A COMPARISON OF THE INTERACTIONS OF CHOLESTEROL AND GRAMICIDIN A WITH LIPID BILAYERS USING AN INFRARED DATA STATION

Manuel CORTIJO and Dennis CHAPMAN

Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, University of London, 8 Hunter Street, London WC1N 1BP, England

Received 7 July 1981

1. Introduction

The interaction between intrinsic proteins and lipids within the bilayer structures of both natural and model biomembranes and the degree to which each perturb the dynamic movement of the other is a subject of considerable interest [1]. Some results using deuterium magnetic resonance indicate that the effect of intrinsic proteins on the lipid chain conformation (above its main transition temperature, T_c) is different from the effect observed by incorporation of cholesterol. The inclusion of cholesterol into the lipid bilayer always induces an increased (static) order of the lipid chain above its T_c temperature [2] as indicated by the deuterium quadrupole splitting measurements. On the other hand, proteins (e.g., cytochrome oxidase, sarcoplasmic reticulum ATPase, myelin proteolipid apoprotein (PLA) and lipophilin) when incorporated into fluid bilayers give deuterium quadrupole splitting values, $\Delta\nu_Q$, associated with the end methyl-group of the hydrocarbon chains, which appear to decrease as the protein concentration increases [3,4].

The deuterium quadrupole data for the polypeptide gramicidin A (an intrinsic polypeptide) included in lipid bilayers shows a more complicated behaviour. In this case, the lipid was labelled in a range of positions along the length of the lipid chain. Small amounts of gramicidin in the bilayer cause an increase in quadrupole splitting and chain order but this trend is reversed with [5] increasing concentration resulting in a decrease in quadrupole splitting and increase in chain disorder, at least near the terminal methyl-group, for lipid/polypeptide molar ratios <15. Even at these high polypeptide contents, a lipid bilayer structure appears to be retained as indicated by X-ray diffraction data [6]. The ^2H NMR results are not however unequivocal in their interpretation due to the possible influence of the vesicle tumbling or the exchange between 'bound' and 'free' lipid molecules which may lead to misleading conclusions [5]. Vibration spectroscopy is a powerful technique for determining the relative population of the *gauche* and *trans* conformers and therefore the order of the lipid chain within lipid bilayer systems [7]. Raman spectroscopy has been very often used for this purpose [7] but the high fluorescence levels obtained at high protein concentration makes for difficulties in the application of this technique. Hydrocarbon chain methylene symmetric and asymmetric stretching vibrations appear in the 2900 cm^{-1} region of the infrared spectra. In [8] it was shown that temperature shifts for these band-maximum frequencies provided convenient probes for monitoring the phase transition of lipid dispersed in water. The frequencies and bandwidths of CH_2 stretching modes have been related to specific molecular properties, i.e., a shift in frequency to the introduction of *gauche* conformers and changes in bandwidth to variations in the rate of librational motions of the chains [9]. We have therefore used IR spectroscopy using a data station to contrast the influence of gramicidin and cholesterol on the lipid chain conformation, both above and below T_c in lipid bilayer structures.

2. Experimental

Gramicidin A, cholesterol, L-1,2-dipalmitoyl-α-lecithin (DPPC) and L-1,2-dimyristoyl-α-lecithin (DMPC) were purchased from Koch-Light Labs., BDH and Fluka, respectively. Samples were prepared by taking stock solutions containing the required propor-

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.50

tions of lecithin and either cholesterol or gramicidin A in benzene/methanol (95:5, v/v) and freeze-dried overnight. The resulting dry powder was dispersed in a buffer mixture containing Hepes 10 mM, sucrose 50 mM, KCl 1 M, pH 7.0 at 50°C by vortex mixing for 5 min. The final lipid concentration was always between 10–20 mg/ml.

The samples were introduced into a thermostatted microcell (Beckmann FH-01CFT) using CaF_2 windows and a pathlength of 7 μm. Buffer alone, lecithins and lipid–polypeptide systems dispersed in the buffer were scanned in a Perkin Elmer IR 561 assisted with a data station as was described in [10]. From 5–10 scans in the 3000–2800 cm^{-1} region were averaged at each temperature using a wide slit and with the maximum noise suppression of the instrument, 16. The temperatures were controlled and changed, always in the increasing direction, by the data station using an interface built by us which will be described later. After the subtraction of buffer spectrum, the difference spectra were amplified ~20-fold, using the Abex subroutine and the band-maximum frequencies measured.

