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# THE INFLUENCE OF POLY(L-LYSINE) ON PHOSPHOLIPID POLYMORPHISM

## EVIDENCE THAT ELECTROSTATIC POLYPEPTIDE-PHOSPHOLIPID INTERACTIONS CAN MODULATE BILAYER/NON-BILAYER TRANSITIONS

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## Summary

$^{31}$P-NMR shows that poly(L-lysine) binding to cardiolipin, phosphatidylserine or phosphatidylglycerol does not affect the macroscopic structure or local order (in the phosphate region) of the phospholipids. In the case of cardiolipin poly(L-lysine) inhibits the ability of $Ca^{2+}$ to induce the hexagonal $H_{II}$ phase. Alternatively, poly(L-lysine) induces the hexagonal $H_{II}$ phase for a fraction of the phospholipids in phosphatidylethanolamine-cardiolipin (2:1) dispersions.

The ability of many membrane lipids to adopt non-bilayer phases suggests a dynamic role of lipids in various functional processes such as fusion and transbilayer transport (for review see Refs. 1 and 2). Among the factors which modulate bilayer/non-bilayer preferences of lipids are: temperature, lipid composition, pH and divalent cations [1,2]. In addition, the different phase behaviour of the lipids in intact membranes and in derived liposomes [3—6] suggests that membrane proteins can also modulate the structure of the phospholipid matrix. In the isolated bovine rod outer segment membrane, for example, the phospholipids are almost exclusively organized in a bilayer, whereas in the hydrated total lipid extract at 37°C the hexagonal $H_{II}$ phase,

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the lamellar phase and many 'lipidic particles' are observed. This suggests a bilayer stabilizing role of the integral membrane protein rhodopsin [3].

More detailed insight regarding the possible effects of proteins on the structure of membrane lipids can be obtained by investigating well-defined reconstituted lipid-protein systems. In this study we report $^{31}$P-NMR results on the effect of poly(L-lysine) on the phase behaviour of various phospholipids. This polypeptide, which appears to be a reasonable model for some extrinsic membrane proteins, experiences strong electrostatic interactions with negatively charged phospholipids [7—11] resulting in an increase in transition temperature [7,11] and heat content [7] of the gel → liquid-crystalline phase transition. Further, in mixed systems it can induce lateral phase separations [12] resulting in the formation of rigid poly(L-lysine)-lipid domains [12,13].

Our results indicate that upon binding of poly(L-lysine) to cardiolipin, phosphatidylserine or phosphatidylglycerol liposomes the lamellar phase is maintained and that the local order in the phosphate region is not affected. Furthermore, in the case of cardiolipin, poly(L-lysine) binding prevents the formation of the hexagonal $H_{II}$ phase by $Ca^{2+}$. In mixed phosphatidylethanolamine-cardiolipin systems poly(L-lysine) induces a hexagonal $H_{II}$ phase formation. Such effects are not observed in mixtures of phosphatidylethanolamine with phosphatidylserine or phosphatidylglycerol. The results are compared and contrasted with the cardiolipin specific formation of non-bilayer structures by cytochrome *c* [14].

Poly(L-lysine) (hydrobromide: degree of polymerization 200; mol. wt. 40 000) and the sodium salt of cardiolipin (bovine heart) were obtained from Sigma (St. Louis, U.S.A.). Phosphatidylcholine was obtained from egg and soya lipids by standard procedures. Phosphatidylethanolamine and phosphatidylserine were obtained from soya phosphatidylcholine and phosphatidylglycerol from egg phosphatidylcholine by the phospholipase D catalyzed base exchange reaction [15]. After isolation the negatively charged phospholipids were converted into their sodium salts [16]. All lipids were chromatographically pure. Aqueous lipid dispersions were prepared by dispersing 50 μmol of lipid at 20°C in 1.0 ml of an aqueous buffer (100 mM NaCl, 10 mM Tris-HCl, 0.2 mM EDTA, pH 7.0) by vortexing [16]. Poly(L-lysine) was either added to the lipids in 0.2 ml of the buffer or the lipids were dispersed in 1.2 ml of the buffer already containing the peptide. Identical results were obtained for both protocols. No pH changes were observed upon addition of poly(L-lysine). $^{31}$P-NMR measurements were done at 81 MHz as described elsewhere [16]. Prior to the NMR measurements 0.1 ml of the $^2H_2O$ analogue of the buffer was introduced to the sample.

