EFFECT OF CYCLOSPORIN-A ON THE ORDER AND DYNAMICS OF DPPC MODEL MEMBRANE SYSTEMS

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ABSTRACT : *Laser Raman spectroscopy has been employed to investigate the effects of cyclosporin- A on the order and dynamics of DPPC (Dipalmytoyl Phosphatidyl Choline) model membrane system. It is shown that the addition of small amount of cyclosporin- A to a DPPC dispersion disturb the system and changes the order/ disorder parameter of the model membrane.*

KEY WORDS : *Dipalmytoyl Phosphatidyl Choline, Cyclosporin- A, Raman Spectroscopy, Membrane Bilayer, Order/Disorder.*

INTRODUCTION :

The mechanism of action of biomembrane systems and their role as molecular barrier in biological cells specially the transport of species into and out of cells is a subject of growing interest both in chemistry and biology [1-8]. The structural diversity and complexities exhibited by membranes preclude the development of a universal model for describing all membrane organizations. However, many of todays unifying principles of membrane structure is based upon the fluid mosaic arrangement of lipid and protein compounds in a membrane bilayer as proposed by *Singer* and *Nicolson* [9].

Phospholipids, including DPPC constitute the lipid composition of most membranes [10]. Phospholipid bilayers prepared in the laboratory may be used as simple membrane models when studying the function of biological membranes. Vibrational spectroscopy and in paticular Raman spectroscopy have proven to be useful and powerful techniques in carrying out such studies [11-13].

In this work we have used pure DPPC bilayer (dispersion) as model membranes, and have investigated their order and dynamics. In the presence of cyclosporin-A molecule. Cyclosporin-A is a cyclic undecapeptide [14] which has been found to have a powerfull immunosup-

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pressive properties[15].The mechanism of action of cyclosporin-A at the biological level is almost unknown.

EXPERIMENTAL :

Dipalmytoyl phosphatidyl choline (DPPC) was obtained from *Sigma (Dorset, England)* and was used without purification. DPPC dispersions were made using a buffer solution of 50mM Tris and 10mM EDTA (pH = 7). About 50%(w/w) lipid/ buffer ratio were mixed in a specially designed optical cell. After initial pre- mixing, the DPPC dispersion was vortexed and centrifuged and then incubated in an oven at 50°C for one hour. Cyclosporine- A was kindly offered by *Leeds St. James* Hospital (Leed, England) and was used without purification. About 1/35 molar ratio of cyclosporin- A was added to DPPC dispersion throught disolving cyclosporin-A in ethanol. Raman spectra were obtained using a SPEX 1401 double monochromator equipped with EMI photon detection system *(Grasbrunn, Germany)*. Excitation frequency at 488.0nm (about 150 - 200mW) was obtained from a Spectra-Physics*(London, England)* 171 Ar^+ laser. All Raman spectra at different temperatures were obtained using a specially designed thermostated Raman Cell. The ratio of peak heights were used to compare the Raman intensities.

RESULTS AND DISCUSSION :

Fig. 1 shows Raman spectra of DPPC bilayer at different temperatures in the wavenumber shift range 1000- 1400cm^{-1}. The Raman bands at 1063, 1095 and 1128cm^{-1} are skeletal C-C stretching modes and reflect the intramolecular trans/gauch conformational changes within the bilayer's hydrophobic acyl chain matrix [16-18]. The Raman band at 1063cm^{-1} is assigned to the out-of-phase skelectal motion [19] and is chain-length insensitive but moderately temperature sensitive. The Raman band at 1128cm^{-1} is assigned to an in- phase C-C motion [19]. Both the 1128cm^{-1} and the weaker band at 1095cm^{-1} are functions of both chain length and temperature.

