Interactions of Cholesterol and Synthetic Sterols with Phosphatidylcholines As Deduced from Infrared CH_2 Wagging Progression Intensities

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Abstract: The intensities of the coupled CH_2 wagging vibrations in the IR spectra of relatively ordered phases of phospholipids have been used to characterize acyl chain conformations in a set of sterol/phosphatidylcholine (PC) mixtures. A series of sterols, with side chain lengths at C-17 varying from 0 to 10 carbon atoms, has been synthesized. The thermotropic behavior of 1,2- dipalmitoylphosphatidylcholine (DPPC) with these sterols has been monitored with Fourier transform IR (FT-IR) spectroscopy. As the sterol side chain lengthens, the *all-trans* conformation of the acyl chains in DPPC persists to progressively higher temperatures. In complementary experiments, cholesterol was mixed with a series of disaturated PCs varying in chain length from C_{12} to C_{22}. Remarkable variations in the thermotropic response of the CH_2 wagging progression intensity of the PCs were noted at molar ratios of 2:1 (PC/cholesterol). For short-chain PCs (C_{12} and C_{14}) the wagging progressions persisted to 35–40 °C above the main phase transition temperature (T_m) for the pure phospholipid. In contrast, mixtures of longer chain PCs with cholesterol displayed progressions which vanished close to their respective T_m values. These trends were unchanged by the presence of a single C=C bond in the acyl chains. A semiquantitative model is used to convert the measured intensity changes into the extent of *gauche* bond formation in the acyl chains. These results are discussed in terms of structural models for cholesterol/PC interaction. The current investigation represents the first systematic evaluation of conformational changes in model membranes in which both the sterol side chain length and the phospholipid acyl chain length have been varied.

Introduction

The widespread occurrence of cholesterol in the plasma membranes of eukaryotic cells has attracted the interest of numerous biophysical investigations. For reviews of the interaction of cholesterol with other membrane components, especially phospholipids, see refs 1 and 2. A reasonably detailed picture has emerged for the phase behavior of cholesterol with two disaturated phosphatidylcholines, namely 1,2-dipalmitoylphosphatidylcholine (DPPC) and 1,2-dimyristoylphosphatidylcholine (DMPC). The thermotropic behavior of these two systems as deduced from high-sensitivity differential scanning calorimetry (DSC)[3–6] is complex. At low cholesterol levels (less than about 25 mol % sterol) two overlapped endotherms are evident, which, when resolved, resemble those of a pure phospholipid phase whose enthalpy diminishes more or less linearly with temperature prior to vanishing at ~25 mol % cholesterol and a broader component representing the melting of cholesterol-rich PC domains. McMullen et al.[6] have recently shown that the broad component has a midpoint temperature that continues to increase as cholesterol is added, with a progressively decreasing enthalpy and cooperativity that each approach zero at cholesterol mole fractions near 50%.

Although high-sensitivity DSC provides an accurate picture of the thermodynamics of PC phase transitions, the molecular nature of the phases present cannot be deduced without spectroscopic data. Vist and Davis[3] have used ^2H NMR spectroscopy in addition to DSC to map the phase boundaries and molecular dynamics properties in a mixture of cholesterol with acyl chain perdeuterated DPPC (DPPC-d_{62}). They noted the existence of the "β" phase characterized by highly ordered acyl chains and rapid axially symmetric molecular reorientation at concentrations higher than 22 mol % cholesterol.

Although characterization of DMPC or DPPC interactions with cholesterol is at an advanced stage, these two systems by no means provide a complete description of the structural role of cholesterol in cell membranes. This point has been emphasized in studies using PCs with one or two unsaturated chains, which undergo very different interactions with cholesterol than their saturated counterparts.[7–9] An investigation of the effects of acyl chain lengths and unsaturation, as well as the effects of varying the side chain of cholesterol, on the phase properties of phospholipids is thus relevant at this time.

The techniques and advantages of Fourier transform infrared (FT-IR) spectroscopy for the study of phospholipid phase transitions are well documented. For recent reviews see refs 10 and 11. Recently, this laboratory has elaborated three IR experiments for the quantitative determination of conformational

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disorder in models for biological membranes. The CD_2 rocking modes of specifically deuterated DPPC were used[12] to measure the extent and position dependence of *gauche* rotamer formation in the L_α phase of DPPC and in the liquid-ordered phase of a 2:1 DPPC/cholesterol mixture at 50 °C.[13] The localized CH_2 wagging modes of disordered phases were used to determine the presence of selected one-, two-, or three-bond conformational states in the L_α phase.[14] A third experiment[15] involved observation of the CH_2 wagging progression intensity characteristic of the *all-trans* conformation to examine slightly disordered acyl chain states. This parameter is well suited to the current work, that is, the determination of disorder in the conformationally ordered, biologically relevant phases that occur at relatively high cholesterol concentrations.

