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Peptide induced polymorphism in model membranes

RATNA S. PHADKE, SUDHA SRIVASTAVA and GIRJESH GOVIL*
Chemical Physics Group, Tata Institute of Fundamental Research, Bombay 400 005, India

Abstract. Lipids in biological membranes generally adopt bilayer structures. However, incorporation of peptides may induce alterations in such structures. We have studied the influence of tryptophan, leucine, Trp-Leu, luteinizing hormone releasing hormone and renin inhibitor peptide on lipid organisation in liposomes. It has been observed that the effect is specific to the peptide molecule as-a whole and does not have direct correlation to the constituent amino acids or the conformation of the molecule.

Keywords. Peptides; phosphorous NMR; polymorphism; electron microscopy; liposomes.

It is well known that the major constituents of biological membranes are lipids and proteins. The relative proportion of these two classes of molecules is closely related to the specific functions of the membrane. For instance, whereas the mitochondrial membranes are rich in protein content, the plasma membranes have preponderance of lipids. In general, lipids provide a fluid matrix while the specific functions of the membranes are dependent to a large extent on the chemistry, structure and dynamics of lipids, proteins and the interactions between these molecules (Singer and Nicolson, 1972). While the membrane lipids are usually organized in bilayer form, morphological and structural studies indicate existence of non-bilayer and/or transient structures (Navarro *et al.*, 1984; Jensen and Schutzbach, 1985) as a consequence of lipid-protein interaction. Such modulations may permit specific processes to occur at discrete sites.

A number of methods have been used to study lipid-protein interactions. X-ray diffraction (Luzzati, 1968), neutron diffraction (Worcester, 1976) and electron microscopy (Deamer *et al.,* 1970) methods have the common drawback that they provide only static information. Spin labelling electron spin resonance (ESR) have been extensively used (Berliner, 1976) but this method has certain limitations. Recently, [^{31}P] nuclear magnetic resonance is being used (Seelig, 1978). The main advantage is that it does not require incorporation of external probes as in ESR spin labelling. Moreover, structural as well as dynamic aspects are reflected in the [^{31}P] resonance pattern.

We have incorporated certain peptides in the normal bilayer structures of dipalmytoyl phosphatidyl choline (DPPC) and have studied polymorphic changes in lipid assemblies brought by such peptides. Since, our aim is to understand the alterations induced by peptides in lipid matrix, we have used higher concentrations of peptides than those encountered in biological systems. A single type of lipid (DPPC) has been used to circumvent difficulties encountered while interpreting the results due to chemical diversity of constituents in real biological membranes.

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*To whom all the correspondence should be addressed.
Abbreviations used: ESR, Electron spin resonance; DPPC, dipalmytoyl phosphatidyl choline; CSA, chemical shift anisotropy; LHRH, luteinizing hormone releasing hormone; RIP, renin inhibitor polypeptide.

A large class of membrane proteins react with the polar and apolar part of lipids and destabilize bilayer structure. For instance, gramicidin can dramatically influence the barrier properties of membranes (Killian and de Kruijff, 1986). Out of the 15 residues of gramicidin, 4 each are tryptophan and leucine. In an earlier communication we have reported that the presence of tryptophan induces nonbilayer structure in liquid crystalline phase of the lipid (DPPC) (Srivastava *et al.*, 1988). Leucine on the other hand stabilizes the bilayer structures when present in 1 : 5 peptide-lipid molar ratio even at high temperatures as judged by

