Biochimica et Biophysica Acta, 436 (1976) 513–522

BBA 77344

RAMAN SPECTROSCOPIC DETECTION AND EXAMINATION OF THE INTERACTION OF AMINO ACIDS, POLYPEPTIDES AND PROTEINS WITH THE PHOSPHATIDYLCHOLINE LAMELLAR STRUCTURE*

L. J. LIS[a], J. W. KAUFFMAN[b] and D. F. SHRIVER[c]

[a]*Department of Materials Science and Engineering,* [b]*Departments of Materials Science and Engineering and Biological Sciences and* [c]*Department of Chemistry, Northwestern University, Evanston, Ill. 60201 (U.S.A.)*

(Received October 3rd, 1975)
(Revised manuscript received February 9th, 1976)

SUMMARY

Raman spectral peaks in the vicinity of 1100 and 2900 cm^{-1} for phosphatidylcholine were found to be sensitive to interactions with amino acids, polypeptides and plasma proteins. The amino acids L-leucine, L-isoleucine, L-tryptophan, L-arginine HCl, L-histidine HCl, L-threonine and L-aspartic acid decreased the dipalmitoyl phosphatidylcholine Raman intensity ratio I_{1064}/I_{1089} indicating an increase in the gauche hydrocarbon chain character of the lipid. The increase in the lipid approx. 2930 cm^{-1} peak intensity in relation to the approx. 2850 and approx. 2890 cm^{-1} peaks upon the addition of the amino acids L-arginine HCl, L-histidine · HCl and L-lysine · HCl to the lipid dispersion indicates that the lipid hydrocarbon chain environment becomes more polar in their presence. The lipid-alamethecin and lipid-valinomycin interactions produced a decrease in the lipid Raman intensity ratio I_{1064}/I_{1089} again indicating an increase in the gauche hydrocarbon chain character of dicyristoyl phosphatidylcholine while producing no change in this ratio for dipalmitoyl phosphatidylcholine. Human fibrinogen and bovine serum albumin were found to increase the I_{2890}/I_{2850} dimyristoyl phosphatidylcholine Raman intensity ratio while decreasing the I_{2850}/I_{2930} dimyristoyl phosphatidylcholine Raman intensity ratio indicating that the lipid underwent a conformational change and that the hydrocarbon chain environment was more polar in the presence of albumin or fibrinogen.

INTRODUCTION

The characterization of the protein-lipid interaction is essential to the study of biomembranes and lipoprotein complexes. It is possible to study protein-lipid interactions and/or study the interactions of lipid and protein subunits such as polypeptides

* Presented at the American Institute of Chemical Engineers 80th National Meeting, September 8–10, 1975, Boston, Massachusetts.

and amino acids. The interaction between lipids and protein subunits can then be used to model the lipid-protein interaction.

The Raman spectra of fatty acids [1] and lipids [2–7] have been reported. Lippert and Peticolas, [2] and recently Spiker and Levin [6] have given assignments to the phosphatidylcholine Raman peaks in the spectral region around 1100 cm^{-1}. The lipid Raman peaks at approx. 1064 and approx. 1128 cm^{-1} were respectively assigned to the symmetric and asymmetric all trans hydrocarbon stretch modes, while the lipid Raman peak at approx. 1089 cm^{-1} was assigned to the random hydrocarbon stretch and O-P-O symmetric stretch modes. The influence of temperature [2–4, 7] cholesterol [2, 3] and ions [8] on the Raman spectrum of phosphatidylcholine has been studied. Upon the addition of cholesterol [2], iodide ion [8] or an increase in temperature [4, 7] the phosphatidylcholine Raman feature at approx. 1089 cm^{-1} shifts to a lower frequency and is broadened. A similar trend is observed when comparing spectra of lipid dispersions of varying hydrocarbon chain length. Fig. 1 shows that the approx. 1089 cm^{-1} band for the short chain lipid dimyristoyl phosphatidylcholine falls at a lower frequency than it does for the longer chain dipalmitoyl phosphatidylcholine dispersion. This shift is thought to result from the increase in the amount of random hydrocarbon conformers. Phillips et al. [9] have also noted that the shorter hydrocarbon chains, as in the case of dimyristoyl phosphatidylcholine tend to have a less stable bilayer structure than do the longer chain hydrocarbons.