The current experiments build upon previous studies of PC/sterol interaction in two directions. First, the effect of systematically varying the side chain length and branching properties of the sterol on the conformational order of DPPC in the biologically relevant liquid ordered phase (at 2:1 PC/sterol molar ratio) is evaluated. Second, the effects of variation in the PC acyl chain lengths and degrees of unsaturation are also probed. In each case, the PC acyl chain conformational order is directly monitored from the intensity of the CH_2 wagging progression in the IR spectrum.

Experimental Section

Materials. A. Lipids. Saturated PCs were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). These compounds were used without further purification and routinely have transition widths for the gel–liquid crystal phase transition of less than 1 °C as determined from DSC analysis. Cholesterol (Sigma Chemical Co., St. Louis, MO) and 5-androsten-3-β-ol (Research Plus, Inc., Bayonne, NJ) were of stated purities greater than 99% and 97%, respectively. These were checked for chemical composition by mass spectrometry. The other sterols were synthesized as outlined below. Unsaturated PCs, also purchased from Avanti Polar Lipids, Inc., were stated to be of >99% purity. The purity of 1,2-dinervonoylPC (di-C24:1-PC) was evaluated by DSC on a Microcal MC-1 calorimeter. Its transition temperature was 27 °C, which is slightly higher than the published value,[16] while the observed DSC half-width and gas chromatography analysis showed it to be greater than 99% pure. The T_m values for 1,2-dierucoyl-PC (di-C22:1-PC) and 1,2-dielaidoylPC (di-C18:1*t*-PC) were each 11 °C as measured by FT-IR, in good accord with the values reported by Caffrey and Feigenson.[16]

B. Synthesis of Sterol Derivatives. The synthesis of sterols with various side chain structures was carried out using the method described by Morisaki et al.[17] 22-(Tosyloxy)bisnorchol-5-en-3β-ol tetrahydropyranyl ether, which was used as the key intermediate, was prepared from bisnorcholenic acid by THP etherification of the C-3 hydroxyl group, reduction of the carboxylic acid group to the alcohol with lithium aluminum hydride, and tosylation of the resulting primary alcohol. From the 22-tosylate, the sterols (except the iso-C3 compound) were obtained by coupling with different alkylmagnesium bromides in the presence of Li_2CuCl_4, followed by acid-catalyzed hydrolysis of the THP ether. Hydrogenolysis of the 22-tosylate intermediate using lithium aluminum hydride gave the iso-C3 compound. The sterols were purified by flash chromatography on silica gel 60 (230–400 ASTM mesh) and then recrystallization from methanol. The reactions were monitored on 0.25-mm-thick silica gel GF TLC plates. The structures were confirmed by NMR spectroscopy.

Sample Preparation. Phospholipid/sterol mixtures (dried from $CHCl_3$ solutions, first under a stream of N_2 gas and then under high vacuum overnight) were dispersed in excess double-distilled H_2O in sealed ampules (usually 2:1 mol/mol PC/sterol) at temperatures well above T_m. Samples were incubated for at least 2–3 h with intermittent agitation on a vortex mixer to ensure complete hydration. For FT-IR thermotropic studies, the aqueous dispersions were contained in a thermostated transmission cell with CaF_2 windows and a Teflon spacer of 6-μm thickness. The temperature was controlled with a circulating water bath and monitored with a digital thermocouple (Physitemp Instruments, Inc., Clifton, NJ) placed adjacent to the focusing point of the IR radiation. The temperature precision is 0.1 °C; the temperature accuracy is estimated to be 0.5 °C.

IR Spectroscopy. FT-IR spectra were acquired on a Digilab FTS-40 spectrometer equipped with a DTGS detector. Spectra were obtained at 4-cm^{-1} resolution under an N_2 purge, by coaddition of 256 interferograms. These were apodized with a triangular function and Fourier-transformed with one level of zero filling to yield data encoded every ~2 cm^{-1}. Data from two or three independent preparations were acquired for each sample. Spectra were corrected for any minor residual absorption bands due to water vapor, using spectra of the latter recorded under the same conditions of instrument aperture and resolution. Following the subtraction process, residual water vapor absorbance in the 1150–1300-cm^{-1} region was less than about 10^{-5}.

Analysis of CH_2 Wagging Modes. For quantitative analysis of the CH_2 wagging modes in the 1150–1300-cm^{-1} region, subtraction of the underlying PO_2^- symmetric stretching band was required. This was accomplished with spectra taken at the highest temperature for that particular sample as a reference spectrum. Subtraction factors were chosen by maximizing the band heights of the progression and choosing a consistent shape for the baseline of the residual contour of the wagging progression components as a function of temperature for a given sample. A flattened baseline was generated for lipid/sterol mixtures for the K = 1–5 progression bands for DPPC by selecting spectral minima at 1280 and 1188 cm^{-1}. Integration of particular progression components was accomplished by transferring Digilab FTS-40 spectra to an off-line microcomputer employing software supplied by D. Moffatt of the National Research Council of Canada. The same end points chosen for baseline leveling were used for integration. Progression intensities were estimated from the integrated area of the K = 4 component alone for DPPC, as this was the feature most easily isolated in the spectrum.

