

RAMAN SPECTROSCOPY OF FATTY ACID
BLODGETT-LANGMUIR MULTILAYER ASSEMBLIES

L. J. Lis, S. C. Goheen and J. W. Kauffman

Biomedical Engineering Center, Northwestern University
The Technological Institute, Evanston, Illinois 60201

Received August 1, 1977

SUMMARY: The Raman Spectra of Blodgett-Langmuir multilayer assemblies made from behenic acid, barium behenate and barium cis-13 erucate are reported. In particular, the peak height intensity ratio of the hydrocarbon chain methylene C-H stretch Raman bands, I_{2890}/I_{2850} , for each multilayer assembly is compared to that of phosphatidylcholine in powders and water dispersions as well as to samples of crystalline hydrocarbon chains. It is found that the fatty acid multilayers are more ordered than the phospholipid samples but less ordered than the crystalline samples. It is suggested that Blodgett-Langmuir multilayer assemblies of lipid might be a useful reference in quantitative studies of packing order in lipid phases.

INTRODUCTION

Recently Gaber and Peticolas (1) and Mendelsohn and co-workers (2) have extended the initial work of Larsson (3) and Larsson and Rand (4) using the lipid C-H stretch Raman modes to determine acyl chain packing structure, to the study of the differences in packing order between unsonicated and sonicated phospholipid dispersions. Larsson and Rand (4) have shown that the peak height intensity ratio of the symmetric methylene C-H stretch modes, I_{2890}/I_{2850} , is sensitive to the packing order of the acyl chains in the liquid crystalline state, decreasing in the order; lamellar liquid crystal > hexagonal or cubic liquid crystal > micellar solution > solution in an organic solvent. Subsequently, Gaber and Peticolas (1) have devised an order parameter describing acyl chain lateral packing from the peak height intensity ratio I_{2890}/I_{2850} as first suggested by Larsson and Rand (4). However, Gaber and Peticolas (1) used the I_{2890}/I_{2850} peak height intensity ratio for crystalline and liquid alkanes (hexadecane) as reference states representing highly ordered and highly disordered acyl chains respectively. This ratio I_{2890}/I_{2850} was given the designation I_{CH_2} in their lateral order parameter:

$$S_{\text{lateral}} = \frac{I_{\text{CH}_2}(\text{sample}) - I_{\text{CH}_2}(\text{liquid hexadecane})}{I_{\text{CH}_2}(\text{crystalline hexadecane}) - I_{\text{CH}_2}(\text{liquid hexadecane})}.$$

Green, et al (5) examined Blodgett-Langmuir multilayers of glycerides and phospholipids of various chain length and suggested that multilayers of lipid should be considered as oriented crystals. Indeed, Kopp, et al (6) has shown that multilayers of fatty acids formed by the Blodgett-Langmuir technique undergo a time dependent structural rearrangement into micro-crystals. In this study, we report, for the first time, the Raman spectra of Blodgett-Langmuir multilayers of various fatty acids and relate their apparent crystallinity as obtained from the acyl chain methylene C-H stretch peak height intensity ratio I_{2890}/I_{2850} to that of phosphatidylcholine in solid and dispersed forms through the use of a recently developed lateral chain packing order parameter (1). These results indicate that Blodgett-Langmuir multilayers of fatty acids generally exhibit a relatively high degree of acyl chain crystallinity as compared to crystalline hexadecane. However, higher order can be obtained by forming a crystalline phase. This and similar multilamellar data can be used for comparing acyl chain packing order in phospholipids. In addition these multilamellar systems may be suitable for examination using Raman spectroscopy for a variety of studies including studies of the effects of radiation on acyl chain structure and packing such as has recently been investigated using Infra-red attenuated total reflection (ATR) spectroscopy (7).

EXPERIMENTAL

Blodgett-Langmuir multilayers (8) were prepared on an epoxy wafer using behenic acid (50 bilayers), barium behenate (40 bilayers) or barium cis-13 erucate (50 bilayers). Due to the time involved in shipping the formed multilayers between laboratories, only aged samples (6) were studied. 1,2 L- α dipalmitoyl phosphatidylcholine (DPL) and 1,2 L- α distearoyl phosphatidylcholine were obtained from Calbiochem and used without further purification (DSL). Crystalline behenic acid was obtained from Sigma and used without further purification. Water was de-ionized and doubly distilled. Unsonicated dispersions were prepared according to techniques which have been described elsewhere for the preparation of unsonicated dispersions of phosphatidylcholines (9).

