

CALORIMETRIC AND FREEZE-ETCH STUDY OF THE INFLUENCE OF Mg^{2+} ON THE THERMOTROPIC BEHAVIOUR OF PHOSPHATIDYLGlycerol

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In the presence of Mg^{2+} ions phosphatidylglycerol shows supercooling which leads to the formation of a metastable gel phase. This contrasts with the behaviour of this negatively charged phospholipid in the presence of Ca^{2+} ions (Biochim. Biophys. Acta 339 (1974) 432). It is demonstrated that the heat content of this phospholipid is dependent on the ionic environment.

I. Introduction

Divalent cations play an important role in determining the temperature of the gel-liquid crystalline transitions of model membranes partly or completely consisting of acidic phospholipids [1–3]. In a similar way divalent cations may affect transitions in biological membranes. In previous studies [3] it was found that Ca^{2+} ions could condense and solidify a negatively charged phospholipid in a bilayer. When Ca^{2+} ions are present in a dispersion of 1,2 didodecanoyl phosphatidyl 1'-sn-glycerol (di-C12-phosphatidylglycerol) at a molar ratio lower than 1 : 2, the negative charge of the phospholipid is neutralized and the transition temperature thereby increased from about +3°C to a maximum of about +20°C. At a ratio > 1 : 2 a Ca-phosphatidylglycerol complex is formed probably by Ca^{2+} binding to phosphatidylglycerol. This complex has a transition at about +75°C and is organized in bilayers wrapped in cylinders.

In previous experiments a deviating thermotropic behaviour of a phosphatidylglycerol was noticed in the presence of Mg^{2+} in comparison to Ca^{2+} [4]. We therefore investigated systematically the thermotropic behaviour and the corresponding morphological changes of phosphatidylglycerol in the presence of Mg^{2+} . Such experiments could elucidate the difference in the Ca^{2+} and Mg^{2+} effects upon this negatively charged phospholipid.

II. Experimental part

Calorimetric measurements were performed on a Perkin Elmer D.S.C.-2B apparatus operating at a heating rate of $5^{\circ}\text{C}/\text{min}$. Di-C12-phosphatidylglycerol was synthesized as described by Tocanne et al., [4]. Samples for Differential Scanning Calorimetry were prepared by dispersing $7\ \mu\text{moles}$ phospholipid in $50\ \mu\text{l}$ of a solution of $40\ \text{mM}$ Tris acetate (pH 7)–ethylene glycol (1 : 1, v/v) as described in detail before [3]. Freeze etch specimens were fractured in a Denton machine at -196°C .

III. Transition temperature

Whereas the Ca-di-C12-phosphatidylglycerol complex exhibits only one transition at about $+75^{\circ}\text{C}$ we found that in the presence of Mg^{2+} (molar ratio $> \frac{1}{2}$) this phospholipid shows transitions at about $+20^{\circ}\text{C}$ and $+75^{\circ}\text{C}$ (fig. 1a). At $+20^{\circ}\text{C}$ an