Results

DPPC Interaction with Sterols. The structures of the synthetic sterols with various side chains at C-17 and their trivial names are shown in Figure 1. The nomenclature and T_m data for the phospholipids used in the current investigation are given in Table I. IR spectra of the CH_2 wagging region at three different temperatures for two sterols that exhibit widely different behavior in their interactions with DPPC, namely iso-C10 sterol and iso-C3 sterol at mole ratios of DPPC/sterol of 2:1, are shown in Figure 2. The spectrum collected at the highest temperature for that sample, usually around 80 °C, has been subtracted in each case, as noted in the Experimental Section. This spectral region is complex, as it contains residual (imperfectly subtracted) contributions from the lipid PO_2^- modes as well the CH_2 wagging progressions of the acyl chains with components (as labeled in the figure) near 1199.8, 1221, 1244.2, 1265.4 and 1288.5 cm^{-1}. These bands arise from the K = 1–5 components of the progression, respectively.[15] In addition, there are modes from the sterol present. In spite of difficulties from overlapping bands, it is feasible to isolate the progression bands in each spectrum as shown in Figure 2 and to quantitatively monitor thermally induced intensity changes. Quantitative differences in the intensities of the band progressions are indeed evident as the temperature is raised. The progression intensity at 40.3 °C for iso-C3/DPPC bilayers is much reduced from its intensity at a similar temperature in iso-C10/DPPC bilayers. This immediately reveals the greater occurrence of *all-trans* conformational order in the latter. In addition, at 60.8 °C, the progression in iso-C3/DPPC bilayers is essentially absent, whereas remnants of the pattern are still evident in the iso-C10/DPPC data at 60.3 °C.

The progression intensities (as monitored from the relatively isolated K = 4 mode) as a function of temperature over the range 20–70 °C are plotted in Figure 3 for all of the sterols studied at a DPPC/sterol molar ratio of 2:1. The progression vanishes very close to T_m for DPPC (41 °C) in the androstene/DPPC sample,

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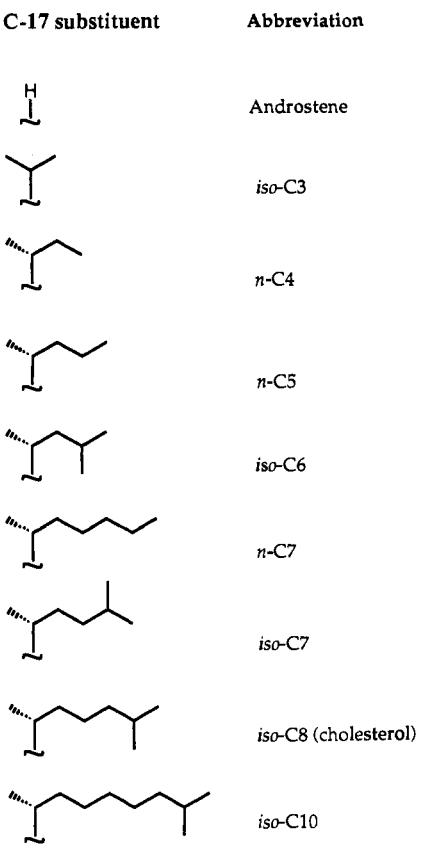

Figure 1. Structures of the C-17 side chain substituted sterols used.

Table I. Phosphatidylcholines Used: Structures, Abbreviations, and T_m Values

chain length: no. of C–C bonds	name of PC	abbreviation	T_m (°C)
12:0	1,2-dilauroyl-PC	DLPC	−1
14:0	1,2-dimyristoyl-PC	DMPC	24.0
16:0	1,2-dipalmitoyl-PC	DPPC	41.5
18:0	1,2-distearoyl-PC	DSPC	54.3
20:0	1,2-diarachidoyl-PC	di-C20-PC	64.1
22:0	1,2-dibehenoyl-PC	di-C22-PC	75
18:1 *trans*	1,2-dielaidoyl-PC	di-C18:1*t*-PC	11
22:1 *cis*	1,2-dieurocyl-PC	di-C22:1-PC	11
24:1 *cis*	1,2-dinervonoyl-PC	di-C24:1-PC	27

while persisting to substantially higher temperatures as the sterol side chain lengthens. Similarly, at temperatures between 25 and 50 °C, a greater relative intensity is observed as the side chain is lengthened. All of the intensity data on the ordinate scale in Figure 3 are normalized at 20 °C. Since the intensity of the sharp progression components reflects specifically the occurrence of *all-trans* conformational order in the acyl chains, the decay in intensity with temperature reflects the addition of *gauche* rotamers to the acyl chains. Conformational order in the DPPC acyl chains clearly persists to higher temperatures as the sterol side chain lengthens. The semiquantitative aspects of these results will be considered in the Discussion.

