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THE INFLUENCE OF pH, Ca^{2+} AND PROTEIN ON THE THERMOTROPIC BEHAVIOUR OF THE NEGATIVELY CHARGED PHOSPHOLIPID, PHOSPHATIDYLGLYCEROL

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Summary

It is demonstrated that the transition temperature from the liquid-crystalline to gel state of a synthetic phosphatidylglycerol is influenced by pH, Ca^{2+} and A_1 basic protein from myelin.

It is known that the fatty acid composition of phospholipids has a great influence on the physicochemical behaviour of artificial and natural membranes [1, 2]. An example of this relationship is the transition undergone by lipids from the liquid-crystalline to the gel state, a phenomenon under intensive investigation in recent years. The phase transition temperature is dependent on the length and degree of unsaturation of the hydrocarbon chains, and on the nature of the polar head groups of the phospholipids [3, 4].

In this study we investigate whether pH, Ca^{2+} and protein can change the transition from the liquid-crystalline to gel state of the negatively charged phospholipid 1,2-didodecanoyl-*sn*-phosphatidyl-1'-*sn*-glycerol (diC12-phosphatidylglycerol). This behaviour is correlated with the morphological state, as detected by freeze-etch electron microscopy and with recent monolayer data [5]. In the course of this study Träuble and Eible [6] informed us of their findings which also demonstrated, by means of a fluorescent probe technique, that pH and divalent cations could change the transition temperature of other negatively charged phospholipids.

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The phase transitions were measured with a Perkin—Elmer 2B differential scanning calorimeter. Synthesis, purification and physicochemical analysis of diC12-phosphatidylglycerol will be described elsewhere [7]. According to thin-layer chromatographic and spectroscopic data, the diC12-phosphatidylglycerol was more than 98% pure. 7 μmoles phospholipid were dispersed in 50 μl of a solution of 40 mM Tris acetate—ethyleneglycol (1:1, v/v) at the desired pH values and containing the relevant ions and protein.

Effect of pH

To investigate the influence of pH on the thermotropic behaviour of diC12-phosphatidylglycerol, the phospholipid was dispersed in 100 mM NaCl. At this NaCl concentration is was found [5] that diC12-phosphatidylglycerol is completely ionized at pH 7. As can be seen from Fig. 1a, diC12-phosphatidylglycerol has a double transition at pH 7 near 0 °C. As the sample is over 98% pure, it must be concluded that this behaviour cannot be caused by an impurity. It is suggested that the influence of the polar head group on the phospholipid thermotropic behaviour is of particular importance in medium-chain phospholipids. A more important feature in the thermotropic behaviour is the considerable increase in transition temperature upon lowering the pH (Fig. 1b). By freeze-etching it is demonstrated (Fig. 2a) that there is no change in morphology, in that only small liposomal structures were visible. The fracture faces of these liposomes are smooth above and below the transition temperature in contrast to those displayed by liposomes of synthetic lecithins [8]. Monolayer studies showed that the area per mole was condensed from 62 Å^2 at pH 7 to 56 Å^2 at pH 3, at a pressure of 30 dynes/cm at 20 °C [5]. So it can be concluded that, upon lowering the pH of the medium, the transition temperature is raised as a consequence of a decrease in surface charge, as indicated schematically in Fig. 3.

Effect of Ca^{2+}

The role of the divalent cations Ca^{2+} and Mg^{2+} is of great importance in membrane physiology. Fig. 1c demonstrates that even a very low concentration of Ca^{2+} (Ca^{2+}:phosphatidylglycerol = 1:100) can markedly raise the transition temperature. There is only a small increase in the transition temperature on raising the Ca^{2+} concentration by a factor of 25 (Ca^{2+}:phosphatidylglycerol = 1:4) (Fig. 1d). However, when the ratio Ca^{2+}:phosphatidylglycerol is greater than 1:2 a new jump in the transition temperature is observed (Fig. 1e). This difference can be seen by the naked eye at 23 °C, where a liposomal milky dispersion and a precipitation are visible below and above the Ca^{2+}:phosphatidylglycerol ratio of 1:2, respectively. This gross difference is reflected also in the freeze-etch replicas. At Ca^{2+}:phosphatidylglycerol ratios $< 1:2$, liposomal structures with smooth fracture faces above and below the transition temperature have been found (Fig. 2b). At higher ratios one finds lamellar

