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EFFECTS OF CHAIN PACKING AND CHAIN MOBILITY ON THE RAMAN SPECTRA OF BIOMEMBRANES

ROBERT G. SNYDER [a,b], JAMES R. SCHERER [b] and BRUCE P. GABER [c]

[a] *Department of Chemistry, University of California, Berkeley, CA 94720,* [b] *Western Regional Research Center, Science and Educational Administration, U.S. Department of Agriculture, Berkeley, CA 94710 and* [c] *Optical Probes Branch, Naval Research Laboratory, Washington, DC 20375 (U.S.A.)*

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Summary

The degree of lateral crystal-like order between hydrocarbon chains in biomembrane systems can be estimated from Raman measurements in the C-H stretching region. Observations on the temperature dependence of the Raman spectra of crystalline *n*-$C_{16}H_{34}$ and the urea clathrate of *n*-$C_{16}H_{34}$ have enabled us to separate to some extent the overlapping effects of chain packing and chain mobility, effects that are normally not distinguished in considering lateral order. The mobility is associated with the freedom of an extended chain to rotate and twist about its long axis. A high degree of such motion must be ascribed to *n*-$C_{16}H_{34}$ in a urea clathrate in order to explain the unusual temperature behavior observed for Raman bands at 2885 and 1174 cm^{-1}. Comparison of the temperature behavior of the Raman spectra of the clathrate with that of crystalline *n*-$C_{16}H_{34}$ permits the effects due to packing and to mobility to be distinguished. The same effects can be expected to be present in the Raman spectra of biomembranes.

Introduction

The C-H stretching region of the Raman spectra of biological and model membranes is widely used to characterize and to monitor changes in the structure of the hydrocarbon component (Ref. 1 and references therein). The peak-height ratio, $I(2885)/I(2850)$, has been found to be particularly sensitive to the physical state of these systems [2,3], its value being affected by both chain conformation and chain packing [4,5]. A parameter that measures 'lateral crys-

tal-like order' in hydrocarbon-chain assemblies has in fact been defined in terms of this ratio [5].

We report here on a more precise definition of lateral order, suggested from observations on the temperature behavior of certain Raman bands of extended *n*-alkanes contained in urea clathrates. The unusual temperature behavior exhibited by these complexes has significant implications concerning the concept of lateral order for hydrocarbon chains in biomembrane systems. Specifically, these findings show that the value of the ratio, $I(2885)/I(2850)$, commonly used as a measure of lateral order, is affected by two different factors: chain packing and chain mobility.

Experimental Procedure

A urea clathrate of hexadecane (n-$C_{16}H_{34}$) was prepared by mixing hexadecane dissolved in decalin with urea dissolved in methanol as described in Ref. 6. The complex was washed several times with isopentane. Raman spectra were measured using a computer-controlled double monochromator (Spex 1401) with a cold photomultiplier (RCA 31034A). The control and photon counting systems have been described [7]. The excitation was the 5145 Å line of an argon-ion laser.

Results

The C-H stretching region of the Raman spectrum of a urea clathrate is essentially devoid of urea bands. This region is shown in Fig. 1 for the n-$C_{16}H_{34}$ complex at five temperatures ranging from 25 to −140°C. While all bands become significantly narrower with decreasing temperature, the narrowing is exceptional in the case of the intense methylene antisymmetric C-H stretching fundamental [4] near 2885 cm^{-1}. The narrowing is accompanied by a shift in the position of the band maximum. The shift is about 4 cm^{-1} to lower frequencies in going from 25 to −140°C. This temperature behavior is to be contrasted with that observed for crystalline n-$C_{16}H_{34}$, of which the spectra in the C-H stretching region are shown in Fig. 2 for the sample at 10 and −140°C. For the 2885 cm^{-1} band, the ratio of its halfwidth at high temperature to that at low temperature is about 1.3, which may be compared with a value near 10 estimated for the clathrate. In the case of crystalline n-$C_{16}H_{34}$, there is only a relatively small downward shift (approx. 1 cm^{-1}) of the peak position of the 2885 cm^{-1} band accompanying a temperature change from 10 to −140°C.