Cholesterol Interaction with Saturated PCs. The remarkable differential effects of cholesterol itself on acyl chain conformational ordering in a series of disaturated PCs (DLPC through di-C22:0-PC) are depicted in Figure 4. The difference spectra of the wagging progression for 2:1 mixtures of DMPC/cholesterol and di-C20:0-PC/cholesterol are shown at various reduced temperatures ($T^* = T - T_m$, where T_m is the phase transition temperature of the pure phospholipid). The persistence of the wagging progression to at least 35–40 °C above the chain melting temperature for DMPC dramatically depicts the ordering ability of cholesterol in mixtures with relatively short acyl chain PCs. In contrast, for longer acyl chain phospholipids, the progression bands vanish at lower values of T^*, as typified by the data at T^* = 11.5 °C for di-C20:0-PC (Figure 4B).

The temperature dependence of the progression intensity is shown in Figure 5. The persistence of the wagging progression to higher reduced temperature as the phospholipid acyl chain length is increased from 12 to 22 carbons is evident. The inset to Figure 5 shows the temperature at which the progression vanishes for each PC. These temperatures were determined from extrapolations of the linear regions of the temperature–intensity plots. The stabilization of the *all-trans* form is greatest for shorter acyl chain lengths, with di-C22:0-PC showing disappearance of the progression closest to its normal (cholesterol free) transition temperature in contrast to the cases of the DLPC or DMPC systems.

Cholesterol Interaction with Unsaturated PCs. The effect of acyl chain unsaturation on the intensity of the progression bands is shown in Figure 6. To the level of accuracy in this study, the effect of unsaturation on the disappearance of the wagging mode progression is insignificant, i.e., the progression intensity for di-C18:1*t*-PC/cholesterol (2:1 mol ratio) vanishes at about 8 °C above T_m, close to the reduced temperature at which the progression vanishes for DSPC. Similar effects are observed for di-C24:1-PC and di-C22:1-PC.

Discussion

The current data reveal substantial differences between the interactions of a DPPC with a series of synthetic sterols bearing different chains at C-17 as well as in the interactions of a series of PCs with cholesterol. In the former case, sterols possessing longer side chains (7, 8, or 10 carbon atoms) show the greatest ability to inhibit *gauche* rotamer formation in the DPPC acyl chains, as monitored by the persistence of the CH_2 wagging progression with increasing temperature. These restrictions to disordering are progressively reduced as the side chain was shortened; for androstene (no side chain at C-17), the progression vanishes close to T_m for pure DPPC.

For the series of PCs of chain length di-C12 through di-C22, the persistence of the progression was enhanced with increasing temperature, when compared on a reduced temperature scale, for the shorter chain length derivatives (Figure 5). The progression persisted, remarkably, to about 40 °C above T_m for DLPC and DMPC.

Qualitative aspects of the above results can be rationalized from the simple structural model presented two decades ago by Rothman and Engelman,[18] as reinforced from the X-ray diffraction studies of Franks[19] and the ideas of "hydrophobic mismatch" proposed more recently by Bloom and co-workers.[20] The structural model[17] proposes a "cholesterol-stiffened" acyl chain region from the vicinity of the ester moiety to a depth of about seven or so carbons into the bilayer in which the PC acyl chains exist in a relatively ordered state resulting from interactions with the sterol ring system. Beyond this region toward the bilayer center, conformational freedom is suggested to increase substantially, because the cross-sectional area of the rigid sterol nucleus is greater than that of the isooctyl side chain which extends from C-17 of cholesterol. Therefore, segments of the phospholipid acyl chains that come into contact with the sterol side chain are less constrained in their packing compared with acyl chain segments closer to the interfacial regions in each monolayer. Thus as the acyl chains shorten, a greater fraction of the CH_2 groups are in contact with the rigid sterol nucleus, permitting the *all-trans* conformation to persist to a higher temperature, as observed.