Spiker and Levin [6] provide convincing arguments for the following assignments for the phosphatidylcholine C-H stretch Raman bands: approx. 2850 cm^{-1} symmetric CH_2 stretch, approx. 2890 cm^{-1} asymmetric CH_2 stretch, approx. 2930 cm^{-1} symmetric CH_3 stretch and approx. 2960 cm^{-1} asymmetric CH_3 stretch. These reassignments are in agreement with the work of Bulkin and Krishnamachari [7] and are based on the Raman C-H stretch assignments for polyethylene. We have found that dimyristoyl, dipalmitoyl and distearoyl phosphatidylcholine have the same intensity ratio I_{2890}/I_{2850} (= 1.2) while the intensity ratio I_{2850}/I_{2930} increases with increasing hydrocarbon chain length in the order: dimyristoyl phosphatidylcholine = 1.6 < dipalmitoyl phosphatidylcholine = 2.2 < distearoyl phosphatidylcholine = 2.8. These results are similar to the reported effect of varying chainlength in polyethylene infrared C-H stretch bands [10].

Brown et al. [4] Larsson [5] and Bulkin and Krishnamachari [7] have noticed a broadening of the C-H stretch vibrations at approx. 2850 and approx. 2890 cm^{-1} in the Raman spectrum of lipid samples when heated. The change in broadening as reflected as a change in the relative intensities of the symmetric and asymmetric C-H stretch with change in temperature is probably associated with the increase in the amount of hydrocarbon chain randomness [7]. This decrease in chain symmetry leads to a relaxation of the selection rules for the C-H stretch modes. In addition, motional broadening is another potential contributor to the linewidths of the C-H stretch modes. Larsson and Rand [11] have noted that the lipid intensity ratio I_{2890}/I_{2850} decreases as the lipid packing becomes looser in the series: lamellar liquid crystal > hexagonal or cubic liquid crystal > micellar solution > solution in an organic solvent; and the approx. 2930 cm^{-1} band becomes more intense in relation to the approx. 2850 and approx. 2890 cm^{-1} bands as the hydrocarbon chain environment becomes more polar. However, the detailed interpretation presented by Larsson and Rand [11] on the physical origin of the environmental effects on the lipid Raman

spectrum must be revised in light of the recent reassignment of these modes for phosphatidylcholine by Spiker and Levin [6].

One criterion used in this study is the I_{1064}/I_{1089} intensity ratio which is diagnostic of the hydrocarbon chain conformation. The O-P-O symmetric stretch which contributes to the approx. 1089 cm^{-1} lipid Raman band is assumed to make a constant intensity contribution at all temperatures and therefore does not seriously affect conclusions drawn about the hydrocarbon chain conformation from the I_{1064}/I_{1089} ratio. The intensity ratio I_{1128}/I_{1089} which was used by others [2, 3] produced general trends similar to the I_{1064}/I_{1089} ratio.

The other criterion used in this study is the ratio of peak height intensity for methylene stretch C-H modes, I_{2890}/I_{2850}, which reflects the degree of conformational and motional broadening in lipids since the broadening of the approx. 2850 cm^{-1} band is much less than that of the approx. 2890 cm^{-1} band. The I_{2850}/I_{2930} intensity ratio was also examined. A change in the I_{2890}/I_{2850} ratio was usually accompanied by a similar change in the I_{1064}/I_{1089} ratio.

In this paper, we study the effect of amino acids, polypeptides and proteins on the Raman spectrum of phosphatidylcholine in the regions around 1100 cm^{-1} and 2900 cm^{-1}. The effect of surface active polypeptides and plasma proteins on the lipid Raman spectrum will be related to their effect on lipid packing and compared with information derived from other sources.

MATERIALS AND METHODS

1,2 L-α-Dimyristoyl phosphatidylcholine, 1,2 L-α-dipalmitoyl phosphatidylcholine, 1,2 L-α-distearoyl phosphatidylcholine, valinomycin (A Grade) and the amino acids (A Grade) were obtained from Calbiochem and used without further purification. Alamethecin was provided by Dr. George B. Whitfield, Jr. of the Upjohn Company. Human fibrinogen fraction I (65 % clottable) and bovine serum albumin fraction V were obtained from the Research Products Division of Miles Laboratories. The water used was doubly distilled and deionized. All solvents and salts were reagent grade with the salts further purified by washing in reagent chloroform. Lipid dispersions were made in a 4 : 1 weight ratio of solvent to lipid.

