Biochimica et Biophysica Acta, 443 (1976) 331–338

BBA 77421

LASER RAMAN SPECTROSCOPY OF LIPID-PROTEIN SYSTEMS

DIFFERENCES IN THE EFFECT OF INTRINSIC AND EXTRINSIC PROTEINS ON THE PHOSPHATIDYLCHOLINE RAMAN SPECTRUM*

L. J. LIS[a], S. C. GOHEEN[a], J. W. KAUFFMAN[a] and D. F. SHRIVER[b]

[a]*Biomedical Engineering Center*, [b]*Department of Chemistry, Northwestern University, The Technological Institute, Evanston, Ill. 60201 (U.S.A.)*

(Received January 27th, 1976)

SUMMARY

Laser Raman spectroscopy is used to examine the interactions of intrinsic and extrinsic proteins with the lipid layer structure. The interactions of cytochrome *c* and cytochrome *c* oxidase with lipids have been well established by others using a variety of techniques. Cytochrome *c* is thought to act as an extrinsic membrane protein while cytochrome *c* oxidase is thought to act as an intrinsic membrane protein. The lipid-cytochrome *c* and lipid cytochrome *c* oxidase systems are used to assist in interpreting the spectral changes due to extrinsic and intrinsic protein interactions. The two types of proteins examined produced differential changes in the lipid hydrocarbon C-H stretch Raman modes for both dimyristoyl and dipalmitoyl phosphatidylcholine. The plasma proteins albumin and fibrinogen were also found to differentially affect the lipid hydrocarbon C-H stretch Raman modes. These proteins appear to interact with lipids in an extrinsic manner different from that of cytochrome *c*.

INTRODUCTION

Work in this laboratory on lipid-protein systems [1] has led us to examine the effect of characterized extrinsic and intrinsic membrane proteins on the phosphatidylcholine Raman spectrum in order to assist in understanding the influence of various protein types on the lipid Raman spectrum. We have noted [1] that the addition of proteins to lipid dispersions significantly lowers the signal of the lipid hydrocarbon chain Raman modes in the region around 1100 cm^{-1} and so have restricted this present study to the examination of the lipid Raman region around 2900 cm^{-1}.

The proteins cytochrome *c* oxidase and cytochrome *c* were chosen for this study because of the large amount of previously reported evidence on their specific interaction with phosphatidylcholine. ESR [2] and electron diffraction studies [3]

* Presented at the Biophysical Society 20th Annual Meeting, February 24–27, 1976, Seattle, Washington

indicate that cytochrome *c* oxidase is a transmembrane intrinsic protein. By contrast, the accumulated evidence suggests that cytochrome *c* interacts in an extrinsic fashion with phosphatidylcholine. For example, Blaurock [4, 5] found from small angle X-ray scattering that the interaction involves attachment of cytochrome *c* at the bilayer. For cytochrome *c* interaction with mitochondrial membranes, Vanderkooi et al. [6] used a spin label on the cysteine 103 and methionine 65 amino acid residues of cytochrome *c* and found from their ESR studies that only the methionine residue was immobilized. The interpretation is that methionine is bound to the mitochondrial membrane with the cysteine residue exposed to the solvent. This extrinsic interaction of cytochrome *c* with the lipid layer structure causes an expansion of the lipid monolayer [7].

MATERIALS AND METHODS

1,2L-α-Dimirystoyl phosphatidylcholine and 1,2 L-α-dipalmitoyl phosphatidylcholine were used as obtained from Calbiochem. Oxidized horse heart cytochrome *c* was provided by Prof. E. Margoliash and prepared in a 0.1 M Tris–HCl buffer at pH 7.0 for a 0.1 mM protien concentration. Cytochrome *c* oxidase prepared by the method of Hartzell, C. R., (unpublished) in a Tris/cacodylate buffer at pH 7.8 with 0.25% Tween added for a 0.15 mM protein concentration, was also obtained from Prof. E. Margoliash. The lipid-protein unsonicated dispersions were prepared by mixing, at room temperature, a 4 : 1 weight ratio of protein solution to lipid. The samples were allowed to equilibrate for approximately 2 h before being examined. This procedure favors the mixing of the proteins with dimyristoyl phosphatidylcholine which is near its liquid crystalline phase transition at room temperature.