Consideration of the effects of the sterol side chain at C-17 is needed to understand their interactions with DPPC, as the sterol

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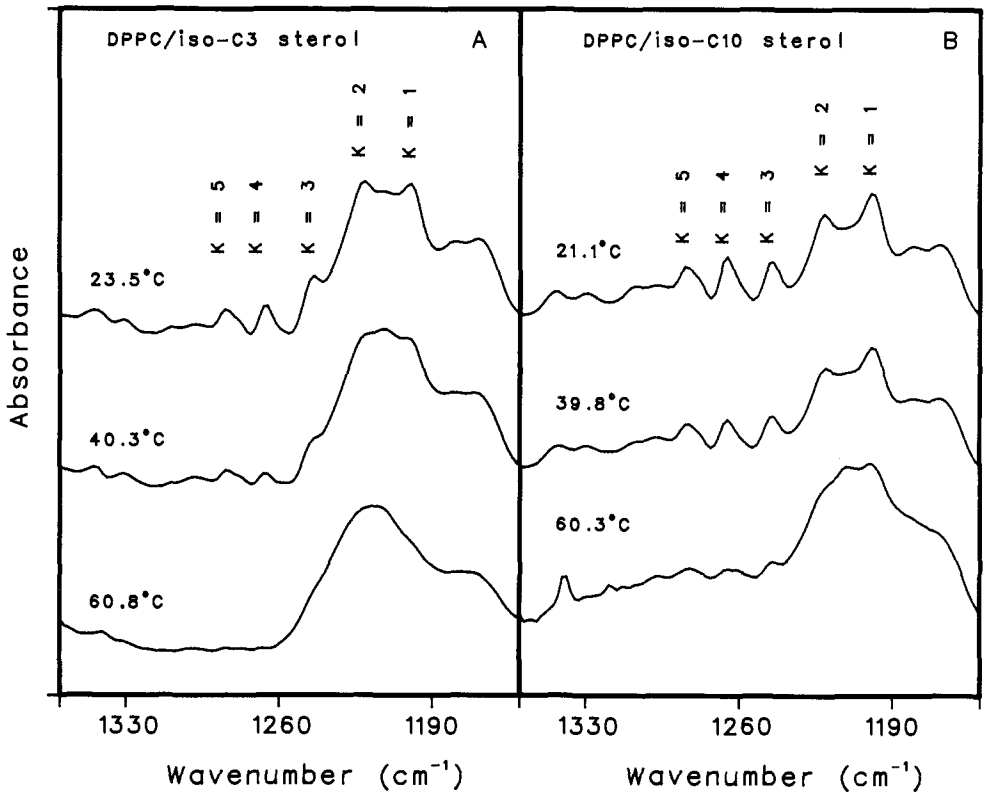

Figure 2. (A) IR spectra of the CH_2 wagging region of a DPPC/iso-C3 sterol (2:1 mol/mol mixture) at three temperatures. The K = 1–5 components of the wagging progression are marked in the 23.5 °C data set. (B) IR spectra of the CH_2 wagging region of a DPPC/iso-C10 sterol (2:1 mol/mol mixture) at three temperatures. The K = 1–5 components of the wagging progression are marked in the 21.1 °C data set. Note the persistence of the progression to higher temperatures in panel B compared with panel A.

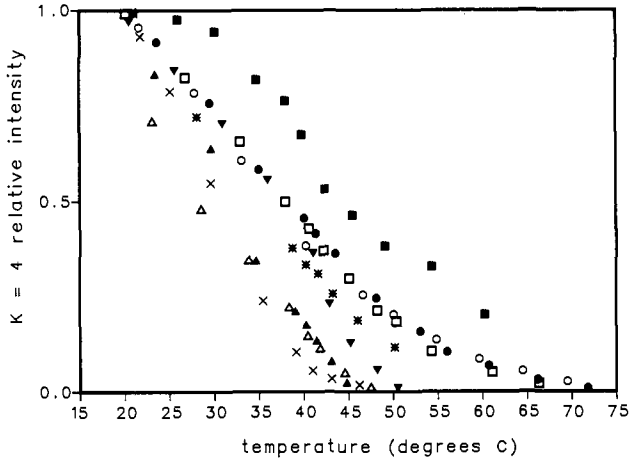

Figure 3. Relative intensities (scaled to 20 °C) of the K = 4 component of the DPPC wagging progression in complexes with sterols (2:1 DPPC/sterol mol/mol) bearing various substituents at the C-17 position: (×) DPPC/androstene; (▲) DPPC/iso-C3; (△) DPPC/n-C4; (*) DPPC/n-C5; (▼) DPPC/iso-C6; (○) DPPC/n-C7; (□) DPPC/iso-C7; (●) DPPC/cholesterol; (■) DPPC/iso-C10.

rings produce (presumably) a fairly constant ordering of the acyl chains in the upper half of DPPC, independent of sterol side chain length. Previous investigations from this laboratory[12,13] have found the following quantitative results on the basis of studies of the CD_2 rocking modes in a series of specifically deuteriated derivatives of DPPC alone and interacting with cholesterol at a 2:1 mole ratio: In the L_α phase of DPPC (50 °C), 3.6–4.2 *gauche* rotamers/chain were formed.[12] In the liquid-ordered phase of a 2:1 cholesterol/DPPC mixture at 50 °C, the extent of conformational disordering was reduced to about 1–1.5 *gauche* bonds/chain.[13] These bonds were nonuniformly distributed along the DPPC acyl chain. For those regions of the acyl chains in contact with the isooctyl side chain of cholesterol, about 12% *gauche* bonds were observed at positions 10, 12, and 13, compared with about 18% in the absence of cholesterol. Thus, formation of *gauche* rotamers is restricted for those regions of the DPPC acyl chains in contact with the side chain of the sterol. The restrictions were much less severe than for those CH_2 groups in contact with the rigid sterol nucleus.[12]

