Biochimica et Biophysica Acta, 640 (1981) 760—766

BBA 79102

EVIDENCE FOR ACYL CHAIN *TRANS/GAUCHE* ISOMERIZATION DURING THE THERMAL PRETRANSITION OF DIPALMITOYL PHOSPHATIDYLCHOLINE BILAYER DISPERSIONS

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(Received September 25th, 1980)

Key words: Bilayer dispersion; Phase transition; Trans/gauche isomerization; Acyl chain; Raman spectroscopy

Summary

In order to clarify, in dipalmitoyl phosphatidylcholine multilayers, the effect of the 34°C thermal pretransition on the acyl chain intramolecular disordering process, Raman spectra of dipalmitoyl phosphatidylcholine gels at 20 and 34°C were compared in the 1000—1200 cm^{-1} skeletal C-C stretching region. In addition to an overall intensity decrease associated with a change in chain packing characteristics, the growth of intensity in the 1080—1090 and 1122 cm^{-1} regions in the (34—20°C) difference spectrum clearly indicates that the thermal pretransition is accompanied by an increase in the population of hydrocarbon chain *gauche* rotamers toward the center of the bilayer.

In describing the molecular origins of the 34°C thermal pretransition of hydrated dipalmitoyl phosphatidylcholine (DPPC) bilayers, both X-ray diffraction and vibrational spectroscopic techniques have provided structural information for defining the bilayer gel phase reorganizations induced by this small thermotropic transition [1—7]. In particular, from X-ray diffraction studies, Janiak et al. [1] concluded that the pretransition is associated with a rippled two-dimensional monoclinic lattice. Furthermore, at the pretransition, the angle of tilt of the fully extended hydrocarbon chains assumes a minimum value with respect to the bilayer normal [1]. In an earlier study, Rand et al. [2], however, suggested that during the pretransition the acyl chains become perpendicular to the bilayer surface. In a Raman spectrocopic study in which the effects of acyl chain *trans/gauche* isomerization were moni-

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tored by following the temperature dependence of the C-C skeletal stretching modes at 1129 and approx. 1090 cm^{-1}, the pretransition region of DPPC was characterized by an increase in *gauche* conformers [3]. The approx. 1090 cm^{-1} *gauche* markers in this study were normalized to a spectrum recorded at −180°C, a temperature for which it is assumed that the chains reflect an all-*trans* conformation. These vibrational data were consistent with the X-ray analysis of Brady and Fein [5] who concluded that the pretransition temperature was accompanied by a partial melting of the acyl chains. In the Raman study by Gaber et al. [6], the *gauche* band, taken at 1080 cm^{-1}, did not increase, however, until after the chain melting phenomenon occurred at the primary phase transition at 41°C. Specifically, the (15—37°C) difference spectrum, where 37°C represented a temperature between the two thermal transitions, indicated an absence of the *gauche* Raman band at 1080 cm^{-1}. In extrapolating infrared intensity results to the Raman experiment, these authors suggested that the absence of the 1080 cm^{-1} feature implied either single *gauche* bonds isolated along the chain or *gauche* bonds located principally near the methyl termini [6]. A recent infrared spectroscopic study demonstrated that the gel phase packing of DPPC bilayers changes from a low-temperature orthorhombic or monoclinic lattice to a near hexagonal lattice at pretransition temperatures [7]. These authors also suggested that the temperature-dependent intensity changes in the Raman spectra previously attributed to differences in the numbers of hydrocarbon chain *gauche* conformers should be carefully evaluated as a consequence of the lattice packing transformations [7].

In view of the caveat stated by Cameron et al. [7] and the varied Raman results regarding the appearance of *gauche* isomers during the thermal pretransition [3,6], we re-examined the 1000—1200 cm^{-1} spectral region of the gel phase of DPPC, at temperatures below and within the thermal pretransition phenomenon, in an effort to assess the use of the 1080—1090 cm^{-1} region for indicating the development of *gauche* conformers in the gel phase environment. Since our Raman spectroscopic experience with pretransition effects particularly for DMPC multilamellar dispersions (Lavialle, F. and Levin, I.W., unpublished observations) indicated the critical nature of the incident laser power, we stress in the present investigation the necessity of low laser power levels (100 mW) and the requirement of signal averaging techniques for obtaining high signal-to-noise ratios in the appropriate difference spectra.