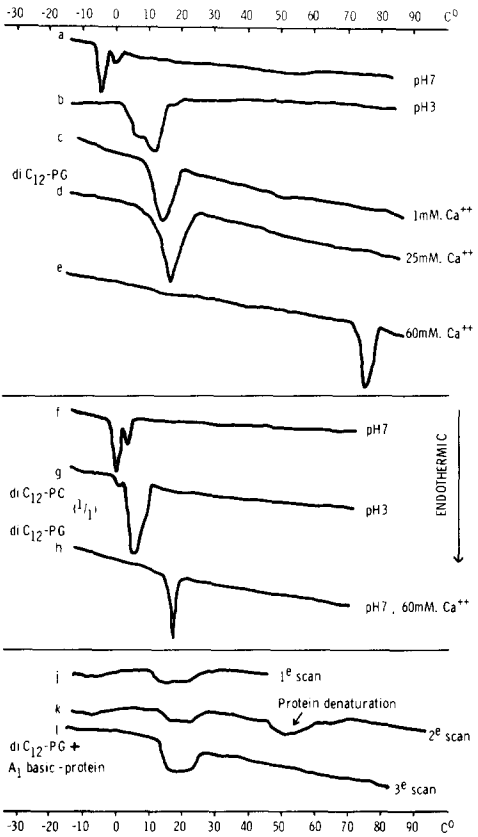

Fig. 1. Influence of pH, Ca^{2+} and A_1 basic protein on the thermotropic behaviour of didodecanoyl-phosphatidylglycerol (diC12-phosphatidylglycerol) and the influence of pH and Ca^{2+} on the thermotropic behaviour of the equimolar mixture of didodecanoyl-phosphatidylglycerol (diC12-phosphatidylglycerol) and didodecanoyl-phosphatidylcholine (diC12-phosphatidylcholine).
a, b: diC12-phosphatidylglycerol + 100 mM NaCl at pH 7 and 3.
c—e: diC12-phosphatidylglycerol with different concentrations of $CaCl_2$ at pH 7.
f, g: diC12-phosphatidylglycerol + diC12-phosphatidylcholine (1:1) + 100 mM NaCl at pH 7 and 3.
h: diC12-phosphatidylglycerol + diC12-phosphatidylcholine (1:1) + 60 mM $CaCl_2$ at pH 7.
j—l: diC12-phosphatidylglycerol + A_1 basic protein + 100 mM NaCl at pH 7.
The samples were buffered with 40 mM Tris acetate—ethyleneglycol (1:1, v/v).

structures which are neither spherical nor elliptical, but rather cylindrical (Fig. 2c). Monolayer data showed that the film is condensed from 62 $Å^2$/mole to 53 $Å^2$/mole by Ca^{2+} at a pressure of 30 dynes/cm at 20 °C [5].

The conclusion is that below the Ca^{2+}: phosphatidylglycerol ratio of 1:2, Ca^{2+} neutralizes the phospholipid charge, which results in an increase of the transition temperature as a consequence of a decrease in intramolecular repulsion (see Fig. 3). It is suggested that at ratios higher than 1:2, a phosphatidylglycerol—Ca—phosphatidylglycerol complex is formed by Ca^{2+} binding to phosphatidylglycerol. This strong interaction is expressed in the high transition temperature and the cylindrical structures.

