Biochimica et Biophysica Acta, 443 (1976) 613—617

BBA Report

BBA 71264

DEUTERATED FATTY ACIDS AS RAMAN SPECTROSCOPIC PROBES OF MEMBRANE STRUCTURE*

R. MENDELSOHN, S. SUNDER and H.J. BERNSTEIN

Division of Chemistry, National Research Council of Canada, Ottawa, Ontario K1A OR6 (Canada)

(Received June 16th, 1976)

Summary

Raman spectra are reported for the C-D stretching region of stearic acid-d_{35} bound in egg lecithin multilayers. The temperature dependence of the spectra shows that the linewidth of the C-D stretching bands is a sensitive and non-perturbative probe of membrane hydrocarbon chain conformation. The utility of this approach for studying lipid conformation in membranes containing a significant fraction of non-lipid component is discussed.

Several recent publications have demonstrated that Raman scattering is a useful technique for studying the hydrocarbon chain conformation in biological membranes and related model systems [1—10]. The two spectral regions most commonly used for this purpose are: (i) The region 2800—3000 cm^{-1} involving the C-H stretching vibrations, and (ii) The region 1000—1150 cm^{-1} involving the C-C stretching modes of the hydrocarbon chains and the symmetric P-O stretching vibration of the phosphate moiety.

The use of these vibrations to probe lipid conformation in complex biological systems containing a substantial amount of non-lipid component is limited by the fact that the non-lipid components also contribute to the scattering in these spectral regions. This renders accurate determination of the lipid contribution to the spectrum extremely difficult. In order to overcome this problem, we have inserted predeuterated stearic acid into a model membrane system and monitored the Raman scattering arising from the C-D stretching vibrations of the probe molecule as a function of temperature above and below the membrane gel-liquid crystal phase transition. It is the purpose of this communication to show that the Raman spectrum of the C-D stretching vibrations, in particular the linewidth of the CD_2 stretching

*N.R.C.C. No. 15393.

modes, is a sensitive and non-perturbative probe of the hydrocarbon chain conformation. As the C-D stretching vibrations occur in a spectral region free from interference from the vibrations of non-lipid membrane components, extension of this approach to complex systems appears feasible.

The model membrane system we have examined consists of an aqueous suspension of egg lecithin lamellae doped with 10 mol% of stearic acid-d_{35}. Egg lecithin (chromatographically pure) was obtained from Lipid Products, Surrey, U.K., and stored at −4°C prior to usage. Stearic acid-d_{35}, the generous gift of Dr. I.C.P. Smith, had been purchased from Merck, Sharpe, and Dohme of Canada. The initial mixture was prepared from $CHCl_3$ solutions of the components. The solvent was evaporated under nitrogen gas and the last traces removed in a vacuum dessicator. Water was added and the aqueous suspension was agitated on a vortex mixer and centrifuged at low speeds (1000 rev./min) in order to concentrate the aggregates. The samples so obtained were sealed in the 1 mm inner diameter capillaries used for Raman studies.

Our Raman equipment and thermostatted cell have been described previously [9]. Excitation in the current experiments was provided by an Ar^+ laser tuned to produce ≈ 350 mW at 5145 Å at the sample. The reported frequencies are accurate to 2 cm^{-1}, and temperatures are accurate to ±2°C. The spectra of the C-D stretching region for stearic acid-d_{35} in the polycrystalline and melt phases are shown in Figs. 1C and 1D. The spectra are very similar to those reported by Larsson [5]. The C-D stretching region for pure stearic acid-d_{35} below its melting point (Fig. 1C) is characterized by an intense,