The proton decoupled $^{31}$P-NMR spectrum of an aqueous dispersion of cardiolipin has a lineshape with a low field shoulder and a high field peak typical [1] of phospholipids organized in extended bilayers (Fig. 1A) in agreement with previous results [17]. The small peak at the 0 ppm position might originate from some small structures present in the sample. Addition of excess $Ca^{2+}$ to the liposomes results in precipitation of the lipids and formation of the hexagonal $H_{II}$ phase [17,18], as evidenced by the characteristic spectrum with a reversed asymmetry and reduced width [1] (Fig. 1c). Alternatively addition of poly(L-lysine) to the liposomes also caused immediate precipitation

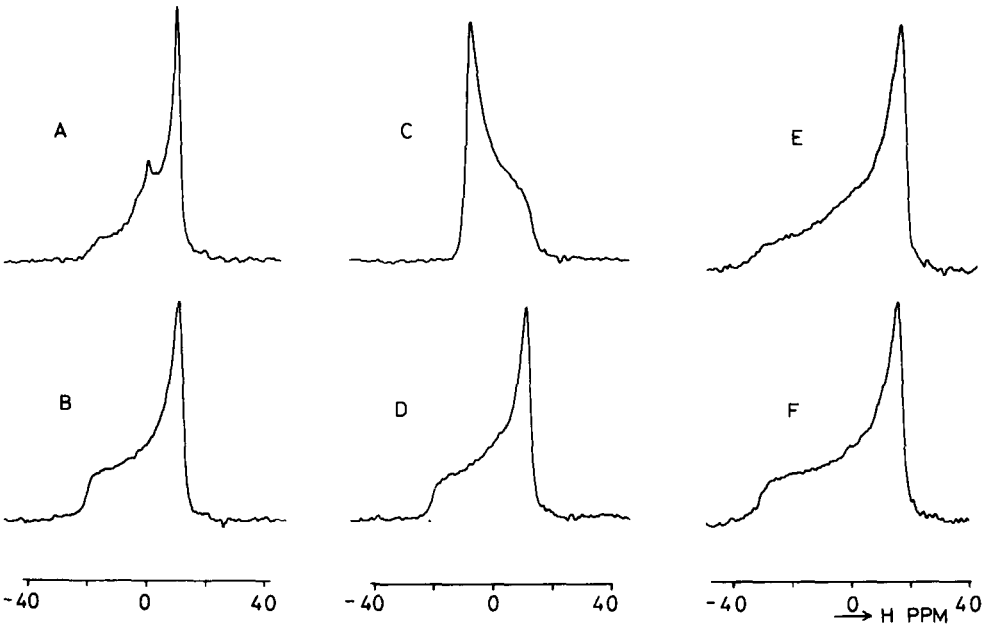


Fig. 1. 81 MHz $^{31}$P-NMR spectra at 30°C of (A) 50 μmol of cardiolipin in 1.0 ml buffer, (B) 50 μmol of cardiolipin in 1.0 ml buffer 5 min after the addition of 40 mg poly(L-lysine), (C) 50 μmol of cardiolipin in 1.0 ml buffer 5 min after the addition of 100 μl 1 M $CaCl_2$ to the sample, (D) 50 μmol of cardiolipin in 1.0 ml buffer to which 40 mg poly(L-lysine) and 100 μl M $CaCl_2$ were added subsequently, (E) 50 μmol of phosphatidylserine in 1.0 ml buffer, (F) 50 μmol of phosphatidylserine in 1.0 ml buffer 5 min after the addition of 40 mg poly(L-lysine). Doubling the amount of poly(L-lysine) (B,F) or incubating the samples for up to 3 h (B,D,F) at 30° C did not affect the $^{31}$P-NMR spectrum. The 0 ppm position in this and the subsequent figure represents the chemical shift of sonicated egg phosphatidylcholine vesicles. All free induction decays were exponentially multiplied resulting in a 50 Hz line broadening.

of the lipids. However, in contrast to the situation observed with $Ca^{2+}$ the cardiolipin remains organized in extended bilayers (Fig. 1B). The disappearance of the isotropic peak and the sharper definitions of the low field shoulder (compare Figs. 1A and B) suggest that either fusion to larger structures has occurred or that the rate of lateral diffusion of cardiolipin in the bilayer is reduced upon interaction with poly(L-lysine). Lateral diffusion can significantly affect the lineshape of phospholipids in relatively large structures, such as unsonicated liposomes [19].