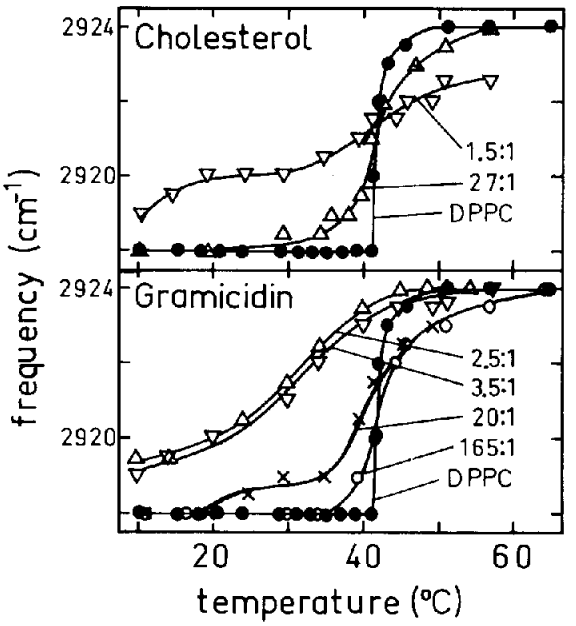

Fig.1. Temperature dependence of the maximum frequency of the CH_2 asymmetric stretching vibration in DPPC (•) and (other symbols) in DPPC/cholesterol or DPPC/gramicidin A at the molar ratios indicated.

3. Results and discussion

The temperature profiles of the band-maximum frequency for the methylene assymmetric stretching vibration of DPPC and DMPC are shown in fig.1 and 2, respectively (•). The results for the pure lipids are similar to those in [8,9]. The main endothermic transition of each lipid results in an abrupt increase in the frequencies of both methylene bands. The symmetric stretching vibrations around 2850 cm^{-1} are not shown, although they give practically the same results as those shown here. The minor overall change of the band-maximum frequencies for the symmetric vibration (only 3–4 cm^{-1}) during the lipid chain melting, makes it less suitable for this type of study due to the greater relative influence of the experimental error in the band position (~0.5 cm^{-1} in our conditions). The main transition is also observed by changes in other band parameters, as the bandwidth and the peak height, in accord with [9]. However, the simplicity of the frequency measurements led us to use them more extensively than in [9]. As is observed by other techniques, slight impurities in the sample make the main transition less sharp. (T_c for pure DPPC is 41°C and for DMPC is 23°C.)

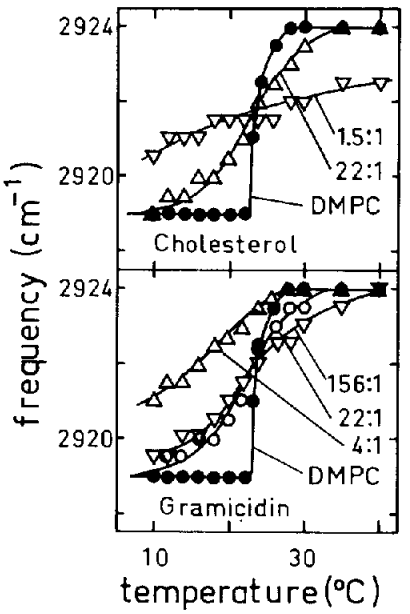

Fig.2. Temperature dependence of the maximum frequency of the CH_2 asymmetric stretching vibration in DMPC (•) and (other symbols) in DMPC/cholesterol or DMPC/gramicidin A at the molar ratios indicated.

The influence of cholesterol on the phase transition of both lecithins is shown in fig.1,2. These results are similar to those obtained in [8,11] by IR spectroscopy and agree with those obtained by others using a great variety of physical techniques [12], i.e., the presence of cholesterol causes shifts in band frequencies which indicate that an increase in the number of *gauche* conformers occurs below T_c (less order) and a decrease in this number above T_c (more order). This effect increases with greater amounts of cholesterol in the bilayer. At very high cholesterol concentrations (see fig.2) almost no change occurs with temperature in the relative population of *gauche* and *trans* conformers of the lipid chains.