Most of the differences between the Raman spectrum of n-$C_{16}H_{34}$ in the clathrate and the spectrum of crystalline n-$C_{16}H_{34}$ may be explained qualitatively in terms of differences between crystal structures. In both structures, the *n*-alkane molecules are fully extended, i.e., are in the all-*trans* conformation. Intramolecular vibrations of the chains are thus essentially the same in both systems. At the intermolecular level the situation is quite different. In the clathrate, the *n*-alkane molecules are separated from one another by urea molecules. The latter form a hexagonal lattice, the 'infinitely' long channels of which are filled with extended hydrocarbon chains packed end to end [8]. For crystalline *n*-alkanes, the chains are in lateral contact [9] so that, relative to the

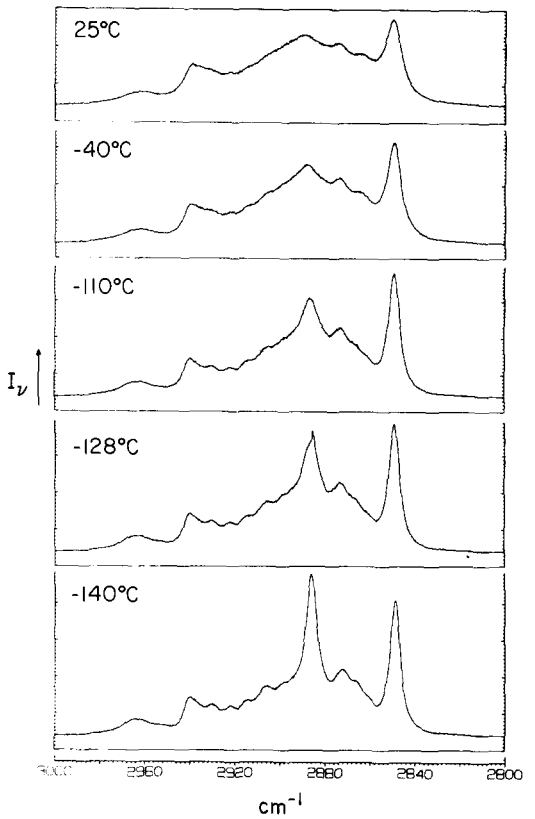

Fig. 1. Raman spectra of the n-$C_{16}H_{34}$: urea clathrate in the C-H stretching region at 25, —40, —110, —128 and —140° C. The resolution is 2 cm^{-1}.

clathrate, intermolecular coupling between vibrational modes is favored while rotational mobility about the long axis of these molecules is severely constrained.

First, we shall consider differences in the spectra arising from differences in intermolecular vibrational coupling. Such differences are evident in the spectra of the clathrate and the crystalline n-alkane in the region between 2900 and 2940 cm^{-1}. The spectrum of the clathrate at —140°C shows a series of bands in this region that have been assigned to methylene scissors overtone vibrations [10] (Fig. 1). The corresponding series of bands in the spectrum of the pure n-alkane at —140°C is not apparent because, in the crystal, these bands are broadened as a result of the dispersion of frequencies of the scissors modes caused by lateral interaction between methylene groups on adjacent chains [4] (Fig. 2). Other differences attributable to intermolecular coupling are found in the region near 2850 cm^{-1}. Here, the clathrate shows a single narrow band while the crystal shows two broader bands separated by about 10 cm^{-1}. We can be certain that the presence of two bands is due to intermolecular effects because they are observed only in the spectra of those n-alkanes, such as n-$C_{16}H_{34}$, that have the triclinic structure and not in the spectra of the orthorhombic or monoclinic forms [4].