Addition of excess $Ca^{2+}$ to the poly(L-lysine)-cardiolipin system did not affect the $^{31}$P-NMR spectrum (Fig. 1D) demonstrating that poly(L-lysine) inhibits the ability of $Ca^{2+}$ to trigger a bilayer-hexagonal $H_{II}$ transition for the cardiolipin.

Poly(L-lysine) addition to phosphatidylserine or phosphatidylglycerol liposomes also caused immediate precipitation of the lipids again with no change in the macromolecular organization of the lipids (Fig. 1E,F; data for phosphatidylglycerol are not shown, but were very similar). In all cases the phospholipids remain organized in extended bilayers. The $^{31}$P-NMR spectrum again shows a better definition of the low field shoulder but no significant change in the distance from the shoulder to the peak, which is a measure of the order of the phosphate region [20], is observed.

Addition of poly(L-lysine) to dispersions of phosphatidylcholine or phosphatidylethanolamine (the bilayer → hexagonal $H_{II}$ transition of this phos-

phatidylethanolamine occurred at 10°C, slightly higher than reported before [21]) did not cause precipitation of the lipids nor was any change in the $^{31}$P-NMR spectrum recorded at 30°C, observed.

The possibility that poly(L-lysine)-lipid interactions modulate bilayer-non-bilayer transitions was further investigated employing lipid mixtures. Mixtures of unsaturated phosphatidylethanolamine with cardiolipin or phosphatidylserine are of special interest in this respect for two reasons:

Firstly, these lipid mixtures appear to be preferentially located in the inner monolayer of various biological membranes. For instance, cardiolipin and phosphatidylethanolamine are mainly localized in the inner monolayer of the inner mitochondrial membrane [22] whereas phosphatidylserine and phosphatidylethanolamine are the main lipids of the inner monolayer of the human erythrocyte membrane [23]. Secondly, the structure of these lipid mixtures is very sensitive to the presence of divalent cations. Whereas cardiolipin and phosphatidylserine stabilize the bilayer configuration of unsaturated phosphatidylethanolamine, $Ca^{2+}$ addition results in bilayer destabilization and formation of an 'isotropic' and hexagonal $H_{II}$ phase [14,24].

Fig. 2A shows that in the soya phosphatidylethanolamine-cardiolipin (2:1) mixture at 30°C cardiolipin stabilizes the bilayer structure for the majority of the phosphatidylethanolamine. Addition of poly(L-lysine) results in a marked and reproducible change in the NMR spectrum (four different experiments provided identical results, see Fig. 2B), in that a shoulder (in the region of

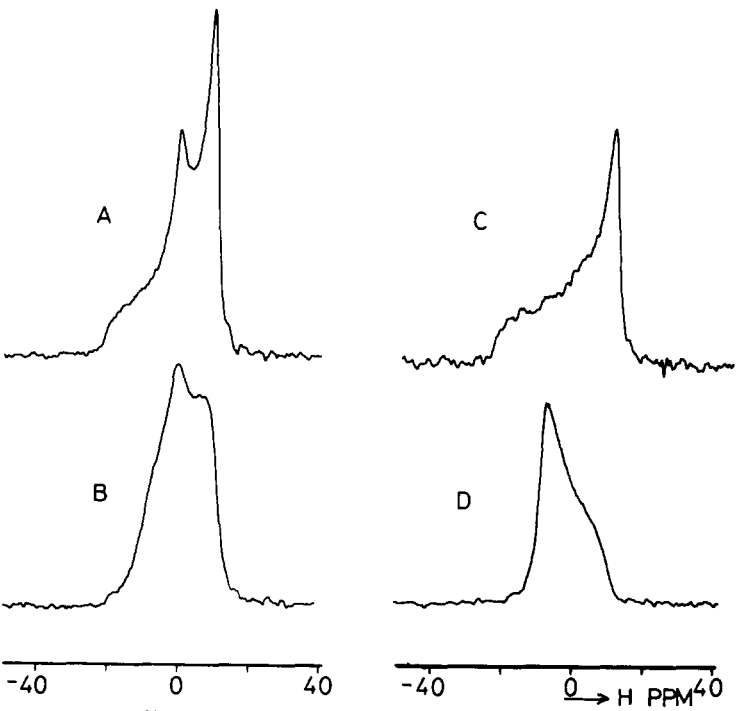


Fig. 2. 81 MHz $^{31}$P-NMR spectra of a dispersion of 50 μmol soya phosphatidylethanolamine-cardiolipin (2:1) in 1.0 ml buffer. (A) at 30° C, (B) at 30° C 5 min after the addition of 40 mg poly(L-lysine), (C) as (B) except recorded at 0° C, (D) as (B) except that 100 μl 1 M CaCl, was added. Doubling the amount of poly(L-lysine) (B,C) or incubating the samples for up to 3 h at 30° C (B,C,D) did not further affect the $^{31}$P-NMR spectrum.