The Raman spectrometer has been described previously [8]. The Raman spectra were obtained with an Argon ion laser operating at 488 or 514.5 nm with a typical power of 0.45 W. All spectra taken for quantitative purposes were obtained with a band pass of 2–5 cm^{-1} and intensities were judged by peak heights. The lipid-protein dispersions were sampled in 1 mm diameter melting point capillary tubes before being placed in a flowing N_2 cell for temperature control. At least four independent runs were made for each system with a maximum difference in peak height intensity ratios of ± 0.1 obtained. Features arising from the resonance Raman spectra of cytochrome *c* [9, 10] or cytochrome *c* oxidase [11] were not observed in the lipid Raman spectra. This indicates that only a small amount of protein is in the lipid phase sampled, since a 0.1 mM solution of either protein will generally produce a resonance Raman spectrum. The resonance Raman spectrum of cytochrome *c* oxidase, however, is generally very weak with the laser lines used.

RESULTS AND DISCUSSION

Bulkin and Krishnamachari [12], and recently Spiker and Levin [13] have presented convincing arguments for the assignments of lipid Raman peaks in the region around 2900 cm^{-1} which are shown in Fig. 1. Bulkin and Krishnamachari [12], Larsson [14], and Brown et al. [15] have observed broadening for the lipid Raman peaks in this region as temperature is increased which Bulkin and Krishnamachari [12] ascribed to motional and/or configurational broadening. In a dimyristoyl phospha-

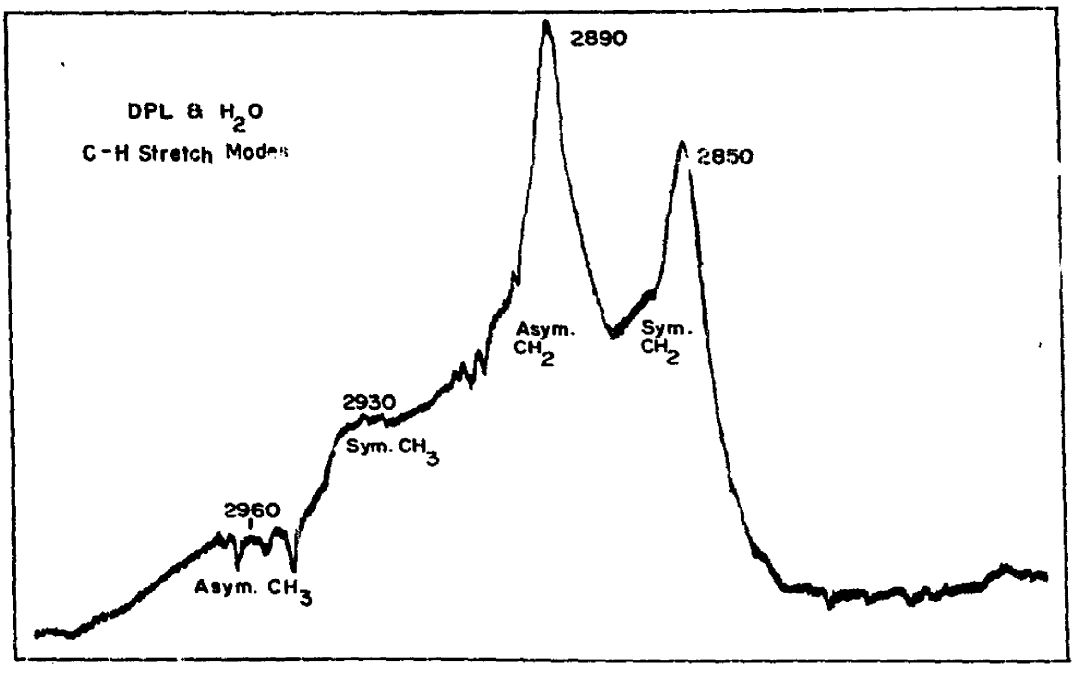

Fig. 1. Assignment of lipid Raman peaks in the region around 2900 cm^{-1}. DPL, dipalmitoyl phosphatidylcholine.

tidylcholine dispersion containing 1 M $CdSO_4$ as a marker, we have observed that although the intensity of the lipid hydrocarbon peaks in the region around 2900 cm^{-1} increases as the temperature of the dispersion is lowered from room temperature to 5 °C, the peak height intensity ratio I_{2890}/I_{2850} remains constant (at 1.2). By contrast, previous data collected above room temperature shows that the peak at approx. 2890 cm^{-1} is differentially broadened in relation to the lipid Raman peak at approx. 2850 cm^{-1} as the lipid dispersion goes through its liquid crystalline phase transition