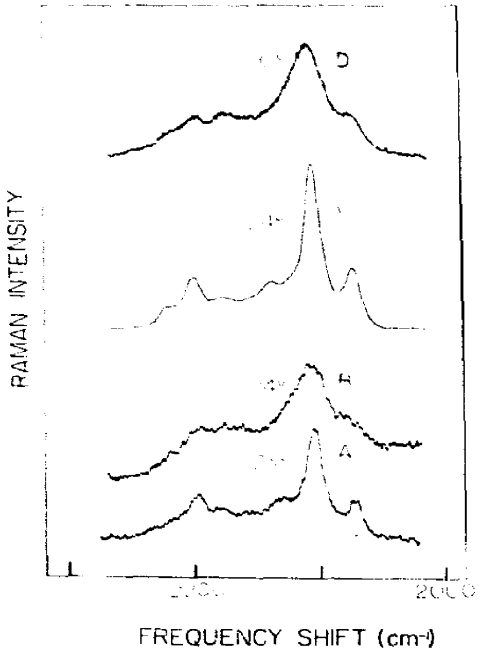

Fig.1. Raman spectra of the C-D stretching region for: (A) Stearic acid-d_{35} bound in egg lecithin lamellae at −23°C; (B) Stearic acid-d_{35} bound in egg lecithin lamellae at 24°C; (C) Polycrystalline stearic acid-d_{35} at 24°C; (D) Stearic acid-d_{35} in the melt phase at 70°C. Spectral parameters-slit width 5 cm^{-1}; time constant 1 s; scan speed 1 cm^{-1}/s, laser power 350 mW at 5145 Å.

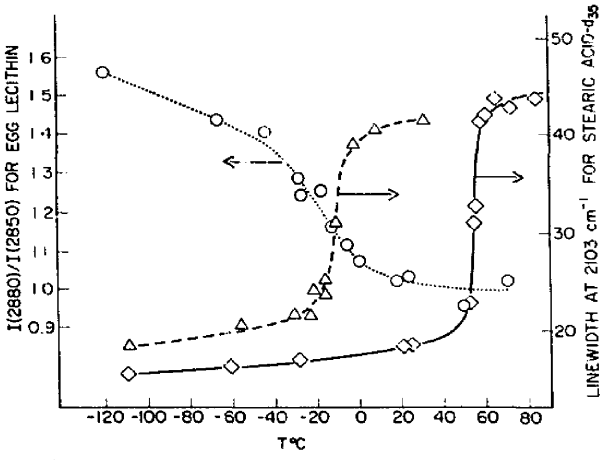

Fig. 2. Temperature dependence of: The linewidth of the 2103 cm^{-1} band for pure stearic acid-d_{35}. (◇); The linewidth of the 2103 cm^{-1} band for stearic acid-d_{35} bound in lecithin multilayers, (△). These curves have their ordinate scale on the right. The linewidth measurement refers to the full width at half maximum. The intensity ratio of the C-H stretching modes at 2880 and 2850 cm^{-1} for egg lecithin multilayers (○), as a function of temperature is shown with its ordinate scale on the left.

fairly sharp feature near 2103 cm^{-1}, assigned to the symmetric CD_2 stretching modes [11]. In addition, there are two medium intensity bands at about 2072 and 2200 cm^{-1} and several weaker features whose origins have been discussed previously [5,12]. In the melt phase at 70°C drastic changes are noted in this spectral region (Fig. 1D). The band at about 2103 cm^{-1} broadens considerably while the other features are smeared out and become overlapped to a large extent. This type of behaviour is typical of changes observed in Raman spectra on going from ordered to disordered phases [13—15] The detailed temperature dependence of the linewidth of the band at 2103 cm^{-1} is shown in Fig. 2 (solid line). At temperatures below about 40°C, the linewidth corresponds to that of the molecule in the all-*trans* chain conformation [16,17]. The sharp increase in linewidth at about 55—57°C indicates a phase transition involving increased disorder and the onset of molecular reorientation in the melt phase compared with the crystalline phase. In addition to these intermolecular effects, it is known that significant formation of *gauche* isomers occurs above T_m [17]. The observed increase in the linewidth is therefore due to a combination of intermolecular and intramolecular disorder and molecular reorientation in the melt*.