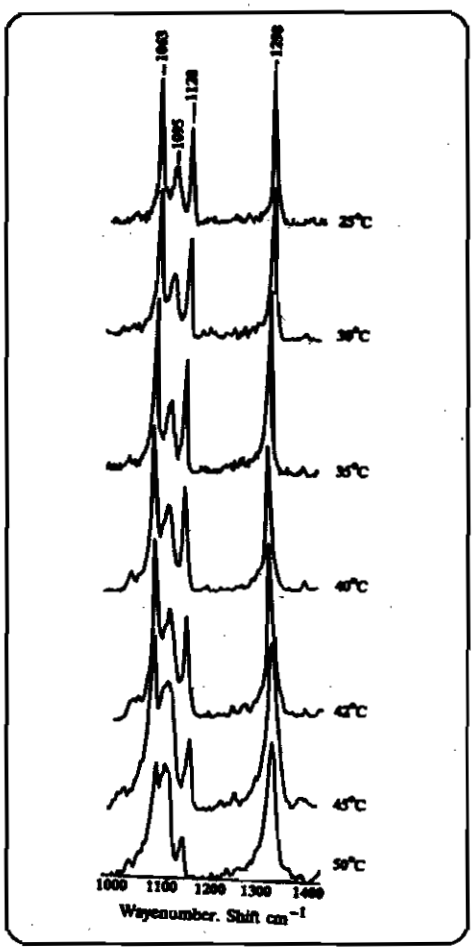

Fig. 1 : Comparison of the Raman spectra of DPPC multilamellar dispersion at different temperature in the wavenumber shift range 1000-1400cm^{-1}.

The behavior of these Raman bands in relation to the changes in the temperature are shown in Fig. 1. It can be seen that as the temperature is increased the intensity of the 1128cm^{-1} band is decreased whereas the intensity of the 1095cm^{-1} band (emergence of gauche conformers), is increased. Using peak height intensity ratios one can construct a so called temperature profile which is discussed later on (Fig. 5). The temperature range in Fig. 1 have been chosen so that it extends from the pre-transition phase into the liquid crystaline phase (the T_m for DPPC bilayer is 41.3°C).

Fig. 2 shows Raman spectra of DPPC bilayer at different temperature in the wavenumber shift range 2700- 3100cm^{-1}.This region of the Raman

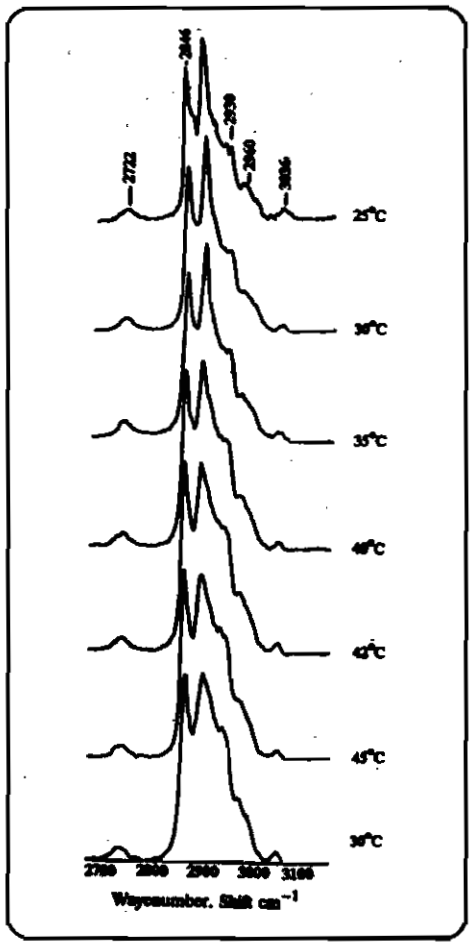

Fig. 2 : Comparison of the Raman spectra of DPPC multilamellar dispersion at different temperature in the wavenumber shift range 2700-3100cm^{-1}.

spectra reflects the C-H stretching modes. The Raman bands at 2846, 2880 and 2930cm^{-1} are assigned to the methylene C-H symmetric stretching, methylene C-H asymmetric stretching and chain terminal methyl C-H symmetric stretching modes respectively [20, 21]. The relative peak height intensity for these bonds provide a convenient means for constructing temperature profiles for membrane assemblies [17] which again is discussed later on (Fig. 6). It can be seen from Fig. 2 that as the temperature is increased (upon which the gel- state DPPC undergoes a transition into the liquid crystaline phase) the intensity of the 2930cm^{-1} band is (increased) where as the intensity of 2880cm^{-1} band is decreased. This feature shows that the gel- state bilayer disorders and enters the liquid crystaline state.

