

RAMAN INTENSITIES OF CARBON-CARBON STRETCHING MODES IN A MODEL MEMBRANE

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Laser-Raman spectra of L- α -dimyristoylphosphatidylcholine (DMPC) liposomes in the spectral range 1000–1200 cm^{-1} were obtained as a function of temperature from -80 to $+50^\circ\text{C}$. The triplet found in this spectral region was resolved into Lorentzian components by means of an iterative computer program. The peak intensities, band widths, and band areas of the resolved 1062 cm^{-1} and 1130 cm^{-1} bands, assigned to C–C stretching vibrations of *trans* segments, were evaluated as a function of temperature. While the peak intensities of the bands decrease substantially with temperature, the band widths show a considerable increase. The change in band areas is therefore smaller than the change in peak heights. Experiments with all *trans* carboxylic acids showed that in these compounds the area of the Raman bands at 1062 cm^{-1} and 1130 cm^{-1} is proportional to the number of *trans* bonds. The variation with temperature of the number of *trans* and *gauche* bonds in the studied phospholipid is reflected by the change of the area of the 1130 cm^{-1} Raman band.

Introduction

Liposomes of 1,2-dimyristoylphosphatidylcholine (DMPC) and 1,2-dipalmitoylphosphatidylcholine (DPPC) exhibit three Raman bands in the spectral region of 1000–1150 cm^{-1} which have been frequently used to study *trans-gauche* isomerism in model membranes [1–4]. Sharp bands close to 1062 and 1130 cm^{-1} have been assigned to C–C stretching modes of *trans* segments, a broader band centering around 1080 cm^{-1} primarily to C–C modes of *gauche* segments [1–5]. The peak intensities (peak heights) of these bands have been used to evaluate the average number of *trans* bonds under a given set of conditions [2] and to estimate thermodynamic parameters associated with *trans-gauche* isomerization [3,6,7]. It is generally understood that integrated Raman intensities (band areas) should be used for such calculations, but peak heights have been employed because of the difficulties involved in resolving the Raman triplet into its constituent bands. Even if band areas are employed, it must be shown that a band is caused by a pure vibrational mode which can be assigned to the stretching of carbon-carbon bonds, and that effects caused by end-groups are small.

We have examined the Raman spectra of a series of crystalline *all-trans* straight chain monocarboxylic acids from C₄ to C₁₈ and measured the peak intensities as well as the areas of the '*trans* bands' at 1062 cm⁻¹ and 1130 cm⁻¹ as a function of the length of the *all-trans* chains. The intensity of the carboxyl band at 1627 cm⁻¹ was used as a reference. The area, but not the peak intensity, of these bands was found to be proportional to the length of the *all-trans* hydrocarbon chains. Subsequently we resolved the above mentioned triplet of the spectrum of DMPC in the 1000 to 1150 cm⁻¹ region into individual components by means of a digital computer and measured the area of the 1062 cm⁻¹ and 1130 cm⁻¹ Raman bands as a function of temperature. Based on these results, we believe that the present data come closer to reflecting *trans-gauche* isomerism in model membranes than those of previous studies.

Materials and methods

High purity DMPC was obtained from Sigma Chemical Company* and its purity was confirmed by thin-layer chromatography. Liposomes were prepared by weighing out phospholipid and distilled water in a 2 : 3 ratio and mixing for 10 min on a vibrator-type mixer above the transition temperature of the phospholipid. Highest purity straight-chain carboxylic acids from C₄ (butyric acid) to C₁₈ (stearic acid) were purchased from Nuchek Preparations, Elysian, Minn. Raman spectra were obtained with a Spex Ramalog System equipped with an RCA C-31034 photomultiplier, photon counting, a Spectra-Physics Model 165-8 Argon ion laser, and a Spex 180° viewing platform. Spectra of DMPC liposomes in the temperature range from -80°C to 0°C were obtained with a Harney-Miller type variable temperature cell [8]. Spectra of the liposomes above 0°C were measured with the help of a brass-jacketed cell constructed for 180°C excitation and connected with a water bath as previously described [9]. The spectra of the two lowest melting carboxylic acids were obtained with the Harney-Miller cell at approx. -40°C. The spectra of the remaining higher homologues were taken with the brass-jacketed cell at 0°C. To insure that the carboxylic acids were all in the same crystalline modification, all samples were recrystallized from the melt in the Raman cell prior to taking their spectra. The laser power was approx. 300 mW at the sample; the 514.5 nm laser line was used for excitation. The Raman spectrometer was interfaced to a Modcomp III digital computer.