The C-D stretching regions of the spectra of stearic acid-d_{35} bound in egg lecithin multilayers at —23 and 24°C are shown in Figs. 1A and 1B, respectively. The spectrum at —23°C resembles that of the pure crystalline solid below its melting point (compare Figs. 1A and 1C). At 24°C, however, the spectrum of the bound probe resembles that of the pure stearic acid-d_{35} in the melt phase, although the temperature is ≈ 40°C below the melting point. The temperature dependence of the linewidth of the 2103 cm^{-1} band for the bound fatty acid is shown in Fig. 2 (dashed lines). The observed sigmoid shaped curve

*The observed discontinuity in the linewidth at 2103 cm^{-1} for pure stearic acid-d_{35} occurs at about 7°C below the visible melting point of 63°C. This may indicate formation of a plastic phase below the melting temperature [26].

demonstrates the presence of a cooperative transition at −11 ± 2°C. This transition temperature of −11°C corresponds reasonably well to that of the gel-liquid crystal phase transition of egg lecithin as measured by calorimetric methods [18]. Thus, the linewidth of the CD_2 symmetric stretching mode used to characterize the transition seems to monitor the hydrocarbon chain conformation of the membrane in which it is bound. At 24°C the chains of egg lecithin are primarily in the *gauche* conformation [3,18]. This is consistent with the observation that the bound fatty acid at 24°C exists in a conformation similar to that of the melt phase of the free molecule at 70°C and contains many *gauche* bends in the chain,

In order to verify that the transition temperature of the model membrane is not significantly perturbed by the probe molecule, the formation of *gauche* isomers in the hydrocarbon chains of egg lecithin was directly measured using the intensity ratio of the 2880 and 2850 cm^{-1} C-H stretching vibrations as a structure indicator. This procedure has been used previously in other phospholipid systems [19]. The temperature dependence of the $I(2880)/I(2850)$ ratio is shown in Fig. 2 (dotted line) and indicates a melting temperature of −14 ± 4°C. The intensity ratios $I(2880)/I(2850)$ shown in Fig. 2 are those for a sample of pure egg lecithin multilayers in H_2O. In addition a virtually identical plot was obtained for egg lecithin in the presence of 10 mol% stearic acid-d_{35}. It is clear from Fig. 2 that the melting temperature is determined less accurately by the intensity ratio measurements than by the linewidth measurements. However, the melting temperatures as determined by the two methods are the same within the experimental error, suggesting that insertion of the probe does not significantly perturb the hydrocarbon chain conformation. This result confirms those previously obtained by a variety of experimental methods, including ESR [20] and ^1H [21,22] and ^2H NMR [23] spectroscopy. In a recent direct study of this problem, Podo and Blaisie [22] have shown that introduction of stearic acid into dipalmitoyl phosphatidyl choline bilayers above the transition temperature does not modify to any significant extent the spin-lattice relaxation rates of either the $N^+(CH_3)_3$ head group or the chains' methylene groups even at phospholipid/fatty acid molar ratios of 2:1. In addition, the present experiments suggest that no strong perturbation occurs below the phase transition temperature due to the addition of fatty acids at the concentration used here, as shown by the following.

The plot of the C-D stretching linewidth vs. temperature for bound stearic acid is independent of the thermal history of the sample over the temperature range studied. That is, the halfwidth value at a given temperature as well as the temperature of the discontinuity in the halfwidth vs. temperature plot is the same whether the data are collected during a heating or cooling cycle. This argues against segregation of the fatty acid on cooling, since if segregation were to occur, the stearic acid C-D stretching linewidth would not be sensitive to the phospholipid phase transition on heating a previously cooled sample. In addition, the $I(2880)/I(2850)$ plot for doped egg lecithin is not only virtually identical to that for pure egg lecithin in H_2O but also independent of the thermal history of the sample. This suggests that the presence of the probe does not perturb the structure of the

phospholipid, insofar as the C-H stretching region is a measure of the structure.

As we have previously noted, in systems containing a significant fraction of non-lipid component, it will be difficult to use the C-H stretching vibrations to directly monitor lipid conformation. The approach outlined here permits that determination in a non-perturbative fashion, as the results clearly illustrate the sensitivity of the CD_2 stretching vibration linewidths of the deuterated probe molecule to conformational change and molecular motion in membrane systems. Although a quantitative analysis [14] of the factors that determine the linewidths in the present system is difficult, the following observations may be pertinent.