Figs. 3 and 4 show similar Raman spectra in the presence of a small amount of cyclosporin-A (molar ratio 1/35).

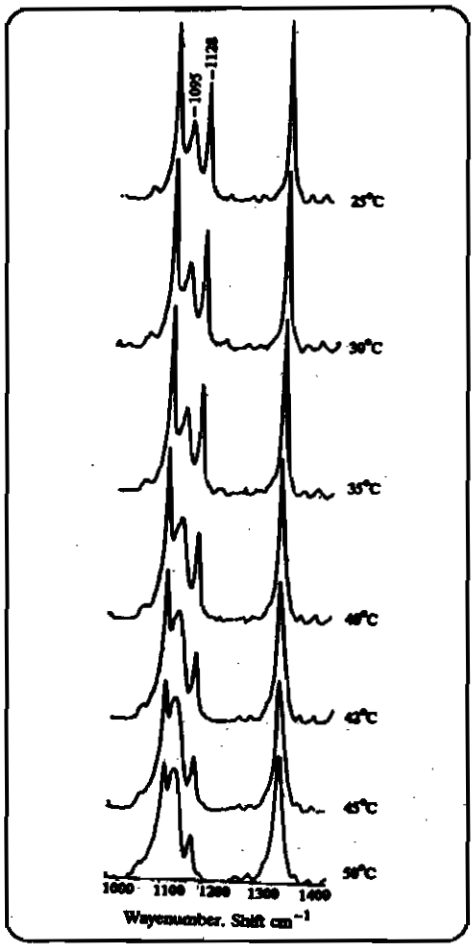

Fig. 3 : Comparison of the Raman spectra of DPPC multilamellar dispersion mixed with cyclosporin- A (molar ratio 1/35) at different temperature in the wavenumber shift range 1000-1400cm^{-1}.

The intensity alterations in the Raman bands in Figs. 3 and 4 relative to Raman bands in the pure DPPC dispersion (Figs. 1 and 2) are compared quantitatively in Figs. 5 and 6.

Fig. 5 shows a plot of the ratio of the intensity of the 1095 cm^{-1} band to that of the 1128cm^{-1} band. This ratio is a convenient means of monitoring the gel-to-liquid crystaline

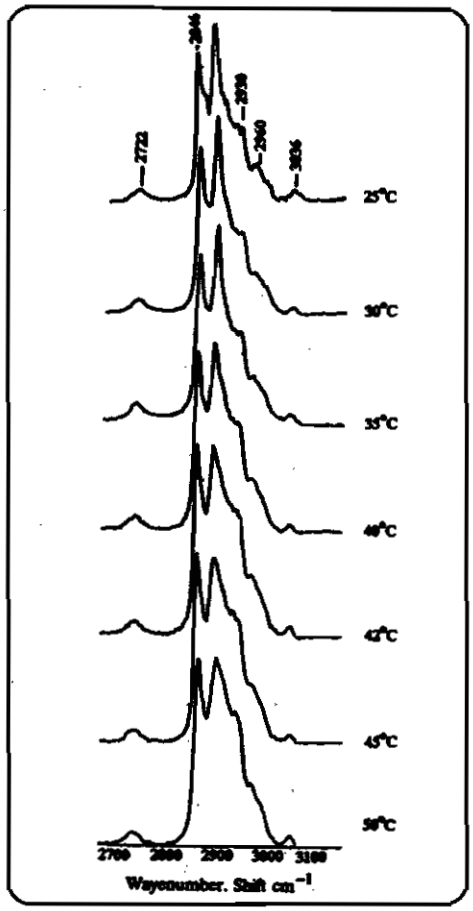

Fig. 4 : Comparison of the Raman spectra of DPPC multilamellar dispersion mixed with cyclosporin-A (molar ratio 1/35) at different temperature in the wavenumber shift range 2700-3100cm^{-1}.

phase transition and for comparing intramolecular chain order/ disorder characteristics in DPPC dispersion [11, 22]. It can be seen from Fig. 5 that below T_m the addition of cyclosporin-A to DPPC dispersion has decreased the order of bilayer, (the number of gauche conformers increases), whereas it increases the order of DPPC bilayer near and above the phase transition.