The observed triplet in the 1000-1150 cm⁻¹ region of the Raman spectra of DMPC was resolved into Lorentzian components by means of a computer program which uses Gauss-Newton iteration. The input information includes the following: (1) The analytical nature (shape) of the resolved bands; a Lorentzian form was found to yield satisfactory agreement between the observed and calculated curves

*Reference to brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

(see below). (2) The number and initially estimated centers of the bands to be resolved. Three bands were assumed, although the actual number may be higher, because the central band is itself likely to be a composite [5] which cannot be easily resolved. (The area of this band is therefore not representative of the intensities of any single Raman mode and is not used for structural calculations).

The iterative computer program seeks the best agreement between observed and calculated curves by varying the slope and intercept of the linear baseline, the peak positions (frequencies), the peak heights (peak intensities), and the half-widths of the resolved bands. The output includes final peak positions, peak heights, and half-widths. (The areas of Lorentzian peaks are proportional to the product of the peak height and half-width.) For each spectrum analyzed, a plotted diagram shows the experimental curve, the calculated curve, and the individual resolved peaks.

The area of the 715 cm^{-1} C-N stretching band, which is independent of temperature [2], was used as an internal intensity reference.

Results and discussion

X-ray diffraction data of single crystals show that even numbered *n*-carboxylic acids crystallize from the melt in the *C* form, with the hydrocarbon chains in an essentially all-*trans* conformation, although the structure is somewhat distorted

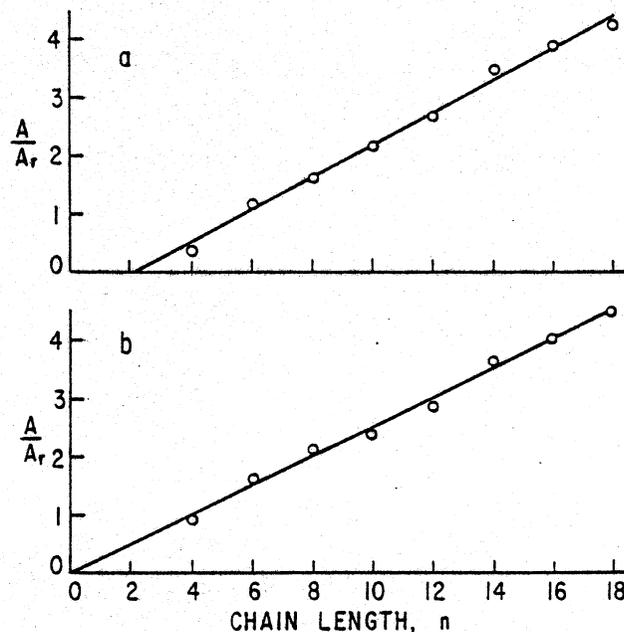


Fig. 1. Dependence of the intensity (a) of the 1130 cm^{-1} Raman band and (b) of the 1060 cm^{-1} Raman band of carboxylic acids on the length of the all-*trans* hydrocarbon chains. Band area divided by band area of reference band ($\text{C}=\text{O}$ stretching mode, 1627 cm^{-1}).