DPPC, stated purity 99%, was purchased from Sigma Chemical Co. and was used without further purification. An aqueous bilayer dispersion was prepared by mechanically agitating a 27% (w/w) lipid/water mixture at 50°C for approx. 10 min. After sealing the sample in a Pyrex capillary, Raman scattered radiation was collected at 90° from the incident 514.5 nm exciting line of a Coherent Radiation CR-3 laser delivering 100 ± 10 mW of power at the sample. The capillary was mounted in a thermostatically controlled holder aligned within the spectrometer sample compartment.

Since spectral subtractions result in the adding of the separate noise levels, we optimized the signal-to-noise ratios for individual spectra by signal averaging 50 scans in the 1000—1200 cm^{-1} spectral region. These data were collected at a scan rate of 1 cm^{-1}/s. Data were acquired, averaged and manipulated with a

Nicolet 1180 computer coupled to a Spex Ramalog 6 spectrometer.

Raman difference spectra for the 20°C gel and 34°C pretransition states are presented in two ways. Fig. 1A shows the data for the gel at 20 and 34°C. Fig. 1B presents the direct difference spectrum for (20—34°C). In order to emphasize the changes arising in the spectrum during the lipid pretransition region, a second difference spectrum was generated in which the 20°C spectrum was subtracted from the 34°C spectrum after normalizing the two spectra to the 1062 cm^{-1} Raman line. These results are displayed in Fig. 2A and B. The difference spectrum in Fig. 2B is expanded by a factor of 4. No computer smoothing routine was applied at any point in the data handling procedures.

The 1062 cm^{-1} band was chosen for normalization of the 1050—1150 cm^{-1} interval in Fig. 2 for several reasons. In general, the use of internal intensity standards in lipid assemblies poses difficulties, since the more intense, isolated features throughout the spectrum tend to manifest line shape, intensity or frequency changes as a function of bilayer perturbations. Since for the comparison of two gel states we are specifically concerned with the introduction of *gauche* bonds along the chains, we seek for normalization purposes a spectral feature, such as the 1062 cm^{-1} C-C stretching mode, that reflects primarily the hydrophobic environment of the bilayer. Normal coordinate calculations

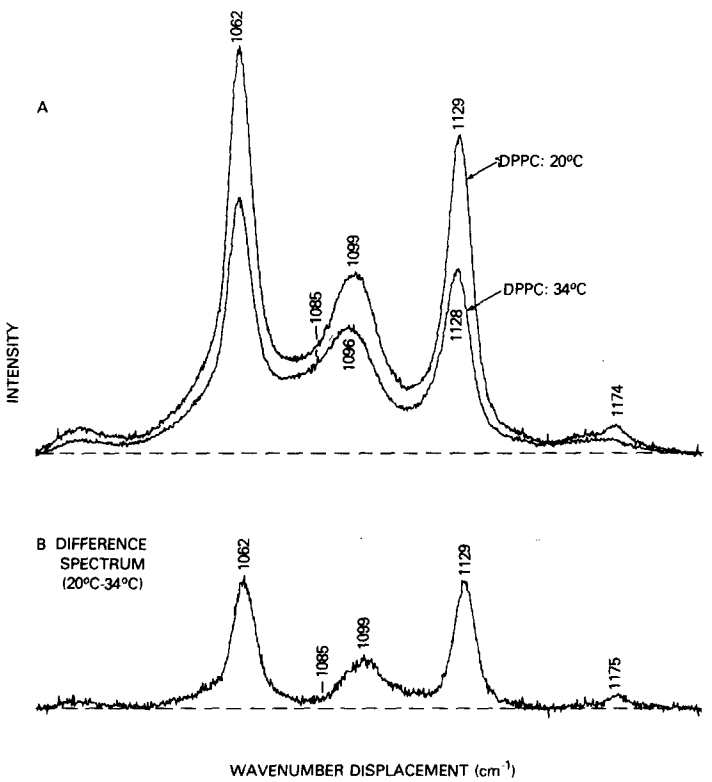

Fig. 1. Raman spectra of DPPC dispersions in the gel state. (A) Superposition of spectra at 20 and 34°C, where 34°C represents the pretransition temperature. Each spectrum represents 50 signal-averaged scans, recorded at rates of 1 cm^{-1}/s. (B) (20—34°C) Difference spectrum.