Fig. 6 presents a plot of I_{2930}/I_{2880} ratio of peak intensities in the C-H stretching region as a function of temperature for pure DPPC bilayer and for a cyclosporin-A-to-lipid molar ratio of 1/35. The I_{2930}/I_{2880} ratio has contributions from both inter and intramolecular disordering of the acyl chains. It can be seen from Fig. 6 that cyclosporin-A brings disorder into the acyl chains both below and above the phase transition temperature. However, the disordering effect is more pronounced below the phase transition temperature.

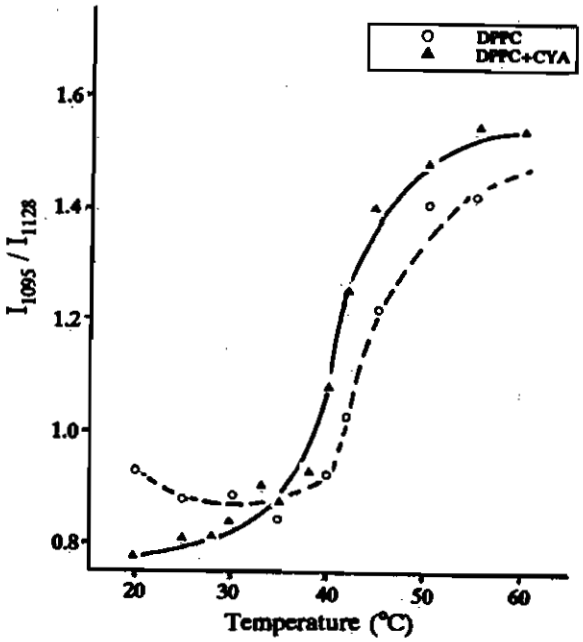

Fig. 5 : Raman temperature profiles of the C-C skeletal stretching region for DPPC multilayer and DPPC mixed with cyclosporin-A (molar ratio 1/35).

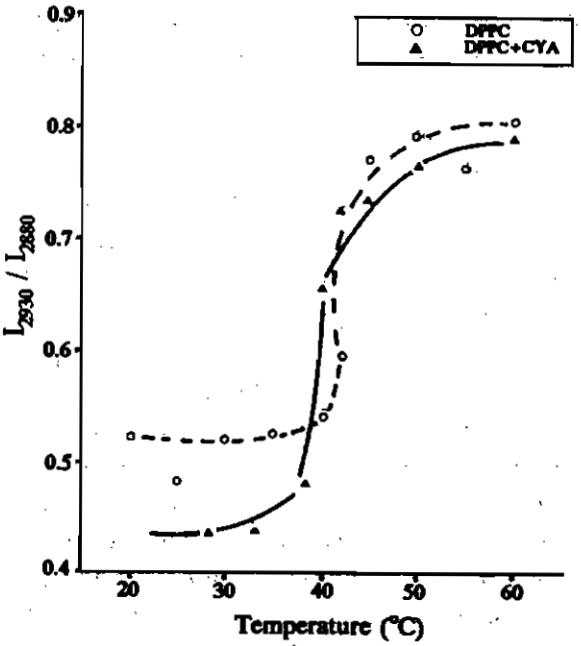

Fig. 6 : Raman temperature profile of the C-H stretching region for DPPC multilayer and DPPC mixed with cyclosporin-A (molar ratio 1/35).

CONCLUSIONS :

Raman spectroscopy offers some new information about the nature of the bilayer system and the structural changes taking place within the hydrocarbon domain of the bilayer above and below the transition temperature.

We conclude that cyclosporin-A interacts with DPPC bilayer through hydrophobic binding. From our discussion we find that it causes disorder in the acyl chains both below and above the phase transction temperature. It may therefore be concluded that this molecule has penetrated the bilayer, but the extent of this penetration can not be discerned.

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