPS, at a mole ratio of Ca^{2+}/PS of 0.2. The effect is largest for DOPS bilayers. In order to see the large effect of cholesterol on POPS and DMPS, the mole ratio of Ca^{2+}/PS may be lower than 0.2. Although cholesterol has a condensing effect on the acyl chains of bilayers in the liquid-crystal state (Demel et al., 1967), the polar groups are further apart than in the absence of cholesterol. Therefore, our result that cholesterol reduces the bandwidth of $\nu_{as}PO_2^-$ may not be an indication of enhanced binding. A recent study (Casal et al., 1990) has shown that the *N*-methyl-DPPS bilayer has a more tightly packed, highly ordered hydrocarbon chain lattice than does DPPS, although the molecular area of *N*-methyl-DPPS is larger than that of DPPS. The polar group is also tightly packed and practically immobilized. The reduced hydrogen bonding and electric repulsion between the negatively charged polar groups by the *N*-methyl residue have been attributed to the tight packing. In our system, cholesterol may function like the *N*-methyl residue in the *N*-methyl-DPPS bilayer. Cholesterol may act as a spacer and reduces the electrostatic repulsion of the adjacent phosphate groups, and hence increases the immobilization.

The large effect of cholesterol on the unsaturated lipids is further indicated by comparing the $\nu_{as}PO_2^-$ bandwidths of the Ca^{2+} complex of DOPS with other more saturated PS such as DMPS. The $\nu_{as}PO_2^-$ bandwidth of DOPS/Chol/Ca^{2+} (1/1/0.2, mol/mol) is 31 cm^{-1}, and that of DMPS/Ca^{2+} (1/0.2, mol/mol) is 30.5 cm^{-1}. They are practically the same. This suggests that the degree of packing in DOPS/Chol/Ca^{2+} (1/1/1, mol/mol) is the same as in DMPS/Ca^{2+} (1/1, mol/mol).

The immobilization of the phosphate group by cholesterol was noticed at all temperatures (Table I). It is interesting to note that the mobility of the cholesterol-containing Ca^{2+} complex at 41 °C is comparable to that of the complex without cholesterol at 30 °C and the cholesterol-containing sample at 24 °C is comparable to that of the complex without cholesterol at −13 °C. The addition of cholesterol lowers the mobility of the phosphate ester group the same as lowering the temperature. The four bands at 1111, 1102, 1074, and 1063 cm^{-1} have been attributed to the antiplanar–antiplanar conformation of P–O bonds in the PS/Ca^{2+} system. The band positions of these four bands are not changed but become sharper in the cholesterol-containing complexes. Cholesterol does not change the P–O conformation, but enhances the immobility of the phosphate group.

Conclusions

Our infrared data have revealed that cholesterol affects the Ca^{2+} and PS molecular system; it (a) perturbs the acyl chain packing, (b) increases the degree of immobilization of the interfacial and phosphate ester groups, and (c) increases the degree of dehydration of the polar group. These results may give a structural basis for many of the functional roles of cholesterol in various biological membrane processes involving Ca^{2+} such as Ca^{2+}-induced membrane fusion.

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Complete Structure of the Cell Surface Polysaccharide of *Streptococcus oralis* C104: A 600-MHz NMR Study†

Chitrananda Abeygunawardana and C. Allen Bush*
Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, Maryland 21228
John O. Cisar
Laboratory of Microbial Ecology, National Institute of Dental Research, Bethesda, Maryland 20892
Received April 12, 1991; Revised Manuscript Received June 14, 1991

ABSTRACT: Specific lectin–carbohydrate interactions between certain oral streptococci and actinomyces contribute to the microbial colonization of teeth. The receptor molecules of *Streptococcus oralis*, 34, ATCC 10557, and *Streptococcus mitis* J22 for the galactose and *N*-acetylgalactosamine reactive fimbrial lectins of *Actinomyces viscosus* and *Actinomyces naeslundii* are antigenically distinct polysaccharides, each formed by a different phosphodiester-linked oligosaccharide repeating unit. These streptococci all coaggregated strongly with both *A. viscosus* and *A. naeslundii* strains, whereas *S. oralis* C104 interacted preferentially with certain strains of the latter species. Receptor polysaccharide was isolated from *S. oralis* C104 cells and was shown to contain galactose, *N*-acetylgalactosamine, ribitol, and phosphate with molar ratios of 4:1:1:1. The ^{1}H NMR spectrum of the polysaccharide shows that it contains a repeating structure. The individual sugars in the repeating unit were identified by ^{1}H coupling constants observed in E-COSY and DQF-COSY spectra. NMR methods included complete resonance assignments (^{1}H and ^{13}C) by various homonuclear and heteronuclear correlation experiments that utilize scalar couplings. Sequence and linkage assignments were obtained from the heteronuclear multiple-bond correlation (HMBC) spectrum. This analysis shows that the receptor polysaccharide of *S. oralis* C104 is a ribitol teichoic acid polymer composed of a linear hexasaccharide repeating unit containing two residues each of galactopyranose and galactofuranose and a residue each of GalNAc and ribitol joined end to end by phosphodiester linkages with the following structure.

$$[\rightarrow 6)\mathrm{Gal}f(\beta 1\rightarrow 3)\mathrm{Gal}_p(\beta 1\rightarrow 6)\mathrm{Gal}f(\beta 1\rightarrow 6)\mathrm{Gal}_p\mathrm{NAc}(\beta 1\rightarrow 3)\mathrm{Gal}_p(\alpha 1\rightarrow 1)\text{ribitol}(5\rightarrow \mathrm{PO}_4^-]_n$$

The viridans streptococci that colonize teeth (Nyvad & Kilian, 1987; Kilian et al., 1989) frequently have cell surface polysaccharides that serve as receptor molecules for the galactose and *N*-acetylgalactosamine reactive lectins of various other oral bacteria including *Actinomyces viscosus* and *Actinomyces naeslundii* (Cisar, 1986). Structural studies of the

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