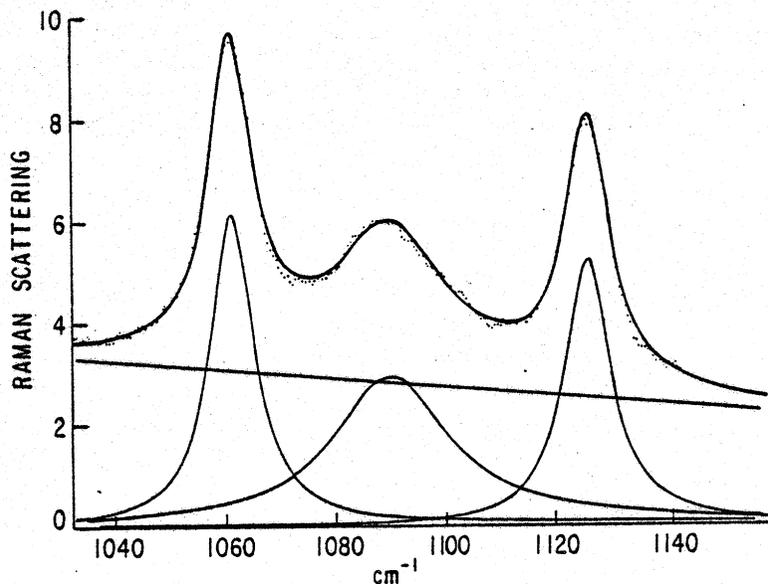


Fig. 2. Upper curves: computer plots of observed (dotted line) and calculated (solid line) Raman spectra of DMPC in the 1020–1150 cm^{-1} range at 0°C. Lower curves: resolved Raman bands.

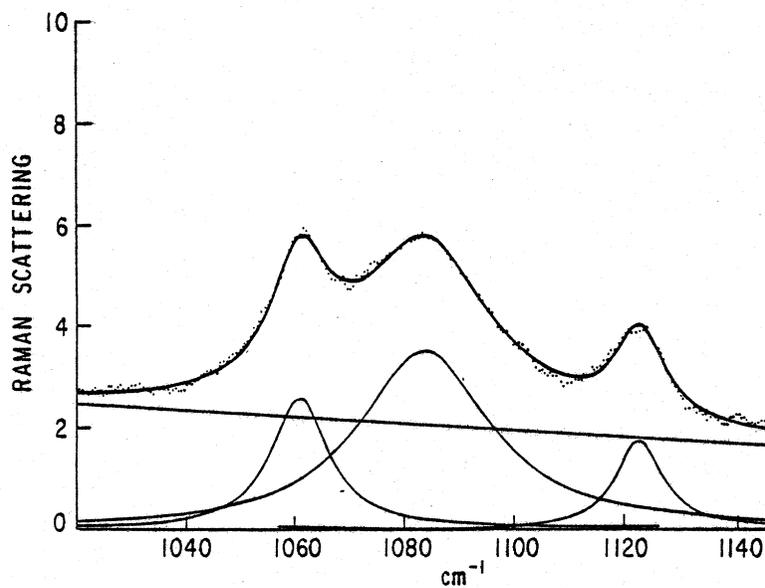


Fig. 3. Upper curves: computer plots of observed (dotted line) and calculated (solid line) Raman spectra of DMPC in the 1020–1150 cm^{-1} range at 30°C. Lower curves: resolved Raman bands.

near the carboxyl group [10, 11]. Similar results have been obtained by Raman spectroscopy, which show that the length of the regular all-*trans* segments for fatty acids with $n < 20$ is $n - 2$, where n is the total number of carbon atoms [12]. There are no *gauche* bonds, however.

Figure 1 gives the intensities of the 1062 cm^{-1} and the 1130 cm^{-1} Raman bands for a series of crystalline all-*trans* monocarboxylic acids as a function of chain length. Straight lines are observed for both bands if band areas are employed, indicating that the vibrations are close to pure stretching modes of *trans* C-C bonds, and that end-group effects are small. (No simple relation could be observed if peak heights were used as a measure of band intensity). The intensity curve of the 1062 cm^{-1} band intersects the abscissa at $n = 0$, that for the 1130 cm^{-1} band, at $n = 2$. Because no '*trans*' bonds can be defined below a chain length of four carbon atoms, the curve for the 1130 cm^{-1} band is more representative of a pure *trans* C-C stretching vibration.

Figure 2 shows the Raman spectrum of DMPC at 0°C in the 1020 to 1150 cm^{-1} region as resolved by the computer into three Lorentzian components. Figure 3 shows the corresponding data obtained at 30°C . The agreement between observed and calculated curves appears satisfactory. Resolved spectra of this kind were obtained for DMPC at regular intervals from -60°C to 50°C .