−7 ppm) and a significant amount of signal on the low field side of the isotropic (0 ppm) peak appear. That this signal intensity originates from part of the phosphatidylethanolamine in the hexagonal $H_{II}$ phase is indicated by the chemical shift of the shoulder (chemical shift of the main peak in the spectrum of the hexagonal $H_{II}$ phase of this lipid mixture in the presence of excess $Ca^{2+}$ is −7 ppm, see Fig. 2D). Furthermore, when the spectrum is recorded at 0°C, at which temperature the phosphatidylethanolamine prefers the bilayer phase, a typical bilayer spectrum (Fig. 2C) is observed. This temperature dependent behaviour was fully reversible. Freeze-fracturing the sample also revealed the presence of extended areas of the hexagonal $H_{II}$ phase (Leunissen-Bijvelt, J., unpublished observations). Subsequent addition of 10 $\mu$l 1 M $CaCl_2$ solution to the liposomes, which in the absence of poly(L-lysine) induces the hexagonal $H_{II}$ phase of most of the lipids (results identical to those described in Ref. 14), did not affect the $^{31}$P-NMR spectrum, again demonstrating that the poly(L-lysine)-cardiolipin interaction inhibits the $Ca^{2+}$-cardiolipin interaction. At much higher $Ca^{2+}$ concentrations, however, $Ca^{2+}$ induces the hexagonal $H_{II}$ phase for all the lipids (Fig. 2D).

Phosphatidylethanolamine-phosphatidylserine (2:1) and phosphatidylethanolamine-phosphatidylglycerol (2:1) liposomes are organized in extended bilayers at 30°C as evidenced by the $^{31}$P-NMR spectrum (data not shown). Addition of equimolar $Ca^{2+}$ (with respect to the charged phospholipid) triggered formation of the hexagonal $H_{II}$ phase as observed before (Ref. 24; and Farren, S.B., unpublished observations). Addition of 40 mg poly(L-lysine) to these liposomes caused precipitation of the lipids but did not affect the $^{31}$P-NMR spectrum demonstrating, in contrast to the situation observed with cardiolipin, that the poly(L-lysine) interaction with phosphatidylserine or phosphatidylglycerol in these mixtures does not result in the formation of hexagonal $H_{II}$ phase.

These results show that poly(L-lysine) can modulate bilayer-non-bilayer transitions in systems containing negatively charged phospholipids in different ways. On one hand poly(L-lysine) stabilizes the bilayer structure of cardiolipin in the presence of $Ca^{2+}$. This suggests that the poly(L-lysine) molecules (which have a random coil structure) cover the surface of the bilayer and prevent $Ca^{2+}$ from approaching the phosphate groups. On the other hand lipid specific bilayer destabilization by poly(L-lysine) is observed. In cardiolipin-phosphatidylethanolamine mixtures the polypeptide induces the hexagonal $H_{II}$ phase for part of the system. This most likely occurs via a process of phase separation of the poly(L-lysine)-cardiolipin complex [12] thereby decreasing the bilayer stabilizing force of cardiolipin and allowing the phosphatidylethanolamine to revert to the $H_{II}$ phase.

It is of interest to compare these results with those obtained for cytochrome *c* in similar systems [14]. This inner mitochondrial membrane protein also binds electrostatically to negatively charged phospholipids but in contrast to poly(L-lysine) it specifically induces the $H_{II}$ phase and possibly inverted micelles in pure cardiolipin and cardiolipin-containing mixtures. It is thought that this nearly spherical basic protein can actually reside in the aqueous compartment present inside these inverted structures [14]. Furthermore, the cytochrome *c*-cardiolipin interaction does not prevent the $Ca^{2+}$-induced formation of the $H_{II}$ phase [14].

In summary, these studies show that electrostatic interactions between polypeptides and membrane lipids can strongly and specifically affect the bilayer/non-bilayer preferences of membrane lipids. Such observations point to the possibility that lipid-protein interactions may play important regulatory roles in membrane processes requiring non-bilayer lipid structures.

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