Lipid and lipid-amino acid dispersions were prepared by heating the mixture to 60 °C for 1 h, air cooling and sampling in 1 mm diameter capillary tubes. Lipid-polypeptide dispersions were made in chloroform. The chloroform was removed under vacuum and water was added. The dispersions were then heated and sampled in the manner of the lipid dispersions. Protein-lipid dispersions were made immediately before use and not heated to prevent protein denaturation. The proteins were prepared at the concentration of 3 mg protein to 1 ml water. The lipid-protein dispersions were also made in a 4 : 1 weight ratio of protein solution to lipid and were sampled in 1 mm diameter capillary tubes. The Raman spectrometer has been previously described [8]. Experiments were run at room temperature (24 °C). At least two independent runs were made for each system and a maximum difference in intensity ratios of ± 0.1 was obtained.

RESULTS AND DISCUSSION

Amino acid-lipid interaction

If one assumes that proteins only interact at their side chains, then as analysis of the effect of various amino acids on lipid conformation or packing should provide useful information about lipid-protein interactions. Bigelow [12] has assigned amino acids in relation to a hydrophobicity index based on Tanford's [13] study of amino acid solubility in solvents of different polarity. The hydrophobicity index ranges from a value of zero for glycine to a value of 3.0 for tryptophan.

Table I shows the effect of the amino acids on the Raman intensity ratio I_{1064}/I_{1089} for dipalmitoyl phosphatidylcholine. The amino acids L-leucine, L-isoleucine, L-tryptophan, L-arginine · HCl, L-histidine · HCl, L-threonine and L-aspartic acid decreased the dipalmitoyl phosphatidylcholine Raman intensity ratio I_{1064}/I_{1089} which indicates that the lipid is forced into a more fluid state by these amino acids. The effect of the solutions of L-leucine, L-isoleucine and L-threonine on the dipalmitoyl phosphatidylcholine Raman spectrum were considered marginal due to the high amino acid concentrations and the closeness of their I_{1064}/I_{1089} to the experimental uncertainty. One of the strongest amino acid interactions with dipalmitoyl phosphatidylcholine occurred using a low concentration of L-tryptophan. The

TABLE I

EFFECT OF VARIOUS AMINO ACIDS ON THE HYDROCARBON CHAIN MODES IN THE DIPALMITOYL PHOSPHATIDYLCHOLINE RAMAN SPECTRUM.

Amino acids	Concentration (M)	I_{1064}/I_{1089}
Nonpolar		
L-alanine	1	2.5
L-valine	0.1	2.2
L-leucine	0.1	2.0
L-isoleucine	0.1	2.0
L-proline	1	2.2
L-methionine	0.1	2.3
L-phenylalanine	0.1	2.3
L-tryptophan	0.01	1.9
Uncharged		
Glycine	1	2.2
L-serine	1	2.5
L-threonine	1	2.0
L-tyrosine	0.01	2.3
Positive		
L-lysine HCl	1	2.4
L-arginine HCl	1	1.5
L-histidine HCl	1	2.1
Negative		
L-aspartic acid	0.01	1.9
L-glutamic acid	0.01	2.4
H_2O		2.4

three positively charged amino acids lead to the following order for the dipalmitoyl phosphatidylcholine Raman intensity ratio I_{1064}/I_{1089}: Arginine · HCl < Histidine · HCl < Lysine · HCl = H_2O. Characteristic Raman peaks due to amino acids were not observed [14]. In addition, L-lysine · HCl, L-arginine · HCl and L-histidine · HCl also decrease the lipid C-H stretch peak intensity ratio I_{2850}/I_{2930} which according to the finding of Larsson and Rand [11] indicates that the hydrocarbon chain environment may be more polar. Since the amino acids affecting the lipid intensity ratio I_{1064}/I_{1089} vary greatly in their hydrophobicity indices as tabulated by Bigelow [12], there is no correlation between this parameter and conformational changes in dipalmitoyl phosphatidylcholine.