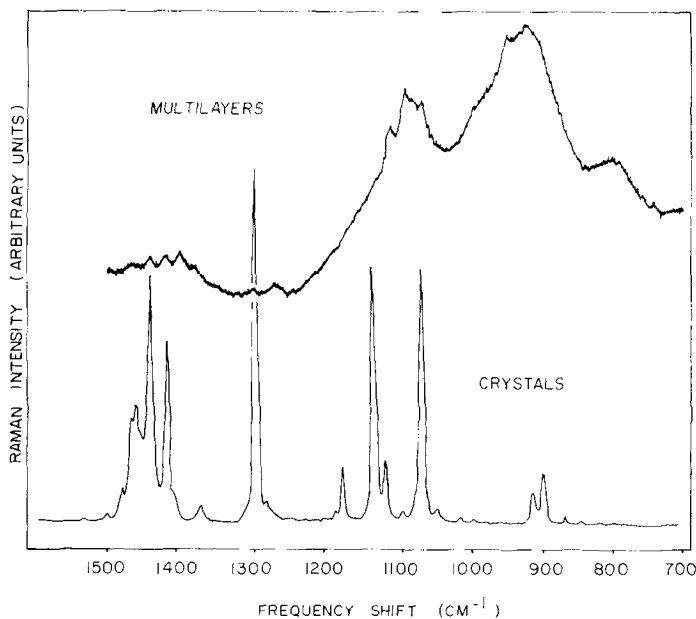


Figure 1: Raman spectrum of behenic acid crystals and Blodgett-Langmuir multilayer assembly in the spectral region 700-1500 cm^{-1} ; band pass, 5 cm^{-1} and 20 cm^{-1} respectively.

The Raman spectrometer and conditions for obtaining spectra of lipid powders and unsonicated dispersions have been described elsewhere (7). The multilayer samples were oriented so that the incident beam was parallel to the multilayer packing and the scattering oblique to the incident beam sampled. The sample was examined at room temperature (24°C) with no provisions for humidity control. Typically, the 514.5 nm Ar^+ laser line was used with a power of 600 mW. The monochromator slits were set at 400μ unless otherwise indicated. Intensities were judged by peak heights with a difference in peak height intensity ratios of less than ± 0.1 for independent runs on the same system.

RESULTS AND DISCUSSION

Figure 1 shows the Raman spectra of behenic acid crystals and multilayers in the spectral region 700-1500 cm^{-1} . The Raman spectra of fatty acid powders have been previously reported (10). In contrast to the fatty acid crystal spectra reported by Lippert and Peticolas (10) which contained narrow Raman peaks, we observe for fatty acid Blodgett-Langmuir multilayers a predominant band at ca. 920 cm^{-1} with small contributions from the methylene vibrations at ca. 1300 cm^{-1} and 1400-1500 cm^{-1} , and C-C stretch vibrations at 1050-1150 cm^{-1} . These differences could be due

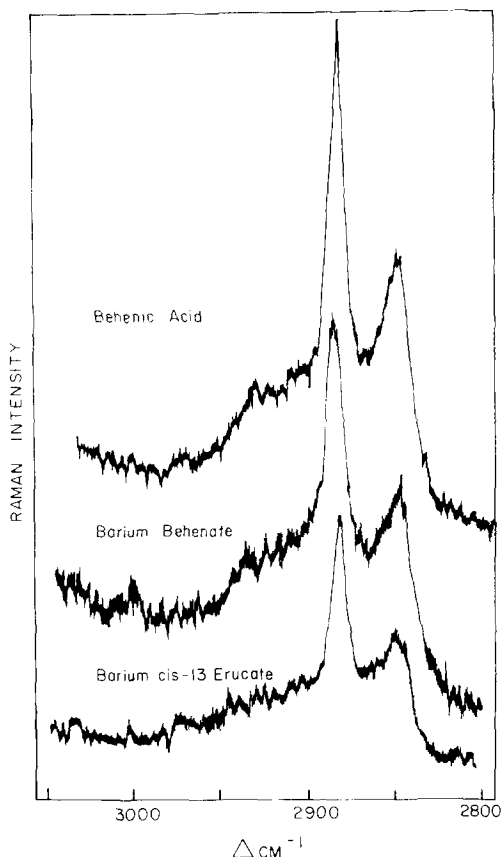


Figure 2: Raman spectra of various fatty acid Blodgett-Langmuir multilayer assemblies in the spectral region around 2900 cm^{-1} due to methyl and methylene C-H stretches.

to preferential excitation of vibrations parallel to the polarization vector of the incoming beam or to differences in acyl chain packing between the crystal and multilayer samples. In addition, the C-C stretch region around 1100 cm^{-1} shows a predominant band at ca. 1085 cm^{-1} which has been assigned to the hydrocarbon chain random C=C stretch with less predominant bands at ca. 1064 cm^{-1} , and ca. 1128 cm^{-1} which have been assigned to the various hydrocarbon chain trans C-C stretches (10-11).