TABLE I

I_{2890}/I_{2850} AND I_{2850}/I_{2930} VALUES FOR LIPID-PROTEIN SYSTEMS UNDER VARIOUS CONDITIONS

Lipid solution	I_{2890}/I_{2850}	I_{2850}/I_{2930}
Dipalmitoyl phosphatidylcholine		
-H_2O	1.2	2.2
-0.1 mM cytochrome *c* (pH 7.0)	1.2	2.2
-0.1 mM cytochrome *c* (pH 7.8)	1.0	1.5
-0.15 mM cytochrome *c* oxidase	1.0	1.3
Dimyristoyl phosphatidylcholine		
-H_2O	1.2	1.8
-0.1 mM cytochrome *c* (pH 6.8) at 24 °C	1.0	1.5
-0.1 mM cytochrome *c* (pH 7.4) at 5 °C	1.3	1.4
-0.15 mM cytochrome *c* oxidase at 24 °C	0.9	1.2
-0.15 mM cytochrome *c* oxidase at 5 °C	1.0	1.2

[15]. The lipid ratio I_{2890}/I_{2850} is therefore used as a measure of the fluidity of the lipid hydrocarbon chains. Larsson and Rand [16] have studied the effect of environment of the lipid Raman peaks in the 2900 cm^{-1} region and their results indicate that I_{2850}/I_{2930} ratio decreases as the polarity of the hydrocarbon chain environment increases.

The lipids dimyristoyl and dipalmitoyl phosphatidylcholine were studied and found at room temperature to have I_{2890}/I_{2850} of 1.2 while having an I_{2850}/I_{2930} of 1.8 and 2.2 respectively (see Table I).

Lipid-cytochrome c interaction

In a slightly basic unbuffered solution of cytochrome *c* (pH 7.8) it was found that the I_{2890}/I_{2850} and I_{2850}/I_{2930} ratios of both dimyristoyl and dipalmitoyl phosphatidylcholine affected as shown in Fig. 2. At pH 7.0, however, the 2900 cm^{-1} region of dipalmitoyl phosphatidylcholine is not affected, whereas the corresponding region of dimyristoyl phosphatidylcholine is perturbed by cytochrome *c*. In addition, as shown in Fig. 3 and Table 1, as the temperature of the dimyristoyl phosphatidylcholine-cytochrome *c* dispersions is lowered to 5 °C, the I_{2890}/I_{2850} ratio returns to

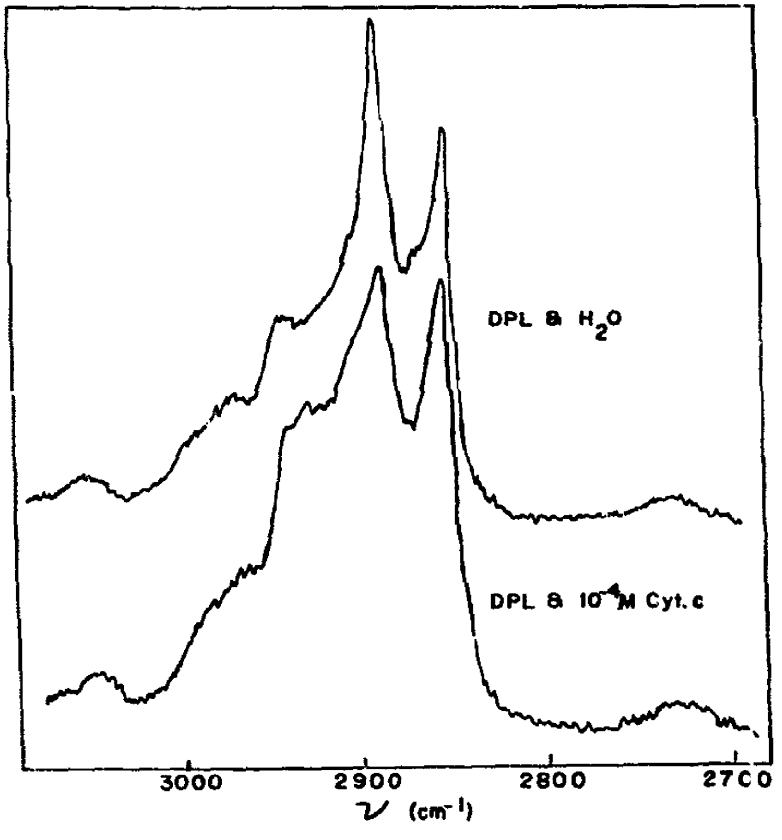

Fig. 2. Raman spectra in the region around 2900 cm^{-1} of dipalmitoyl phosphatidylcholine (DPL)-cytochrome *c* (pH 7.8) dispersion and dipalmitoyl phosphatidylcholine-H_2O dispersion