These considerations are relevant to the current results for sterol/DPPC interaction. As the sterol side chain at C-17 is shortened, there is the progressive removal of the constraints to conformational disordering presented by the side chain. Although these constraints are much smaller than those induced by the sterol rings, they nevertheless are sufficient to produce the substantial effects seen in Figure 3.

To convert the progression intensities to a more quantitative measure of chain conformation, several approximations are needed. First, it is assumed that the sharp K = 1–5 progression components measured arise only from the *all-trans* chain acyl chain conformation. The presence of a *gauche* bend anywhere in the chain is assumed to destroy the coupling between oscillators that produces the progression. The justification for this follows. Although it might be anticipated that a *gauche* bend moving along the chain could produce a broad envelope that is the sum of a series of overlapped progressions, each characteristic of the number of CH_2 groups between the *gauche* bend and the C–O bond,[21] our subtraction protocols would tend to minimize any contribution of these relative to the measured intensity of the sharp and relatively well-isolated K = 4 component of the progression. With this assumption, the measured intensity is of the form

$$I \propto \prod_{i}^{N} (p_{trans})_i \qquad (1)$$

where p_{trans} is the *trans* probability at the ith bond and N is the number of bonds (excluding terminal bonds) in the acyl chain so

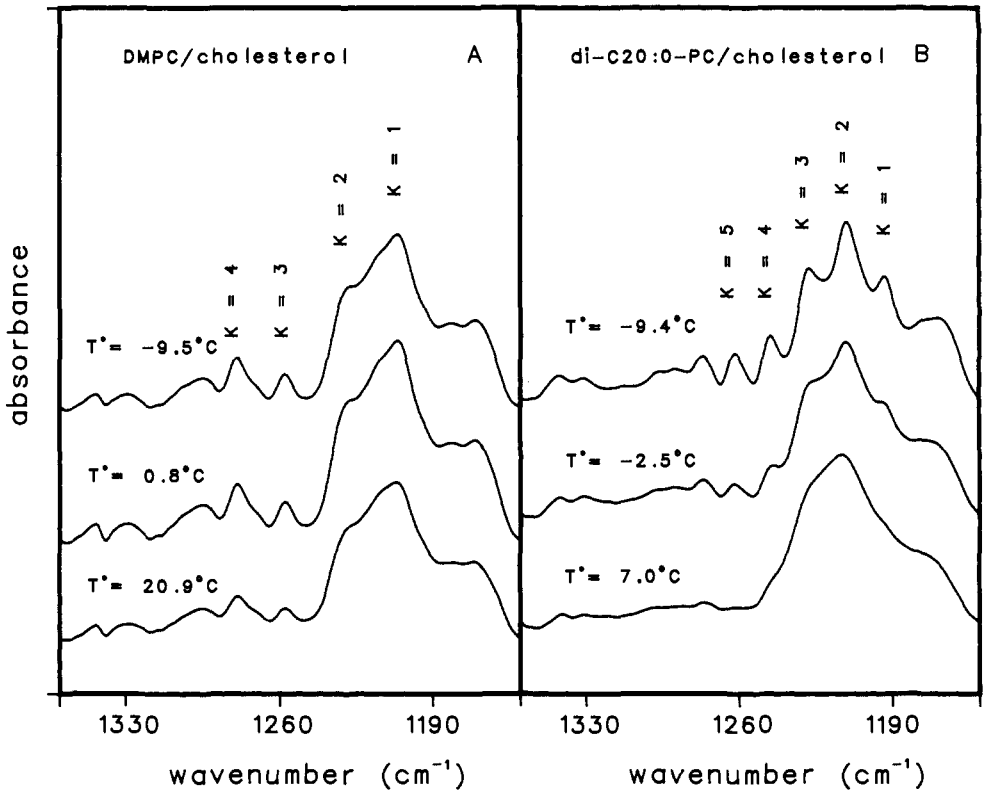

Figure 4. (A) IR spectra of the CH_2 wagging region of a DMPC/cholesterol (2:1 mol/mol mixture) at three reduced temperatures where $T^* = T - T_m$. The $K = 1$–4 components of the wagging progression are marked in the $T^* = -9.5$ °C data set. (B) IR spectra of the CH_2 wagging region of a di-C20:0-PC/cholesterol (2:1 mol/mol mixture) at three reduced temperatures. The $K = 1$–5 components of the wagging progression are marked in the $T^* = -9.4$ °C data set. Note the persistence of the wagging progression components in the DMPC/cholesterol system (panel A) to a much higher T^* than in the di-C20:0-PC/cholesterol system (panel B).