It is concluded that the amino acid-dipalmitoyl phosphatidylcholine interaction is highly specific and lacks any simple correlation with amino acid polarity. It is most likely that lipid-protein interactions depend not on the polarity of the individual amino acids but on the collective influence of the amino acids on the structure and properties of the protein.

Polypeptide-lipid interaction

The surface active polypeptides alamethecin and valinomycin have been shown to have significant effects on the electrical properties of black lipid membranes [15]. Valinomycin has been proposed as an alkali metal carrier [16], while the high levels of conductance observed with alamethecin indicate that it may act in some way as a path for ions through lipid bilayers [17]. Mueller and Rudin [18] have shown that the bimolecular lipid membrane is in a non-conducting state, even in the presence of alamethecin, until an outside voltage is applied. At that time the alamethecin molecule is given the impetus to penetrate the lipid layer, which Eisenberg et al. [17] have observed to cause conductance changes in a series of five distinct levels. When this result is coupled with other electrical measurements, the former authors [17] suggest that alamethecin acts as a pore for ion transport. Recently, Chan and co-workers [19–21] have used delayed Fourier transform NMR to study the effects of these polypeptides on unsonicated dispersions of phosphatidylcholine. These authors found that the addition of either polypeptide did not affect the fatty acid methylene or terminal methyl signals for dipalmitoyl phosphatidylcholine, but did for dimyristoyl phosphatidylcholine above its liquid crystalline phase transition (T_m). On the basis of this evidence and the broadening of the lipid choline methyl signal for dipalmitoyl and dimyristoyl phosphatidylcholine induced by the presence of more than 2 mol % valinomycin (50 molecules lipid to 1 molecule valinomycin), Chan et al. [19–21] therefore concluded that the polypeptide interaction is confined to the lipid head group with negligiable effect on the hydrocarbon chains.

The Raman spectral results extend the NMR findings of Hsu and Chan [19] for the valinomycin-phosphatidylcholine system. The Raman data taken at room temperature shows no change in the intensity ratio I_{1064}/I_{1089} for dipalmitoyl phosphatidylcholine dispersions (below T_m) while showing a decrease in the same ratio for dimyristoyl phosphatidylcholine dispersions (above T_m) for all valinomycin concentrations used (Fig. 2). The decrease in I_{1064}/I_{1089} for dimyristoyl phosphatidylcholine-valinomycin dispersions is due to the increase in the amount of gauche hydrocarbon conformers present (Fig. 1 versus Fig. 3) and may be caused by either the penetration of valinomycin into the less stable dimyristoyl phosphatidylcholine layer

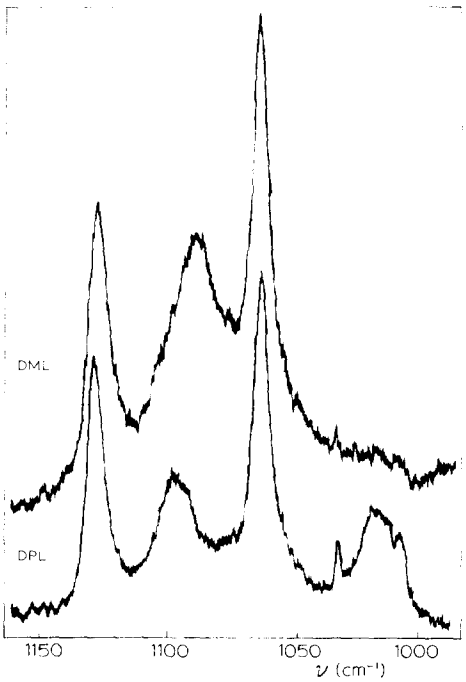

Fig. 1. Raman spectra of dimyristoyl (DML) and dipalmitoyl (DPL) phosphatidylcholine-water dispersions in the region 1000–1150 cm^{-1}.