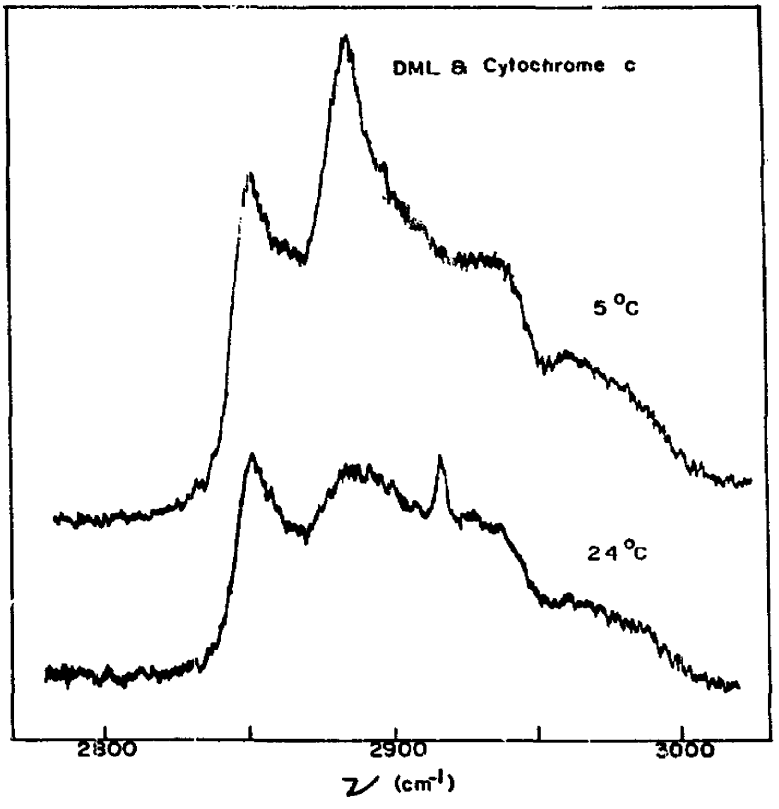

Fig. 3. Raman spectra in the region around 2900 cm^{-1} of dimyristoyl phosphatidylcholine (DML)-cytochrome *c* dispersions at different temperatures.

the value of a normal dimyristoyl phosphatidylcholine-H_2O system at room temperature indicating that cytochrome *c* lowers and/or broadens the lipid liquid crystalline transition temperature as has been observed by Papahadjopoulos, et al. [17] using differential scanning calorimetry. The lowering of the temperature, however, has no effect on the lipid I_{2850}/I_{2930} ratio which is consistently lower than that of dimyristoyl phosphatidylcholine-H_2O solution at room temperature. This result, according to the emperical evidence presented by Larsson and Rand [16] indicates that the lipid hydrocarbon chains are now in a more polar environment. This interpretation is somewhat difficult to reconcile with the extrinsic nature of cytochrome *c*. One possibility is that the spreading of the lipid layer which arises upon interaction with cytochrome *c* [7] leads to the simultaneous entry of water into the layer. An alternative interpretation, which is less attractive because of the evidence for extrinsic interaction, is that the protein has penetrated the lipid layer.