Figure 4 shows the peak intensity and the half-width of the resolved 1130 cm^{-1} '*trans*-band' of DMPC as a function of temperature from 0°C to 50°C . The peak

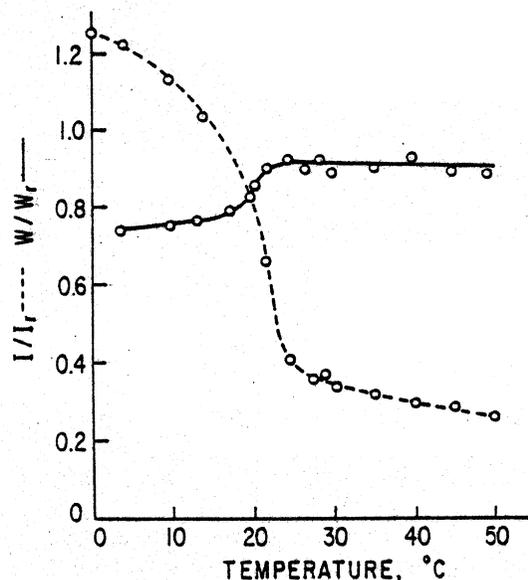


Fig. 4. Change with temperature in the peak height (dotted line) and half-width (solid line) of the 1130 cm^{-1} Raman band of DMPC. The 715 cm^{-1} Raman band was used as internal reference.

intensity and band width of the 715 cm^{-1} Raman band, which do not change with temperature, are used as internal references with an assigned value of 1. The peak intensity of the 1130 cm^{-1} band decreases considerably with increasing temperature and shows a sudden decrease at the transition temperature of 21°C . At the same time, the band width shows a marked increase with increasing temperature, with a particularly steep rise around the transition temperature. The change in the integrated intensity, which for Lorentzian peaks is proportional to the product of peak height and half-width, is thus much smaller than the change in peak height. The change in band width indicates a loosening of the *trans* domains of the hydrocarbon chains, which evidently takes place in addition to the *trans-gauche* isomerism. The half-width of the 1130 cm^{-1} Raman band attain a constant value at about -50°C .

Figure 5 shows the change of the area of the 1130^{-1} Raman band with temperature in the 0 – 50°C range as a fraction of the maximum area as obtained at -50°C . Assuming that at temperatures below -50°C the hydrocarbon chains are in an all-*trans* conformation [6] and designating this maximum area as A_0 , the quantity of A/A_0 at any given temperature reflects the average fraction of C–C bonds which are in the *trans* conformation. It is seen that a sudden decrease of integrated intensity takes place at the transition temperature, but the change is considerably smaller, than the change observed in band heights (cf. Figs. 4 and 5).

(We have not been able to observe the pretransition of DMPC with Raman spectroscopy, although it is easily detected by differential scanning calorimetry [9].)

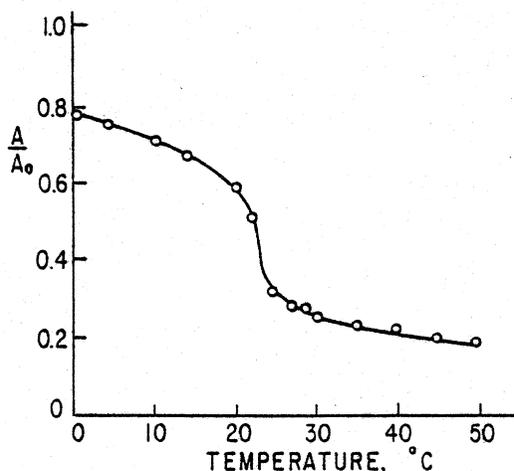


Fig. 5. Change with temperature of the integrated area of the 1130 cm^{-1} Raman band of DMPC. At approx. -50°C the band area shows no further change. If we denote this value as A_0 , and assume that all bonds are in a *trans* conformation below -50°C , A/A_0 reflects the fraction of C–C bonds in the *trans* conformation.