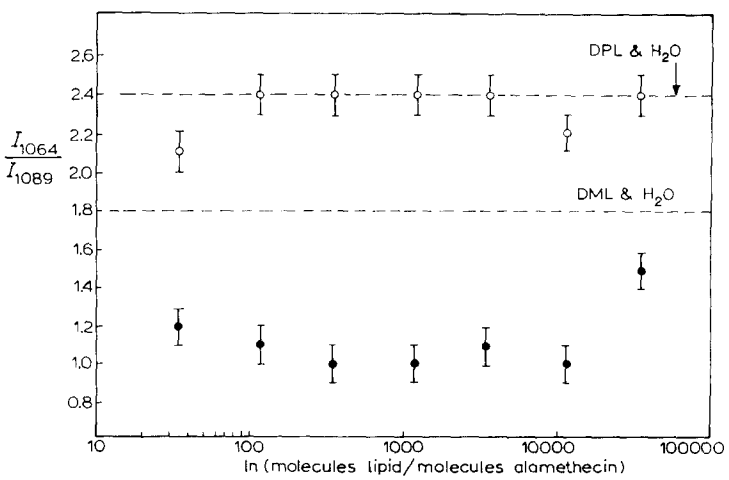

Fig. 2. The Raman intensity ratio I_{1064}/I_{1089} for dipalmityol (DPL) and dimyristoyl (DML) phosphatidylcholine with various mole ratios of valinomycin.

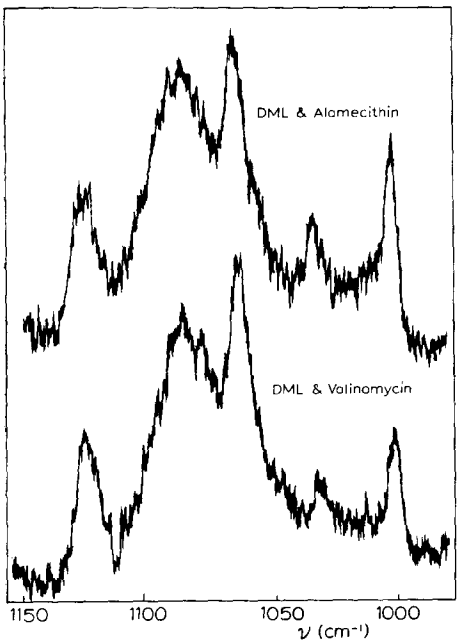

Fig. 3. Raman spectra of dimyristoyl phosphatidylcholine (DML) and 10^{-4} M alamethecin or valinomycin in the region 1000–1150 cm^{-1}.

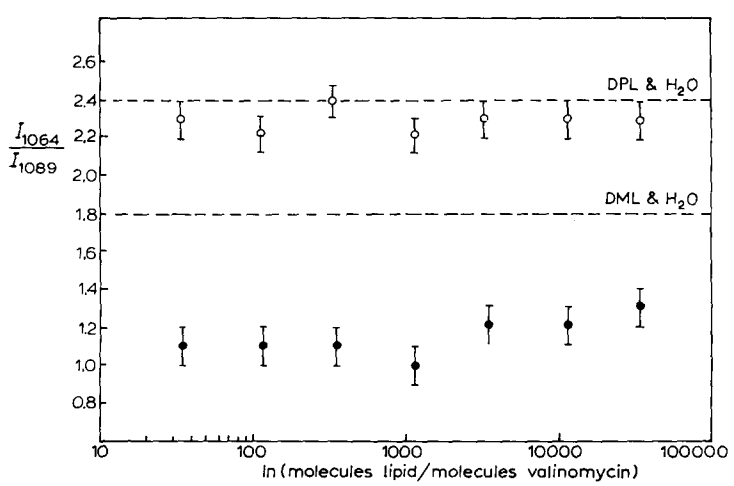

Fig. 4. The Raman intensity ratio I_{1064}/I_{1089} for dipalmitoyl (DPL) and dimyristoyl (DML) phosphatidylcholine with various mol ratios of alamethecin.

structure or interaction with the lipid head groups causing a spreading of the lipid layers. Upon the addition of valinomycin to either lipid, we observe no change in the frequency or half band width for the approx. 720 cm^{-1} band which arises from the lipid head group C-N stretch [4, 6]. Either the interaction of the polypeptide with the choline moiety is extremely weak or else the vibrational modes are insensitive to the interaction. We observe no additional change in the effect of 10^{-3} M valinomycin on the Raman spectrum of either dimyristoyl or dipalmitoyl phosphatidylcholine when 1 M KCl is added to the system.