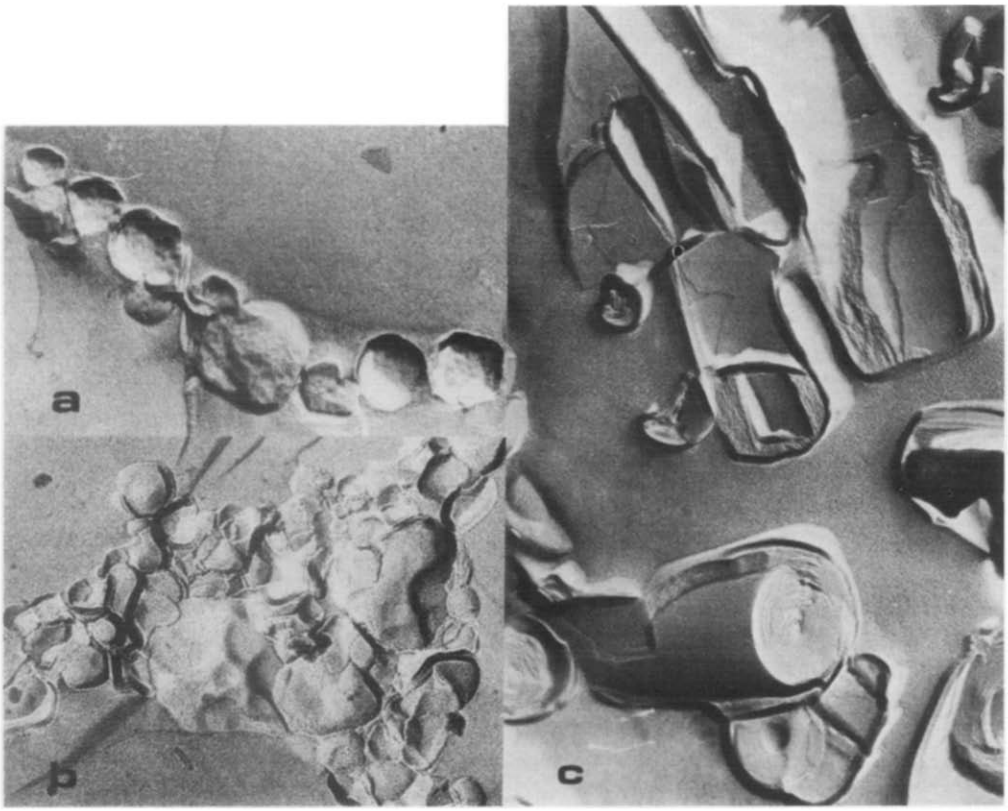

Fig. 2. Freeze fracture faces of:
a, diC12-phosphatidylglycerol + 100 mM NaCl at all pH values (Magnification × 90 000; reproduction factor 7/9).
b, diC12-phosphatidylglycerol at Ca^{2+}:phosphatidylglycerol ratios < 1/2 (pH 7).
c, diC12-phosphatidylglycerol at Ca^{2+}:phosphatidylglycerol > 1/2 (pH 7).
Buffer: 40 mM Tris acetate—ethyleneglycol (1:1, v/v).

Phosphatidylglycerol—phosphatidylcholine mixture

Equimolecular mixtures of diC12-phosphatidylglycerol and diC12-phosphatidylcholine were used to investigate how the negatively charged phospholipid phosphatidylglycerol behaves with changing pH and upon addition of Ca^{2+}, in the presence of a neutral phospholipid. The thermotropic behaviour of this mixture will not be influenced by the apolar moiety of the phosphatidylcholine.

From Fig. 1f, g and h, it is clear that both pH and Ca^{2+} increase the transition temperature of the mixture; pH by lowering the surface charge and Ca^{2+} by neutralizing the negative charge of phosphatidylglycerol in the mixture. With freeze-etching, only smooth liposomes have been found in all phosphatidylglycerol—phosphatidylcholine mixtures. No cylinders were

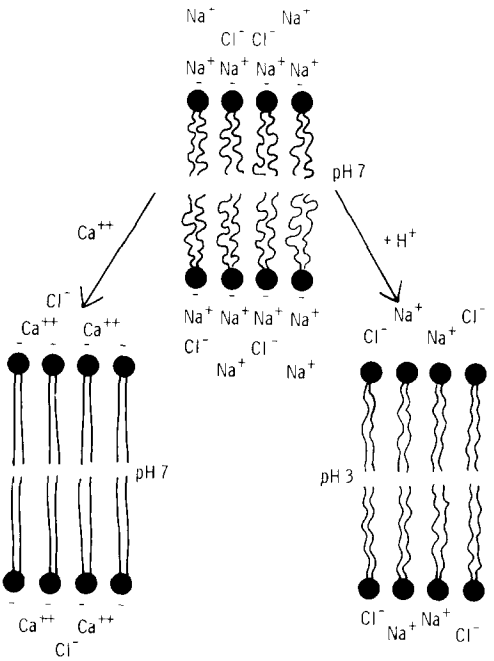

Fig. 3. A hypothetical model illustrating the effects of pH and Ca^{2+} on phosphatidylglycerol bilayers. The top bilayer represents diC12-phosphatidylglycerol in 100 mM NaCl at pH 7. At the bottom right the influence of lowering the pH is presented and the bottom left the influence of Ca^{2+} on phosphatidylglycerol is visualized.

formed in the case of high concentration of Ca^{2+}, indicating that spatial separation of charge by phosphatidylcholine inhibits the formation of a phosphatidylglycerol—Ca—phosphatidylglycerol complex.

The transition temperature of negatively charged phospholipids can also be changed by positively charged proteins. A_1 basic protein from myelin isolated and purified by London [9] was mixed with diC12-phosphatidylglycerol at a diC12-phosphatidylglycerol:A_1 basic protein ratio of 14:1. As can be seen from Fig. 1j, k and l, this protein induced an increase in the transition temperature when compared with that of diC12-phosphatidylglycerol in 100 mM NaCl. After denaturation of the protein during a second scan, the transition was even more apparent.

The most important conclusion of this work is that Ca^{2+} or a positively charged protein can condense and solidify a negatively charged phospholipid in a bilayer, when the phospholipid is in a pure state and in a mixture with a neutral lipid, at constant temperature. These phenomena may have far-reaching implications for the biological membrane.

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