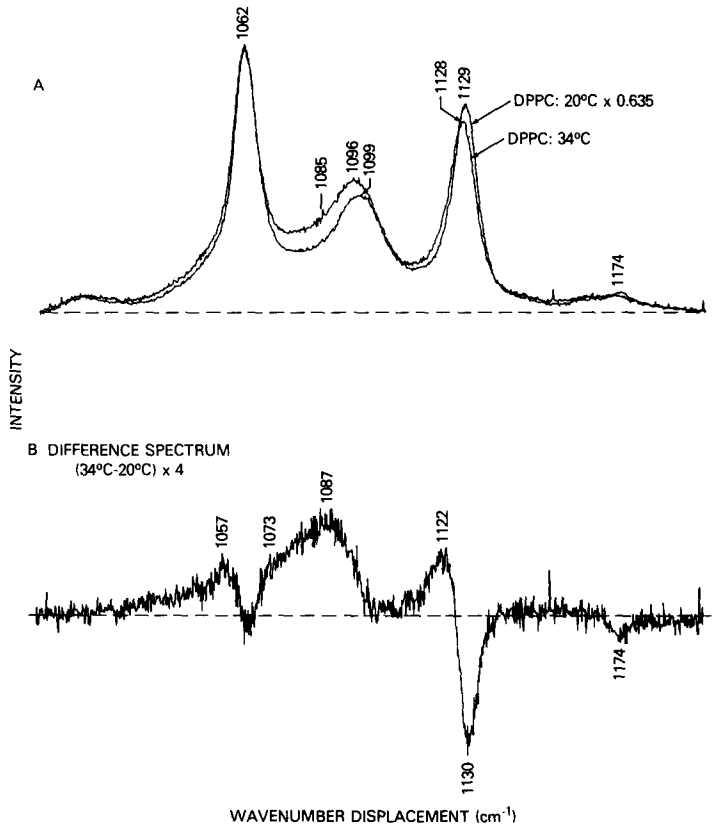

Fig. 2. Raman spectra of DPPC dispersions. (A) Superposition of the 20 and 34°C spectra normalized to the frequency invariant 1062 cm^{-1} feature. (B) Resulting (34—20°C) difference spectrum expanded by a factor of 4.

[9] indicate that the 1062 cm^{-1} C-C stretching vibration is coupled to the methylene wagging coordinates, while in contrast, the 1129 cm^{-1} C-C stretching mode (the other prominent feature in the C-C stretching region) couples significantly to the skeletal chain C-C-C angle bending and terminal methyl rocking coordinates. Thus, the gel-phase intensity changes and frequency shifts for the 1129 cm^{-1} mode will reflect both crystal structure changes and the introduction of *gauche* bonds, since the latter conformational modifications directly alter the chain C-C-C bond angles. The frequency invariant 1062 cm^{-1} C-C stretching mode, however, undergoes intensity changes which suggests a response only to differences in lateral interactions arising from lattice rearrangements. The different behaviour of the 1062 and 1130 cm^{-1} features to intra- and intermolecular interactions is further supported by the observed discrepancies in intensity and band width changes induced in dimyristoyl phosphatidylcholine bilayers by temperature perturbations (Susi, H., Byler, D.M. and Damest, W.C., personal communcation). In particular, the half-width changes in the 1062 cm^{-1} band are considerably greater on increasing temperature than those for the 1130 cm^{-1} feature. This behaviour suggests that the 1062 cm^{-1} mode more sensitively reflects the differences in chain

mobility that arise as the lateral interactions, which characterize the lipid lattice, are decreased. Since the decrease in spectral intensity in going from 20 to 34°C is generally uniform throughout the 1050—1150 cm^{-1} C-C stretching region and since the 1062 cm^{-1} line provides a marker primarily for observing the effect of changes in lateral interactions on the skeletal C-C stretching modes, the normalization of the two spectra to the 1062 cm^{-1} feature essentially corrects for the decrease in Raman scattering efficiency as the lattice packing characteristics change. The remaining changes in the spectrum are then attributed to differences in intramolecular *trans-gauche* isomerization.

Fig. 1A illustrates that the peak height intensities in the 1000—1200 cm^{-1} C-C stretching region spectrum of the DPPC gel at its 34°C pretransition temperature are approx. 35—40% less intense than the transitions for the gel phase spectrum at 20°C. This effect has been observed before by ourselves and others [6] and is probably associated with the alterations in chain packing that were observed by infrared techniques [7].