The effects observed by incorporation of gramicidin into the bilayer are more complex than those due to the influence of cholesterol. It can be seen in fig.1,2 that gramicidin at molar lipid/polypeptide ratios >10 (~20% wt) show frequency–temperature profiles similar to those obtained by addition of cholesterol (fig.1,2), i.e., the presence of the polypeptide is to cause a decrease of the average number of *gauche* isomers above the T_c temperature and an increase in the average number of *gauche* isomers below the T_c temperature. It can be noted that, at temperatures far above the T_c transition temperature, the ordering or disordering effects due to the presence of gramicidin are compensated by the higher extreme temperatures examined. However, when higher polypeptide concentrations are included within the lipid bilayer, the transition profile changes dramatically. At temperatures higher than T_c we see that now an increase in the average number of *gauche* conformers population occurs reaching the same level as that of the pure lipid alone.

This increase cannot be due to the presence of the methylene groups of the amino acid side-chains of the polypeptide. The relative number of these groups is <15% for the case with greatest relative amount of gramicidin. Neither could we observe any clear shoulder at the high frequency side of the asymmetric band in the spectra scanned at low temperature as occurs with other lipid systems with a very high proportion of protein (unpublished). Thus the result of the vibrational spectroscopy indicate that similar ordering and disordering effects occur on a much shorter time scale (10^{-14} s) to that corresponding to the ^{2}H NMR time scale (10^{-5} s). The infrared spectra will not be influenced by factors which can affect the ^{2}H NMR spectra, such as vesicle tumbling or lipid exchange processes. It hence provides clear independent evidence of the effect of an intrinsic polypeptide on the average *gauche* isomers of the lipid chains.

A model has been proposed [13] to explain the deuterium NMR data which envisages that various populations of lipids occur:

(i) Those not adjacent to a polypeptide;
(ii) Those which are adjacent to a polypeptide; and
(iii) Those which are trapped between 2 or 3 polypeptides.

The deuterium data is interpreted in terms of these lipid populations whose concentration varies with the concentration of the intrinsic polypeptide in the lipid bilayer. Lipid chains adjacent to one polypeptide are envisaged to be more ordered (statistically) whilst lipid chains 'trapped' between 2 or more polypeptides are more disordered than that of the pure lipid system.

The infrared spectroscopic data is consistent with this model. It also indicates that even when an ordering effect is caused by the polypeptide a further increase in temperature brings an additional increase in the average number of *gauche* isomers. We are presently applying this technique to intrinsic protein–lipid systems.

Acknowledgements

We wish to acknowledge support from the Wellcome Trust. M. C. is a Visiting Welcome Fellow. We also wish to thank the Humane Research Trust for their support. We thank Dr W. Gratzer for the IR microcell used in this work.

References

[1] Chapman, D., Gómez-Fernández, J. C, and Goñi, F. M. (1979) FEBS Lett. 98, 211–223.
[2] Mabrey, S., Mateo, P. L. and Sturtevant, J. M. (1978) Biochemistry 17, 2464–2468.
[3] Kang, S. Y., Gutowsky, H. S., Hsung, J. C., Jacobs, R., King, T. E., Rice, D. and Oldfield, E. (1979) Biochemistry 18, 3257–3267.
[4] Rice, D. M., Meadows, M. D., Scheinman, A. O., Goñi, F. M., Gómez-Fernández, J. C., Moscarello, M. A., Chapman, D. and Oldfield, E. (1979) Biochemistry 18, 5893–5903.
[5] Rice, D. and Oldfield, E. (1979) Biochemistry 18, 3272–3279.
[6] Chapman, D., Cornell, B. A., Eliasz, A. W. and Perry, A. (1977) J. Mol. Biol. 113, 517–538.

[7] Wallach, D. F. H., Verma, S. P. and Fookson, J. (1979) Biochim. Biophys. Acta 559, 153 208.
[8] Asher, I. M. and Levin, I. W. (1977) Biochim. Biophys. Acta 468, 63–72.
[9] Cameron, D. G., Casal, H. L. and Mantsch, H. H. (1980) Biochemistry 19, 3665–3672.
[10] Chapman, D., Gómez-Fernández, J. C., Goñi, F. M. and Barnard, M. (1980) J. Biochem. Biophys. Methods 2, 315–323.
[11] Umemura, J., Cameron, D. G. and Mantsch, H. H. (1980) Biochim. Biophys. Acta 602, 32–44.
[12] Quinn, P. J. and Chapman, D. (1980) CRC Crit. Rev. Biochem. 1–117.
[13] Pink, D. A., Georgallas, A. and Chapman, D. (1981) Biochemistry in press.