(i) At −110°C, where effects due to thermal motion are expected to be small, the linewidth of the bound stearic acid is significantly greater than for the pure stearic acid-d_{35} (Fig. 2), indicating that the bound stearic acid is in a less ordered environment. This disorder is due in part to the wide distribution of hydrocarbon chain lengths in egg lecithin and relatively imperfect packing of the chains even at such low temperature.

(ii) The 2103 cm^{-1} band monitored in the current study involves contributions from 16 CD_2 groups of the stearic acid. Consequently, the current observations refer to an average over all chain positions. We are now investigating the spectra of bound stearic acids deuterated at specific chain positions in order to understand the variation of conformation and molecular motion through the membrane bilayer. The results will allow a direct comparison with observations recently reported from ^2H NMR spectroscopy [23—25] and ought to lead to a more detailed understanding of hydrocarbon chain conformation and motion.

We thank Dr. I.C.P. Smith for supplying the stearic acid-d_{35}.

References

1 Lippert, J.L. and Peticolas, W.L. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1572—1576
2 Lippert, J.L. and Peticolas, W.L. (1972) Biochim. Biophys. Acta 282, 8—17
3 Mendelsohn, R. (1972) Biochim. Biophys. Acta 290, 15—21
4 Larsson, K. and Rand, R.P. (1973) Biochim. Biophys. Acta 326, 245—255
5 Larsson, K. (1973) Chem. Phys. Lipids 10, 165—176
6 Verma, S.P., Wallach, D.F.H. and Schmidt-Ullrich, R. (1975) Biochim. Biophys. Acta 394, 633—645
7 Lippert, J.L., Gorczyca, L.E. and Meiklejohn, G. (1975) Biochim. Biophys. Acta 382, 51—57
8 Milanovich, F.P., Yeh, Y., Baskin, R.J. and Horney, R.C. (1976) Biochim. Biophys. Acta, 419, 243—250
9 Mendelsohn, R., Sunder, S. and Bernstein, H.J. (1975) Biochim. Biophys. Acta 413, 329—340
10 Mendelsohn, R., Sunder, S. and Bernstein, H.J. (1976) Biochim. Biophys. Acta 419, 563—569
11 Jones, R.N. and Ripley, R.A. (1964) Can. J. Chem. 42, 305—325 and references therein
12 Sunder, S., Mendelsohn, R. and Bernstein, H.J. (1976) Chem. Phys. Lipids in the press
13 Hornig, D.F. (1949) J. Chem. Phys. 17, 1346
14 Bertie, J.E. and Sunder, S. (1973) J. Chem. Phys. 59, 3853—3855
15 Bertie, J.E. and Sunder, S. (1974) Spectrochim. Acta 30A, 1373—1384
16 Phillips, M.C. (1972) in Progress in Surface and Membrane Science, Vol. 5., Academic Press, New York
17 Snyder, R.G. (1967) J. Chem. Phys. 47, 1316—1360
18 Chapman, D. (1973) in Form and Function of Phospholipids (Ansell, G.B., Dawson, R.M.C. and Hawthorne, J.N., eds.), p. 127, Elsevier Scientific Publishing Co., Amsterdam
19 Brown, K.G., Peticolas, W. and Brown, E. (1973) Biochem. Biophys. Res. Commun. 54, 358—364
20 Saitô, H., Schreier-Muccillo, S. and Smith, I.C.P. (1973) FEBS Lett. 33, 281
21 McLaughlin, A., Podo, F. and Blaisie, J.K. (1973) Biochim. Biophys. Acta 330, 109—121
22 Podo, F. and Blaisie, J.K. (1976) Biochim. Biophys. Acta 419, 1—18
23 Stockton, G.W., Polnaszczek, C.F., Leitch, L.C., Tulloch, A.P. and Smith, I.C.P. (1974) Biochem. Biophys. Res. Commun. 60, 844—850
24 Seelig, A. and Seelig, J. (1974) Biochemistry 13, 4839—4845
25 Stockton, G.W., Polnaszczek, C.F., Tulloch, A.P., Hasan, F. and Smith, I.C.P. (1976) Biochemistry 15, 954—966
26 McClure, D.W. (1968) J. Chem. Phys. 49, 1830—1839