For the gel state, three acyl chain all-*trans* C-C stretching modes are assigned at 1129, 1099 and 1062 cm^{-1} [4,6]. As the population of *gauche* conformers increases, the contour and intensity of the 1099 cm^{-1} feature change due to the appearance of a feature in the 1085—1090 cm^{-1} region [4,8]. Weak contributions to the 1062 and 1070—1080 cm^{-1} regions from underlying C-O and symmetric PO_2^- stretching modes are relatively unimportant when considering the C-C stretching modes in this spectral region. In comparing the 1085—1100 cm^{-1} region of the 20 and 34°C spectra in Fig. 1A, the asymmetry of the contour toward low frequencies in the 34°C spectrum, as well as a 3 cm^{-1} shift in the peak maximum, indicates definite growth in the intensity at approx. 1085 cm^{-1}. Another way of viewing the effect is to note that the slope of the contour in the 1085—1090 cm^{-1} region increases in the 34°C spectrum relative to the 20°C spectrum. The difference spectrum (20—34°C) (Fig. 1B) implies that the pretransition 34°C spectrum exhibits a significantly greater intensity in the 1085 cm^{-1} region in comparison to the lower temperature spectrum. That is, the return of the contour to the baseline at approx. 1085 cm^{-1} in the difference spectrum indicates growth of the 1085 cm^{-1} *gauche* bond marker in the 34°C spectrum relative to that of the lower temperature spectrum.

While the spectrum of Fig. 1B demonstrates all of the changes which occur as DPPC is heated from 20°C to the pretransition state at 34°C, the difference spectrum shown in Fig. 2B, based upon normalizing the two spectra to the 1062 cm^{-1} feature (Fig. 1A), renders more apparent the intramolecular spectral changes between the pretransition and low-temperature gel states. For Fig. 2B the low-temperature spectrum is subtracted from the high-temperature spectrum such that for visual convenience the intensity above the base line reflects growth of Raman intensity accompanying the temperature increase.

Fig. 2B indicates a substantial intensity increase for the *gauche* marker at approx. 1087 cm^{-1}. The derivative shape of the 1122—1130 cm^{-1} portion of the difference spectrum indicates the frequency shift of the 1129 cm^{-1} feature on the introduction of *gauche* conformers. In addition, a comparison of Fig. 2A and B suggests the appearance of additional intensity at approx. 1120—1222 cm^{-1}. The appearance of the approx. 1122 cm^{-1} feature, which has been

discussed previously [8], has been interpreted on the basis of normal coordinate treatments [9] as an acyl chain C-C stretching mode for a *gauche* bond involving the terminal methyl group. A decrease in the population of all-*trans* chain segments is reflected by the additional intensity decrease for the 1129 cm^{-1} C-C stretching mode after approximately compensating for the effect of intermolecular changes on the vibrational mode.

Other features of the difference spectrum (Fig. 2B) reflect increased intensities at 1057 and approx. 1073 cm^{-1}, assigned to the ester C-O and symmetric PO_2^- stretching modes, respectively, and a decreased intensity at 1174 cm^{-1}. Support of the 1073 cm^{-1} assignment to the phosphate stretching mode comes from several lipid systems. In particular, the symmetric PO_2^- stretching mode has been assigned at 1081 cm^{-1} in the Raman spectra of polycrystalline lysophosphatidylcholine samples at ambient temperatures [10]. Raman spectra for anhydrous DL-DPPC samples recorded at liquid N_2 temperatures show a doublet with features at 1077 and 1073 cm^{-1} which are assigned to crystal field components for the phosphate mode (Hill, I.R. and Levin, I.W., unpublished data). Analogous Raman splittings for L-DPPC at liquid N_2 temperatures appear at 1082 and 1074 cm^{-1} (Hill, I.R. and Levin, I.W., unpublished data). The additional intensity and suggested derivative nature of the curve at 1057 and 1073 cm^{-1} in the 34°C spectrum may result from increases in mobility about the lipid headgroup and interfacial regions which, in turn, induce band broadening effects. Finally, the 1174 cm^{-1} Raman line has been assigned to a chain CH_2 rocking mode coupled to the CH_3 rocking mode [4,9].

In summary, a comparison of the 1000—1200 cm^{-1} acyl chain C-C stretching intervals of DPPC gels at 20 and 34°C, the pretransition temperature region, shows two general spectral effects. First, an overall 35—40% intensity decrease occurs for the spectrum reflecting the pretransition. This effect is associated with a gradual change in chain packing as the lattice undergoes a transformation from the orthorhombic or monoclinic state to the near hexagonal state at pretransition temperatures [7]. Second, the (34—20°C) difference spectrum shows a significant increase in the 1087 cm^{-1} *gauche* bond marker which indicates that intramolecular chain disordering occurs during the thermal pretransition. The simultaneous appearance of a feature at approx. 1122 cm^{-1} suggests that the *gauche* rotamers involve the terminal methyl groups toward the center of the lipid bilayer.

Acknowledgement

We wish to acknowledge several interesting discussions with Dr. David Cameron.

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