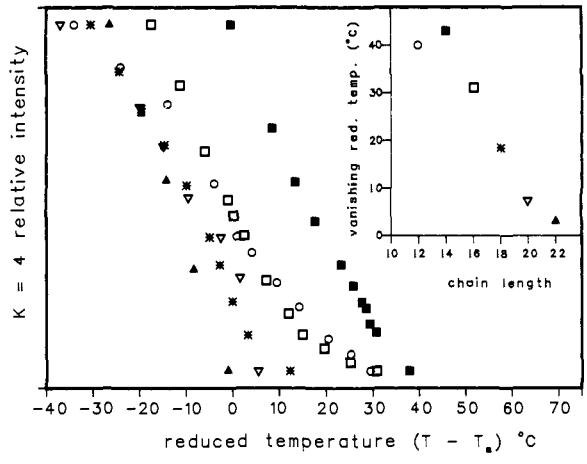

Figure 5. Relative intensities of the $K = 4$ components of the wagging progressions as a function of reduced temperature for a series of PC/cholesterol complexes at 2:1 PC/cholesterol molar ratios, as follows: (○) DLPC/cholesterol; (■) DMPC/cholesterol; (□) DPPC/cholesterol; (*) DSPC/cholesterol; (▽) di-C20:0-PC/cholesterol; (▲) di-C22:0-PC/cholesterol. Nomenclature is given in Table I. Insert: ordinate scale, temperature at which the measured progression intensity vanishes, as determined by extrapolation of the linear regions; abscissa, phospholipid acyl chain length.

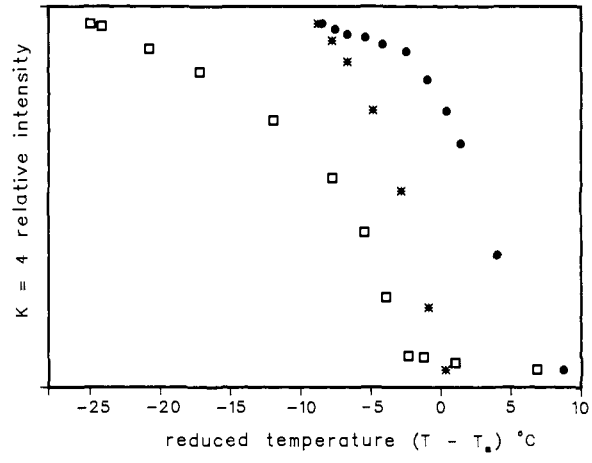

Figure 6. Relative intensities of the $K = 4$ components of the wagging progressions as a function of reduced temperature for a series of unsaturated PC/cholesterol complexes at 2:1 PC/cholesterol molar ratios, as follows: (●) di-C18:1*t*-PC/cholesterol; (*) di-C22:1-PC/cholesterol; (□) di-C24:1-PC/cholesterol.

that $N = 13$ for DPPC. To simplify eq 1 in the absence of data about the position dependence of the *trans* probability for the series of systems studied, a uniform probability of *gauche* bonds along the chain is assumed, leading to

$$I/I_o = (p_{trans})^N \qquad (2)$$

thus, $I = I_o([1 - p_{gauche}])^N$ and the number of gauche bonds in the chain is Np_{gauche}, where $p_{gauche} = 1 - p_{trans}$. Equation 2 is employed assuming a reference intensity (fully ordered state) at 20 °C. Since there may be slight disorder already present in the acyl chains at 20 °C, it is most accurate to interpret the loss of progression intensity at temperatures >20 °C as an *increase* in the number of *gauche* bonds; we anticipate that this will be close to the *actual* number of *gauche* bonds present. The present

(21) Chia, N.-C.; Mendelsohn, R. *J. Phys. Chem.* **1992**, *96*, 10543. It is shown that the presence of acyl chain unsaturation destroys the coupling between the sets of CH_2 groups in ordered phases and produces band progressions with frequencies characteristic of the number of CH_2 groups between the position of the *gauche* bend and the C–O bond, which confers intensity to the progression.

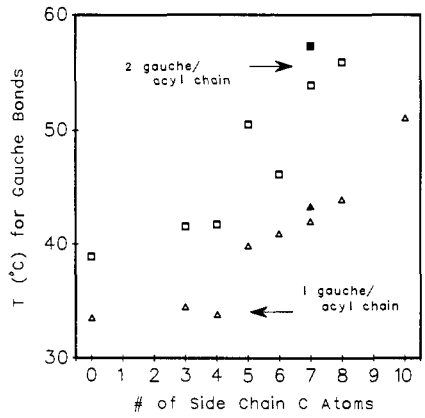

Figure 7. Plot of the temperature needed to introduce *gauche* rotamers into DPPC acyl chains vs sterol side chain length. Ordinate scale, temperature at which one (triangles) or two (squares) additional *gauche* rotamers/chain are found in the DPPC acyl chains; abscissa, sterol side chain substituent length. The doubled points at a side chain length of 7 depict data for the n-C7 (▲) (1 *gauche* bond; (■) two *gauche* bonds) and iso-C7 ((△) 1 *gauche* bond; (□) two *gauche* bonds) sterol derivatives.

calculation also assumes that the *trans* probability is uniform along the chain.