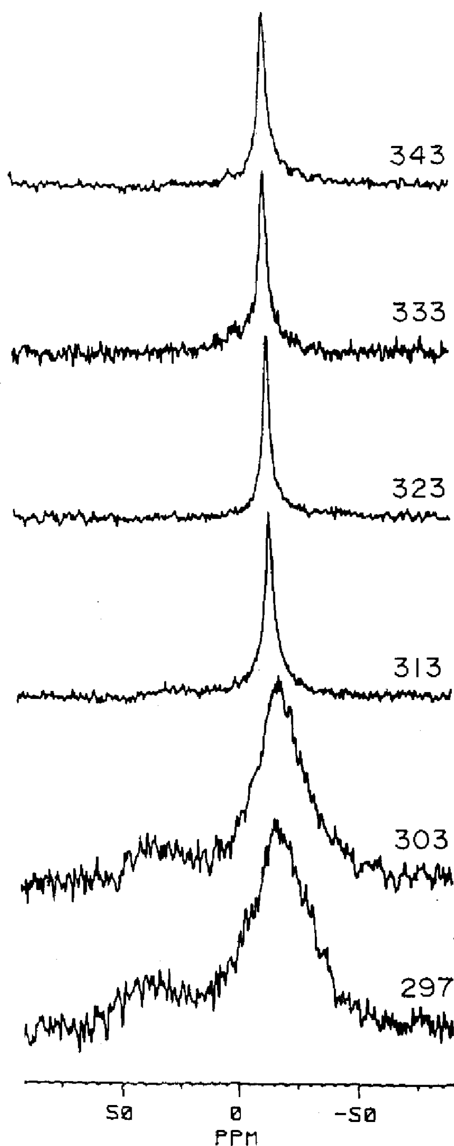

Figure 1. [^{31}P]CSA pattern at 202·457 MHz with broad band decoupling for DPPC (50 mM) +leucine (10 mM). Temperatures are indicated in Kelvin. The zero of the scale corresponds to sharp peak of sonicated DPPC at 323 K.

[^{31}P] chemical shift anisotropy (CSA) pattern (figure 1). In the gel phase (T< 313K) the characteristic bilayer pattern consisting of an intense peak separated by a broad shoulder is clearly visible ($\Delta\sigma$ = 48 ppm). With increasing temperatures, the width of the intense peak decreases, the broad shoulder becomes less prominent and the CSA ($\Delta\sigma$) value decreases. Such behaviour is expected because higher temperatures increase overall tumbling rates of the vesicles and also increase rotational as well as segmental motions of the lipid molecules. All these add to decrease anisotropy experienced by the phosphate moiety. Additional support regarding structural