For the dipalmitoyl phosphatidylcholine-alamethecin interaction our Raman spectral data again complement the NMR study made by Chan and co-workers [20, 21]. We find no change in the lipid intensity ratio I_{1064}/I_{1089} for dipalmitoyl phosphatidylcholine-alamethecin dispersions while observing a decrease in this same ratio for dimyristoyl phosphatidylcholine-alamethecin dispersions (Fig. 4). A comparison of Fig. 1 and 3 shows that this decrease is due to the increase in the amount of gauche conformers of dimyristoyl phosphatidylcholine in the presence of alamethecin. No change was found in the spectral wavelength or half band width for the C-N stretch mode for any lipid-alamethecin dispersion studied.

It is concluded on the basis of our present study and the previous NMR work [19–21] that for unsonicated lipid dispersions, polypeptide interaction with phosphatidylcholine causes conformational changes in the hydrocarbon chains, which may arise from interactions between the phospholipid head groups and the polypeptides.

Protein-lipid interactions

The plasma proteins albumin and fibrinogen have been found to be associated

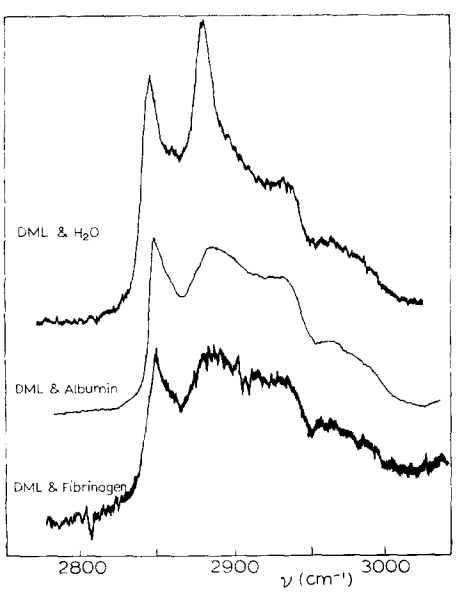

Fig. 5. Raman spectra of dimyristoyl phosphatidylcholine (DML) with H_2O, human fibrinogen or bovine serum albumin in the region around 2900 cm^{-1}.

TABLE II

EFFECT OF HUMAN FIBRINOGEN AND BOVINE SERUM ALBUMIN ON THE RAMAN C-H STRETCH PEAKS IN THE VICINITY OF 2900 cm^{-1} OF DIMYRISTOYL PHOSPHATIDYLCHOLINE.

Protein	I_{2890}/I_{2850}	I_{2850}/I_{2930}
Fibrinogen	0.9	1.3
Albumin	0.9	1.3
H_2O	1.2	1.8

with both lipids and blood cells. Human and bovine serum albumin have been found to alter the geometry and dimensions of human erythrocytes [24]. Rand [25] has found that extended bovine serum albumin affects the packing of cardiolipid/phosphatidylcholine mixtures using X-ray diffraction techniques, while Jonas [26] has recently reported that radioactively labelled phosphatidylcholine will bind to unextended bovine serum albumin. In addition, phospholipids act as catalysts for various steps in the blood coagulation process including the formation of thrombin which in turn catalyzes the fibrinogen to fibrin monomer reaction [27]. It is also believed that fibrinogen forms the first layer of adsorbed material on implanted prosthetics [28] and later reacts with blood cells to form a thrombus [29].

Dimyristoyl phosphatidylcholine was used for the protein studies because it is fluid enough at room temperature to allow mixing with the protein. In most cases, the protein-lipid dispersions produced broad signals in the lipid Raman spectrum around 1100 cm^{-1} while a protein spectrum was not observed in the region 1400–1700 cm^{-1} [30]. Thus there can be little interference to our spectral analysis from the protein spectrum. The broad lipid C-C signal makes quantitative analysis of the protein interaction with the lipid in the Raman spectral range 1000–1150 cm^{-1} not feasible. Fortunately, the lipid Raman spectral region around 2900 cm^{-1} proved to be very informative in relation to protein-lipid interactions. As shown in Fig. 5, fibrinogen and albumin cause the lipid methylene C-H band at approx. 2890 cm^{-1} to broaden indicating that both proteins have caused the lipid to undergo a conformational change. The lipid methyl C-H bands also increase in peak height intensity in relation to the methylene C-H stretch bands with the addition of either protein. As shown in Table II, the Raman intensity ratios I_{2890}/I_{2850} and I_{2850}/I_{2930} are approximately the same for the fibrinogen-dipalmitoyl phosphatidylcholine dispersion and the albumin dimyristoyl phosphatidylcholine dispersion. It appears that albumin and fibrinogen penetrates the hydrocarbon chain. This conclusion could explain the alteration in erythrocyte dimensions and geometry by albumin which was observed by Jay [24].