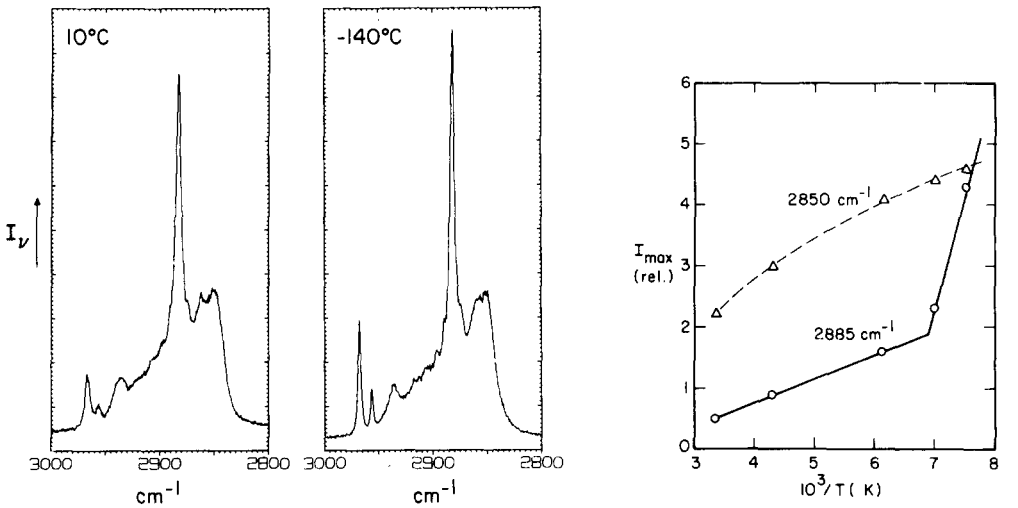

Fig. 2. Raman spectra of crystalline n-$C_{16}H_{34}$ in the C-H stretching region at 10 and —140° C. The resolution is 1 cm^{-1}.

Fig. 3. Plot of observed peak intensities of the 2885 and 2840 cm^{-1} bands of the n-$C_{16}H_{34}$: urea clathrate vs. $1/T$(K) (the 2885 cm^{-1} band resides on a broad background (Refs. 4 and 10); data from Fig. 1).

We now turn to the effects of chain mobility. We attribute the extraordinary breadth of the 2885 cm^{-1} band observed for the clathrate at room temperature to this factor. The breadth appears to be a consequence of the proclivity of the hydrocarbon chain to librate (rotate and twist) about its long axis. Considerable freedom for librational motion is indicated from NMR [11] and thermodynamic [12] studies. That this type of motion contributes heavily to the observed broadening is suggested by the fact that this mode and another at 1175 cm^{-1}, which displays an analogous temperature behavior (to be discussed later), both belong to the symmetry species, B_{1g}, that contains the rigid rotation of the molecule about its long axis, (modes have been classified into symmetry species under the factor-group, D_{2h}, of the infinite chain according to the species conventions contained in Ref. 13). The observed peak intensity of the 2885 cm^{-1} band is plotted against $1/T$ in Fig. 3 (this band resides on a broad background which was measured in Ref. 10). The data show a discontinuity near —128°C, a temperature about midway between the temperature (—121°C) of the principal order-disorder transition and that (—138°C) of a secondary transition reported for this clathrate from calorimetric measurements [12]. The nature of these transitions is not yet clear. X-ray studies [14] indicate that in going to lower temperatures, the lattice goes from hexagonal to orthorhombic at the principal transition and then undergoes an asymmetric contraction of its channel dimensions. Such changes would restrict the freedom of the n-alkane to undergo librational motion and would account in part for the more rapid narrowing of the 2885 cm^{-1} band that occurs below the transition temperature.

The temperature dependence of the peak height of the 2850 cm^{-1} band is indicated in Fig. 3 for comparison. This band is a component of the methylene C-H symmetric stretching vibration and belongs to the totally symmetric sym-

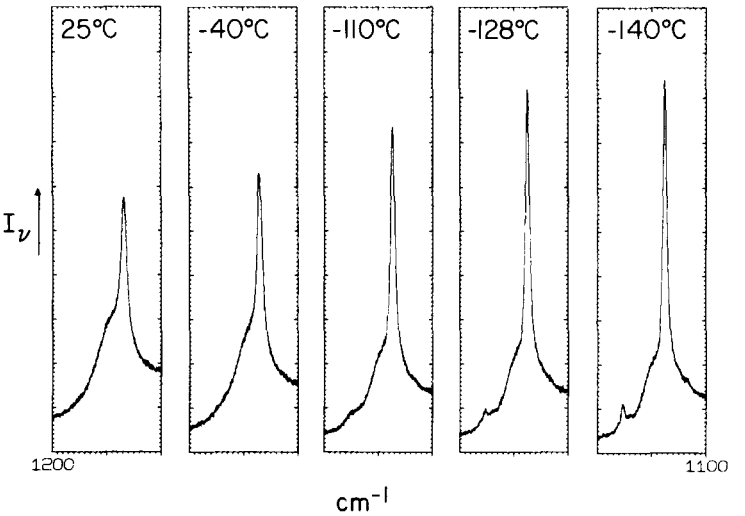

Fig. 4. Raman spectra of the n-$C_{16}H_{34}$: urea clathrate in the methylene rocking (1175 cm^{-1}) and symmetric C-C stretching (1140 cm^{-1}) fundamental region (1200—1100 cm^{-1}) at 25, —40, —110, —128 and —140°C. The resolution is 2 cm^{-1}.

metry species, A_g [4,10]. Unlike the 2885 cm^{-1} band, the temperature behavior of this band displays no discontinuity in passing through the phase transition.