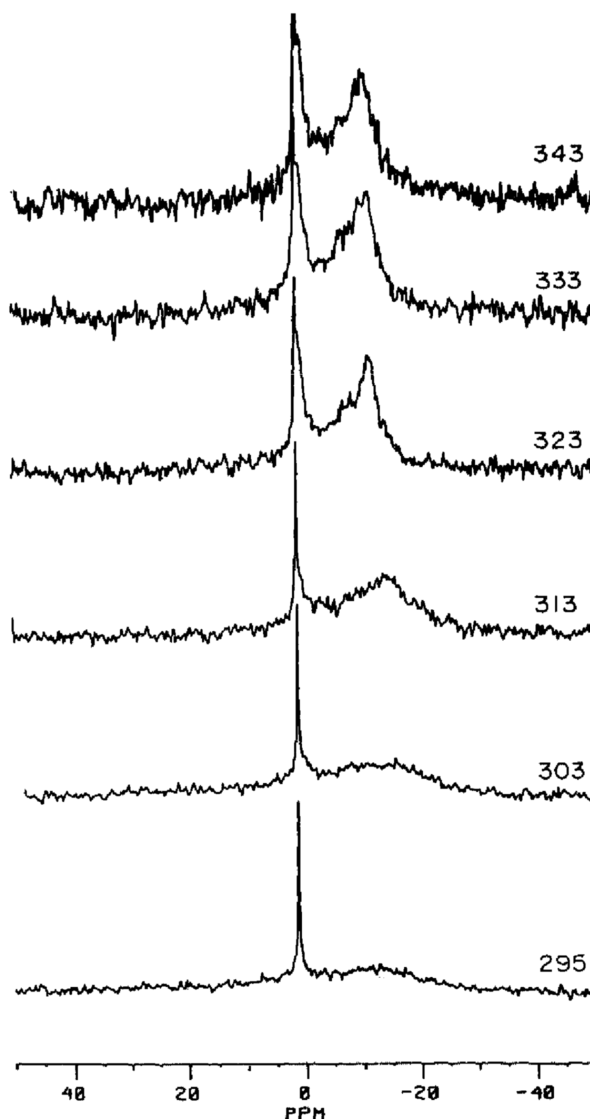

Figure 2. [^{31}P]CSA pattern at 202·457 MHz with broad band decoupling for DPPC (50mM) + RIP (25mM) at different temperatures (in Kelvin). The zero of the scale corresponds to sonicated DPPC at 323 K.

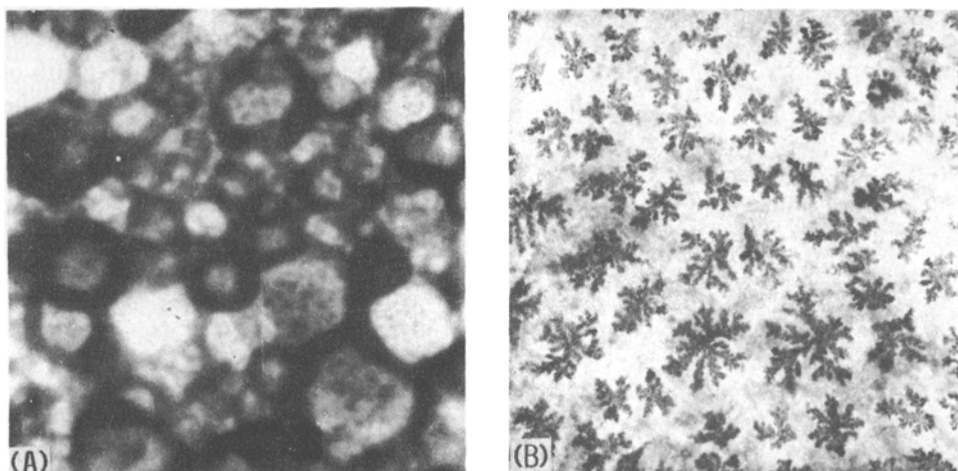

Figure 3. Electron micrograph (× 10,000) of DPPC-peptide system (2:1 molar ratio) indicating a change in lipid packing. (**A**), DPPC; (**B**), DPPC + RIP.

aspects is obtained by electron microscopy which shows that the normal circular liposomal organization remains unchanged on incorporation of leucine.

Exactly identical behaviour have been observed for liposomes containing dipeptide Trp-Leu. Gramicidin, on the other hand, has been reported to induce hexagonal phase formation for lipids with phosphocholine head group (Killian and de Kruijff, 1986). This indicates that the polymorphism of limpid assemblies is specific to the peptide molecule as a whole and does not totally depend on constitutent amino acids.

It has been reported earlier that the shape of the peptide molecules plays crucial role in inducing polymorphism (de Kruijff *et al*., 1985). Our earlier studies indicate that luteinizing hormone releasing hormone (LHRH) (Srivastava, 1986) and renin inhibitor polypeptide (RIP) assume random coil conformations when in aqueous solution and ordered structures in limpid phase. We have incorporated these hormones in DPPC liposomes. In the presence of LHRH the normal bilayer structure remains unaltered as indicated by [^{31}P]CSA pattern but RIP seems to induce mixed phase formations. In the latter case, the CSA pattern in gel phase consists of a sharp peak at 0·4 ppm and a broad peak centered around -20 ppm (figure 2). The CSA sign is reverse to that for bilayer structure and the value is almost Ralf. With increasing temperatures the broad peak builds up and CSA ($\Delta\sigma$) decreases. Figure 3A shows normal bilayer assemblies which in the presence of RIP transform into complex patterns consisting of rods joined together (figure 3B). Thus, one observes that although both peptides assume similar shapes they induce different organizations in DPPC liposomes indicating that shape of the peptide is not the sole decisive criteria.

Acknowledgement

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