ACKNOWLEDGEMENTS

We thank the Northwestern University Materials Research Center for the use of the Raman facilities.

REFERENCES

1 Lippert, J. L. and Peticolas, W. L. (1972) Biochim. Biophys. Acta 282, 8–17

2 Lippert, J. L. and Peticolas, W. L. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1572–1576
3 Mendelsohn, R. (1972) Biochim. Biophys. Acta 290, 15–21
4 Brown, K. G., Peticolas, W. L. and Brown, E. (1973) Biochem. Biophys. Res. Commun. 54, 358–364
5 Larsson, K. (1973) Chem. Phys. Lipids 10, 165–176
6 Spiker, R. C. and Levin, I. W. (1975) Biochim. Biophys. Acta 388, 361–373
7 Bulkin, B. J. and Krishnamachari, N. (1972) J. Am. Chem. Soc. 94, 1109–1112
8 Lis, L. J., Kauffman, J. W. and Shriver, D. F. (1975) Biochim. Biophys. Acta 406, 453–464
9 Phillips, M. C., Williams, R. M. and Chapman, D. (1969) Chem. Phys. Lipids 3, 234–244
10 Snyder, R. G. (1967) J. Chem. Phys. 47, 1316–1360
11 Larsson, K. and Rand, R. P. (1973) Biochim. Biophys. Acta 326, 245–255
12 Bigelow, C. B. (1967) J. Theoret. Biol. 16, 187–211
13 Tanford, C. (1962) J. Am. Chem. Soc. 84, 4240–4247
14 Koenig, J. L. (1972) J. Poly. Sci. Part D, 59–177
15 Haydon, D. A. and Hladky, S. B. (1972) Quart. Rev. Biol. 2, 187–282
16 Eisenman, G., Szabo, G., Ciani, S., McLaughlin, S. and Krasne, S. (1973) Prog. Surf. Membrane Sci. 6, 139–241
17 Eisenberg, M., Hull, J. E. and Mead, C. A. (1972) J. Membrane Biol. 14, 143–176
18 Mueller, P. and Rudin, D. O. (1968) Nature 217, 713–719
19 Hsu, M. and Chan, S. I. (1973) Biochemistry 12, 3872–3876
20 Chan, S. I., Sheetz, M. P., Seiter, C. H. A., Feigenson, G. W., Hsu, M., Lau, A. and Yau, A. (1973) Ann. N.Y. Acad. Sci. 222, 499–522
21 Lau, A. L. Y. and Chan, S. I. (1974) Biochemistry 13, 4942–4948
22 Rothschild, K. J., Asler, I. M., Anastassakis, E. and Stanley, H. E. (1973) Science 182, 384–386
23 Rothschild, K. J. and Stanley, H. E. (1974) Science 185, 616–618
24 Jay, A. W. L. (1975) Biophys. J. 15, 205–222
25 Rand, R. P. (1971) Biochim. Biophys. Acta 241, 823–834
26 Jonas, A. (1975) Biochem. Biophys. Res. Commun. 64, 1003–1008
27 Bruck, S. D. (1974) Blood Compatible Synthetic Polymers, pp. 69–71, Charles C. Thomas, Springfield, Illinois
28 Baier, R. E. and Dalton, R. C. (1969) J. Biomed. Mater. Res. 3, 191–206
29 Andrade, J. D. (1973) Med. Instrument. 7, 110–120
30 Bellow, A. M., Lord, R. C. and Mendelsohn, R. (1972) Biochim. Biophys. Acta 257, 280–287