Lipid-cytochrome c oxidase interaction

Cytochrome *c* oxidase, as shown in Fig. 4 and Table I, affects both the dipalmitoyl and dimyristoyl phosphatidylcholine Raman spectrum in the region around 2900 cm^{-1}. In particular, the I_{2890}/I_{2850} lipid fluidity ratio is lowered in both cases, indicating that the lipid hydrocarbon chains have become more fluid. The change in

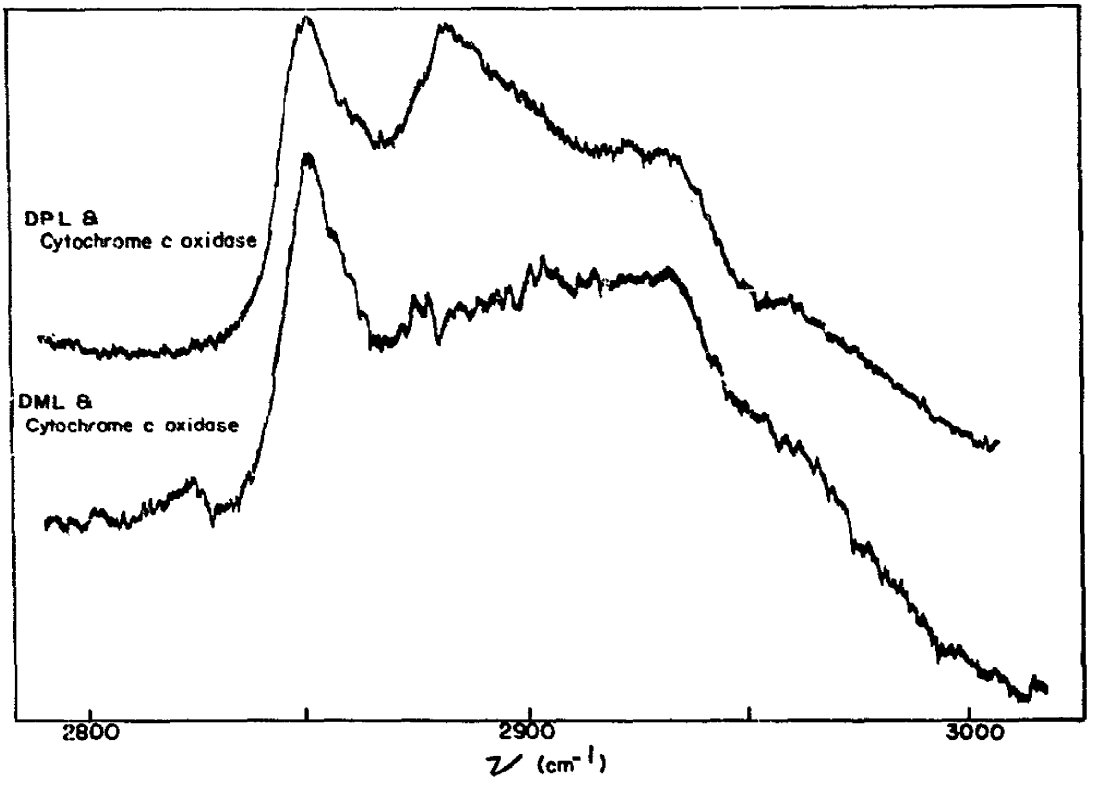

Fig. 4. Raman spectrum of the effect of cytochrome *c* oxidase in dimyristoyl phosphatidylcholine (DML) and dipalmitoyl phosphatidylcholine (DPL) dispersions in the region around 2900 cm^{-1}.

lipid hydrocarbon fluidity with the addition of cytochrome *c* oxidase is more dramatic for dimyristoyl phosphatidylcholine in which case $I_{2890}/I_{2850} = 1.0$. This ratio does not change when a dimyristoyl phosphatidylcholine-cytochrome *c* oxidase dispersion is lowered in temperature from room temperature to 5 °C which indicates that the equilibrium state for the lipid-cytochrome *c* oxidase mixture is more fluid than the equilibrium state for the dimyristoyl phosphatidylcholine-H_2O dispersion. The thermal instability of cytochrome *c* oxidase prevents the direct observation of the lipid T_m. However, the observation of no change of I_{2890}/I_{2850} ratio value from room temperature to 5 °C for the dimyristoyl phosphatidylcholine-cytochrome *c* oxidase dispersions implies a structural change of the low temperature lipid phase. This result agrees with the general observation of Papahadjopoulos, et al. [17] that transmembrane proteins only affect ΔH_m of lipid dispersions and not T_m. In addition, the I_{2890}/I_{2850} lipid intensity ratio is lowered when cytochrome *c* oxidase is added to dimyristoyl and dipalmitoyl phosphatidylcholine dispersions and remains so in the temperature range 5–24 °C.