Figure 2 shows the Raman spectra in the C-H Stretch region, $2800\text{-}3000\text{ cm}^{-1}$ for a number of fatty acid multilayered assemblies. The limited amount of sample available in each case, restricted our attempts to

Table I: Raman peak height intensity ratio I_{2890}/I_{2850} for various lipid and fatty acid samples.

Sample	I_{2890}/I_{2850}
DSL powder	1.6
DSL-H ₂ O dispersion	1.2
DPL powder	1.5
DPL-H ₂ O dispersion	1.2
behenic acid multilayer	1.9
barium behenate multilayer	1.8
barium cis-13 erucate multilayer	1.9
behenic acid powder (crystals)	2.2

increase the signal in relation to the noise present. Table 1 compares the I_{2890}/I_{2850} values for the multilayer assemblies used to the values found for phosphatidylcholine powders and water dispersions in this laboratory. In most cases, this peak height intensity ratio is highest for the fatty acid multilayer assemblies. However, the I_{2890}/I_{2850} ratio for behenic acid crystals (powder) is higher than the corresponding intensity ratio from behenic acid multilayers. In addition the lateral chain packing order parameter ($S_{\text{lateral}} = \frac{I_{\text{CH}_2 \text{ sample}}^{-0.7}}{1.5}$) devised by Gaber and Peticolas

(1) is ca. 0.8 in our ordered multilayer systems, which is lower than the 1.0 of crystalline hexadecane (or alkanes in general) and behenic acid crystals but greater than the values for phospholipid dispersions and powders, 0.33 and 0.53, respectively. This finding suggests that for at least some fatty acids, the crystalline phase contains much greater lateral order than the multilamellar state

We suggest that Blodgett-Langmuir multilayers of fatty acid or phos-

pholipids can be used as references to determine the degree of acyl chain packing in phospholipids. However, it appears that these systems are less ordered than either crystalline alkanes or crystalline fatty acids, i.e. behenic acid.

ACKNOWLEDGEMENTS

We thank the Northwestern University Materials Research Center for use of the Raman facilities. We also thank Dr. R. C. Waldbillig of the Duke University Medical Center for the fatty acid multilayer samples used in this study, and Drs. B. P. Gaber and W. L. Peticolas for providing us with preprints of their paper. This work was partially funded through the Office of Naval Research.

REFERENCES

1. Gaber, B. P. and Peticolas, W. L. (1977) *Biochim. Biophys. Acta* 465, 260-274.
2. Mendelsohn, R., Sunder, S. and Bernstein, H.J. (1976) *Biochim. Biophys. Acta* 419, 563-569.
3. Larsson, K. (1973) *Chem. Phys. Lipids* 10, 165-176.
4. Larsson, K. and Rand, R. P. (1973) *Biochim. Biophys. Acta* 326, 245-255
5. Green, J. P., Philips, M. C. and Shipley, G. G. (1973) *Biochim. Biophys. Acta* 330, 243-253.
6. Kopp, F., Fringeli, U. P., Muhlethaler, K. and Gunthard, H. H. (1975) *Biophys. Struct. Mech.* 1, 75-96.
7. Baumeister, W., Fringeli, U. P., Halin, M., Kopp, F. and Seredynski, J. (1976) *Biophys. J.* 16, 791-810.
8. Waldbillig, R. C., Robertson, J. D. and McIntosh, T. J. (1976) *Biochim. Biophys. Acta* 448, 1-14.
9. Lis, L. J., Kauffman, J. W. and Shriver, D. F. (1975) *Biochim. Biophys. Acta* 406, 453-464.
10. Lippert, J. L. and Peticolas, W. L. (1972) *Biochim. Biophys. Acta* 282, 8-17.
11. Spiker, R. C. and Levin, I. W. (1975) *Biochim. Biophys. Acta* 282, 8-17.