Other models, extending our limited experimental data, will eventually permit a more accurate description of conformational disordering. For example, a possible model in accord with our DPPC/cholesterol CD_2 rocking data[13] might assume that $p_{trans} = 0.96$ at bonds 1–7, $p_{trans} = 0.88$ at acyl chain positions in contact with the sterol side chains, and $p_{trans} = 0.82$ (possibly higher) at other chain positions.[12] As information about the depth dependence of conformational disorder in these sterol/PC mixtures becomes available, eq 1 can be employed.

In Figure 7 the results of the simplest (constant order) model are plotted as the temperature at which an additional one (▲) or two (□) *gauche* rotamers is (are) introduced into the DPPC acyl chains as a function of the sterol side chain length. A reasonably monotonic correlation occurs; the longer the sterol side chain, the higher the temperature required to introduce additional phospholipid acyl chain conformational disorder.

There are few studies extant which address systematically the effects of variation in sterol side chain length on PC conformational order. Singer and Finegold, on the basis of relatively low-sensitivity DSC data, have suggested[22,23] that no differences are evident between the interactions of androstene and cholesterol with DPPC. The current results, in contrast, show that cholesterol provides a much stronger impediment to *gauche* rotamer formation in the DPPC acyl chain. Differences in sensitivity between the DSC experiments of Singer and Finegold and the current FT-IR approach may be responsible for the observed discrepancies. The DSC approach measures the overall enthalpy change during the main endotherm, which presumably includes the contribution of *gauche* rotamer formation. The IR approach measures specifically and sensitively the formation of a small number of *gauche* bonds. High-sensitivity DSC scans[6] measure enthalpies of about 2 kcal mol^{-1} for 2:1 DPPC/cholesterol (mol/mol). While the energy difference between *trans* and *gauche* states of a single *gauche* rotamer forming in a sterol environment is unknown, it is probably less than 2 kcal, as the value in gaseous butane is 508 cal.[24] Since the *gauche* bond may form over a wide range of temperature, this source of enthalpy change in the DSC endotherm may be difficult to extract from the background. The results of recent[25] DSC experiments by McElhaney and co-workers on the interaction of cholesterol and androstene with saturated PCs (carried out at much higher sensitivity than that used by Singer and Finegold) are completely consistent with our current IR observations. They observe that cholesterol increases the main transition temperature of DPPC while androstene decreases it, consistent with greater androstene-induced disordering as we have clearly shown in Figure 3.

The current results for the series of saturated PCs with cholesterol may be directly compared with the studies of McMullen et al.[6] They observed that the phase transition midpoint temperature of the broad endotherm (arising from cholesterol/PC domains) is progressively shifted to higher reduced temperatures as the hydrocarbon chain length of the PC decreases below 17 carbons, with a lesser and eventually opposite effect (although of reduced magnitude) as the chain length increases above 17 carbons. These results are consistent with the observation (Figure 5) of restricted conformational disordering in the shortest PCs, with few restrictions to disordering at the longer chain lengths.

The current results may be also considered within the concept of hydrophobic mismatch. The direction and magnitude of the temperature shifts in the gel–liquid crystal phase transition, when phospholipids are mixed with molecules that possess hydrophobic regions, are expected to be dependent on the extent of the mismatch between the hydrophobic length of the sterol compared with that of the phospholipid hydrocarbon chains.[6,20] Introduction of disorder at T_m effectively shortens the chains. For a constant PC acyl chain length, sterols with greater hydrophobic (i.e. longer side chain) length would be expected to stabilize the bilayer gel phase and produce higher transition temperatures or, in the context of the current FT-IR measurement (Figure 3), restricted conformational disordering.

The IR approaches presented here and in earlier studies provide a powerful direct means to examine conformational order in model membrane systems. We have focused on the CH_2 wagging progression intensity, which we recently have been able to detect in live cells of myristate-, palmitate- and stearate-enriched *Acholeplasma laidlawii B*.[26] Thus the spectra–structure correlations which serve as background for the current work appear to be applicable to a hierarchy of membranes of increasing complexity, including viable cells.

Note Added in Proof: The surface behavior of the synthetic sterols has recently been investigated: Jungeo, M., Vilchèze, C.; Bittman, R. *Biochim. Biophys. Acta*, in press.

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