As mentioned earlier, there is a second B_{1g} Raman-active vibration that shows basically the same temperature behavior as the 2885 cm^{-1} band. This band, which is the methylene rocking fundamental [13], appears weakly at 1175 cm^{-1}. Its temperature dependence may be compared in Fig. 4 with that of the intense C-C stretching A_g fundamental at 1140 cm^{-1}. At room temperature, the B_{1g} band at 1175 cm^{-1} is extremely broad and appears to be lost in the background. After the sample is cooled to a temperature in the vicinity of the transition, this band comes into evidence and at —140°C is quite narrow. In contrast, the halfwidth of the A_g band at 1140 cm^{-1} undergoes a much smaller and more continuous change.

Discussion

The temperature phenomena reported here indicate that the term, 'lateral crystal-like order', as it has been used earlier in describing the environment of the fully extended chain in membrane structures includes two separate factors, the first associated with chain packing, i.e., with the static structure of the system, and a second associated with chain mobility, i.e., with the dynamic behavior. While the existence of static and dynamic factors has been suggested [5], their effects on vibrational spectra have not previously been distinguished even though they can be quite different.

Static effects are present in the spectra at all temperatures, but are most clearly in evidence at low temperature. They are manifest in large part in the complexities in band structure that result from intermolecular interaction in the crystalline state [15] as we have seen here for crystalline n-$C_{16}H_{34}$.

Dynamic effects are associated with vibrational relaxation and tend to be

more highly temperature dependent than static effects. They are manifest primarily in determining band shapes as observed here for the 2885 and 1175 cm^{-1} bands of the n-$C_{16}H_{34}$ clathrate near room temperature.

For hydrocarbon assemblies such as those existing in membranes, the value of the intensity ratio, $I(2885)/I(2850)$, is affected both by static and by dynamic factors. That this ratio is sensitive to one type of static factor, intermolecular coupling, has been demonstrated earlier through isotopic dilution experiments [4,5]. Thus, when a hydrocarbon chain is isolated in an environment consisting of its perdueterated analogue, the value of this ratio is changed significantly relative to its value in the undiluted crystal. This occurs because intermolecular coupling with neighboring chains is essentially eliminated so that the 2850 cm^{-1} band is narrowed (and hence the peak height, $I(2850)$, is increased) while the 2885 cm^{-1} band is relatively unaffected. In fact, the Raman spectrum of the isotopically isolated n-alkane in the CH stretching region is similar to that of the clathrate at low temperature: in both cases the effects of intermolecular coupling are small. On the other hand, in going to higher temperatures, the spectrum of the isotopically isolated n-alkane is relatively unaffected while that of the clathrate shows the effects of mobility in that the 2885 cm^{-1} band is broadened.

The degree of mobility of extended segments of hydrocarbon chains in a typical biomembrane system will lie somewhere between that for the chain in the clathrate and in the crystalline n-alkane. Between these extremes there can be considerable variation as is indicated, for example, by the difference in the values of $I(2885)/I(2850)$ for phospholipid dispersions and vesicles as measured by Gaber and Peticolas [5]. Certainly, as the authors argue, the lower value associated with the vesicles indicates a more open structure and hence a greater chain mobility. This difference in packing structure between the dispersion and vesicle states implies, however, differences in intermolecular coupling as well. The quantitative significance of this ratio, or an order parameter derived from it, is made ambiguous by the presence of two influencing factors. Thus, while the usefulness of the ratio for characterizing the environmental state of a system remains, this ratio by itself should not be presumed to yield a unique quantitative measure of lateral order.

Before quantitative significance can be attached to the band intensities and band shapes observed for these systems, additional understanding is clearly needed. For this reason, we are continuing the present study of environmental effects on the intensities and shapes of bands in the Raman and infrared spectra of those hydrocarbon systems that have relevance to biomembrane bilayer structures.

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