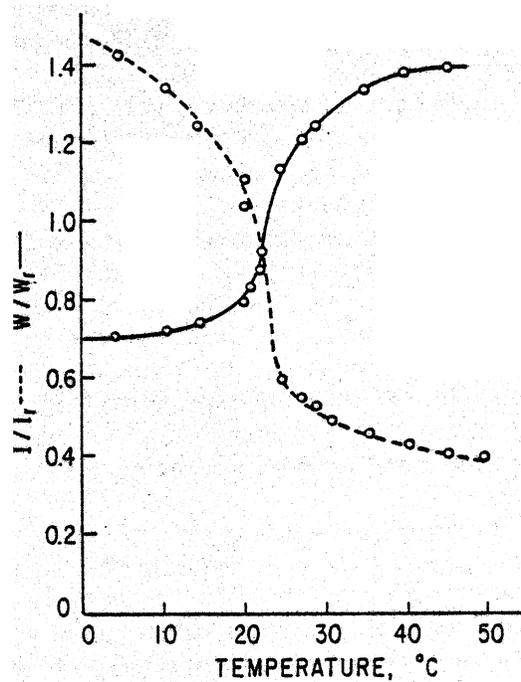


Fig. 6. Change with temperature of the peak height (dotted line) and half-width (solid line) of the 1062 cm^{-1} Raman band of DMPC. The 715 cm^{-1} band was used as internal reference.

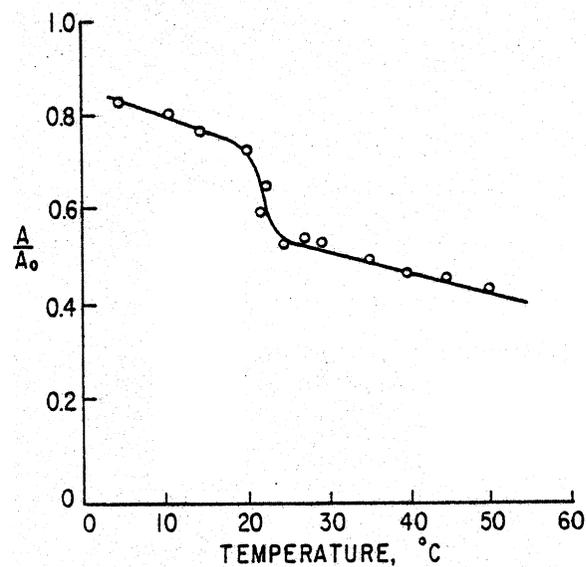


Fig. 7. Change with temperature of the integrated area of the 1062 cm^{-1} Raman band of DMPC. A_0 is the area at -50°C , A the area at any given temperature.

Figures 6 and 7 give analogous results observed with the 1062 cm^{-1} band. The change of half-width of this band is considerably larger than the corresponding change in the 1130 cm^{-1} band (cf. Figs. 4 and 6) and the relative change in band area is smaller (cf. Figs. 5 and 7).

It is not immediately obvious what causes the discrepancy in the change of band widths and band areas of the two bands. They are commonly assigned to in-phase (1130 cm^{-1}) and out-of-phase (1060 cm^{-1}) [14] C-C stretching modes of *trans* segments of hydrocarbon chains [1-4,13]. The results with carboxylic acids (Fig. 1) suggest that the 1130 cm^{-1} band is a better measure of the length of *trans* C-C segments than the 1063 cm^{-1} band. The relatively small change of the width of the 1130 cm^{-1} band over the studied temperature range also suggests that we are dealing with well-defined vibrations, i.e., the in-phase symmetric CC stretching modes of *trans* segments. For pure C-C vibrations the frequency of this mode should be independent of chain length [13]. This has been verified by studies on *n*-paraffins [14]. By contrast, for the out-of-phase C-C stretching modes of *n*-hydrocarbons, normal coordinate calculations [15] do not reveal such a stable band.

In general, we conclude that for both investigated bands, the change with temperature of the peak height is different from the change of the band area, because of the inverse change of the band width; that the temperature variation of these three band parameters differs for the two bands; and that the area of the 1130 cm^{-1} band probably provides the best measure of *trans* bonds in the sample.

Acknowledgment

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