Lipid-plasma protein interaction

The effects of fibrinogen and albumin on the dimyristoyl and diplamitoyl phosphatidylcholine Raman spectrum at room temperature has been previously reported [1]. We have recently extended these studies to find the effect of temperature

on the plasma protein-dimyristoyl phosphatidylcholine interaction. As the temperature of the dimyristoyl phosphatidylcholine-plasma protein dispersions is lowered to 5 °C, the dimyristoyl phosphatidylcholine spectrum in the region around 2900 cm^{-1} returns to that of the dimyristoyl phosphatidylcholine-H_2O dispersions at room temperature for both fibrinogen and albumin.

CONCLUSIONS

It was shown that the intrinsic protein cytochrome *c* oxidase, the extrinsic protein cytochrome *c* and the plasma proteins fibrinogen and albumin produce differential changes in the Raman spectra of both dimyristoyl and dipalmitoyl phosphatidylcholine in the hydrocarbon C-H stretch region around 2900 cm^{-1}. The interaction of these three types of proteins can thus be used to define the interactions of proteins of unknown type on the Raman spectra of lipids. In particular, it was shown that intrinsic transmembrane protein cytochrome *c* oxidase causes the lipid I_{2890}/I_{2850} Raman intensity ratio to decrease. This ratio remains constant below the lipid liquid crystalline transition, in agreement with differential scanning calorimeter studies [17]. The extrinsic membrane protein cytochrome *c*, which speads the lipid layer, was found to decrease the ratio I_{2850}/I_{2930} both above and below the liquid crystal transition temperature. In addition, the I_{2890}/I_{2850} intensity ratio was temperature dependent in the presence of cytochrome *c*. The presence of the two plasma proteins (another type of extrinsic protein), fibrinogen and albumin, causes the lipid Raman peak intensity ratios I_{2890}/I_{2850} and I_{2850}/I_{2930} to decrease at room temperature. At 5 °C both of these ratios have returned to the values for a standard dimyristoyl phosphatidylcholine-water dispersion. It is therefore inferred that, under our conditions, the proteins fibrinogen and albumin have very little, if any, effect on the dimyristoyl phosphatidylcholine bilayer at low temperatures; whereas cytochrome *c* at low temperatures retains some influence on the environment of the hydrocarbon chains but not on their conformation.

ACKNOWLEDGEMENT

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

REFERENCES

1 Lis, L. J., Kauffman, J. W. and Shriver, D. F. (1976) Biochim. Biophys. Acta 436, 513–522
2 Griffith, O. H., Jost, P., Capaldi, R. A. and Vanderkooi, G. (1973) Ann. N.Y. Acad. Sci. 222, 561–573
3 Vanderkooi, G., Senior, A. E., Capaldi, R. A. and Hayashi, H. (1972) Biochim. Biophys. Acta 274, 38–48
4 Blaurock, A. E. (1973) Biophys. J. 13, 281–289
5 Blaurock, A. E. (1973) Biophys. J. 13, 290–298
6 Vanderkooi, J., Erecinski, M. and Chance, B. (1973) Arch. Biochem. Biophys. 157, 531–540
7 Morse, P. D. and Deamer, D. W. (1973) Biochim. Biophys. Acta 398, 769–782
8 Lis, L. J., Kauffman, J. W. and Shriver, D. F. (1975) Biochim. Biophys. Acta 406, 453–464
9 Loehr, T. M. and Loehr, J. S. (1973) Biochem. Biophys. Res. Commun. 55, 218–223
10 Strekas, T. C. and Spiro, T. G. (1972) Biochim. Biophys. Acta 278, 188–192

11 Salmeen, I., Rima, L., Gili, D., Yamamoto, T., Palmer, G., Hartzell, C. R. and Bennett, H. (1973) Biochem. Biophys. Res. Commun. 52, 1100–1107
12 Bulkin, B. J. and Krishnamachari, N. (1972) J. Am. Chem. Soc. 94, 1109–1112
13 Spiker, R. C. and Levin, I. W. (1975) Biochim. Biophys. Acta 388, 361–373
14 Larsson, K. (1973) Chem. Phys. Lipids 10, 165–176
15 Brown, K. G., Peticolas, W. L. and Brown, E. (1973) Biochem. Biophys. Res. Commun. 54, 358–364
16 Larsson, K and Rand, R. P. (1973) Biochim. Biophys. Acta 326, 245–255
17 Papahadjopoulos, D., Moscarello, M., Eylar, E. H. and Isac, T. (1975) Biochim. Biophys. Acta 401, 317–335