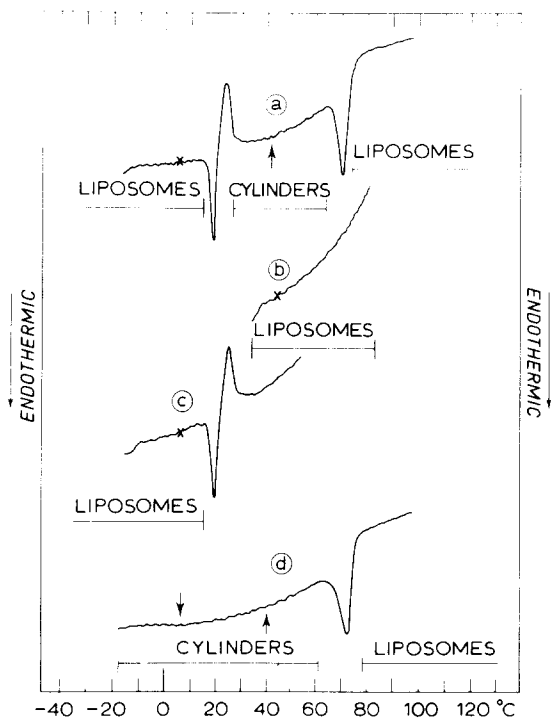


Fig. 1. Calorimetric experiments are recorded in heating operations. (a) Calorimetric scanning of 1,2 didodecanoyl phosphatidyl 1'-sn-glycerol in the presence of Mg^{++} (Mg^{++} : phosphatidyl glycerol $> \frac{1}{2}$). (b) Rescanning from $+30^{\circ}\text{C}$ to $+90^{\circ}\text{C}$ after cooling to $+30^{\circ}\text{C}$. (c) Heating curve of the sample recorded between -20°C and $+50^{\circ}\text{C}$. (d) Rescanning from -20°C to $+90^{\circ}\text{C}$ after cooling the sample from $+50^{\circ}\text{C}$ to -20°C .

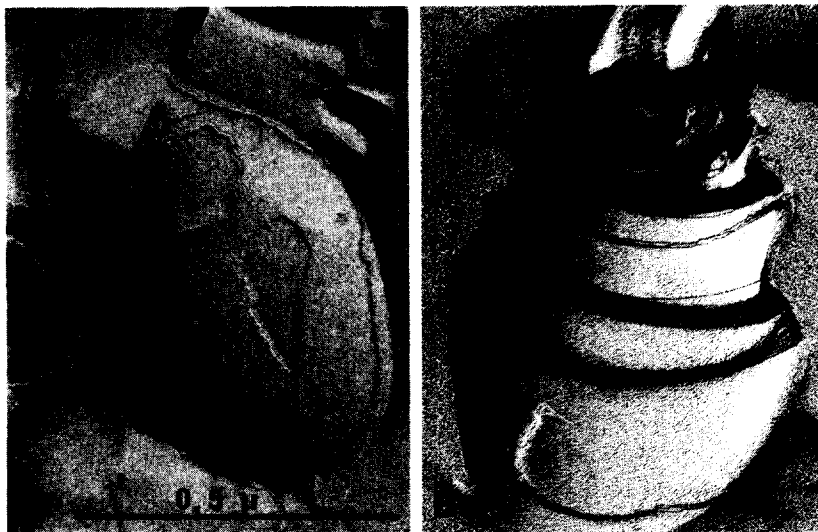


Fig. 2. (a) Electronmicrograph of freeze-etch replica of di-C12-phosphatidylglycerol in the presence of Mg^{2+} : phosphatidylglycerol $> \frac{1}{2}$) quenched from points in the temperature region indicated with X: liposomes. 80,000 \times . (b) Electronmicrograph of freeze-etch replica of di-C12-phosphatidylglycerol in the presence of Mg^{2+} (Mg^{2+} : phosphatidylglycerol $> \frac{1}{2}$) quenched from points in the temperature region indicated with +: cylinders. 80,000 \times .

endothermic heat change is followed immediately by an exothermic change. Because such behaviour may reflect a metastable transition we performed calorimetric experiments to prove the metastable character of this phase.

(1) Cooling of the lipid from $+80^{\circ}\text{C}$ to $+30^{\circ}\text{C}$ did not produce a gel phase, since no endothermic peak in the subsequent heating scan was observed at $+75^{\circ}\text{C}$ (fig. 1b). Thus the lipid remained in a liquid crystalline state exhibiting a marked supercooling.

(2) When the lipid was heated from 0°C to $+30^{\circ}\text{C}$, thus passing through the region of supposed metastability (fig. 1c), and thereafter cooled to below 0°C , rescanning to $+80^{\circ}\text{C}$ gave the following picture (fig. 1d). No transition could be detected at $+20^{\circ}\text{C}$, but there was a peak at $+75^{\circ}\text{C}$. This indicates that after cooling from $+30^{\circ}\text{C}$ the phospholipid exists in a stable gel state up to $+75^{\circ}\text{C}$. At that temperature the material passes into the liquid crystalline state.

Interpreting the calorimetric results we encountered three phases (1) a metastable gel phase below $+20^{\circ}\text{C}$; (2) a stable gel phase between $+20^{\circ}\text{C}$ and $+75^{\circ}\text{C}$; (3) a liquid crystalline phase which can exhibit substantial supercooling. We then investigated the structure of these phases by quenching the material rapidly from different points in the heating or cooling procedure (see fig. 1). It was found that the metastable gel phase and the liquid crystalline phase showed liposomal structures. On the other hand the stable gel phase was characterized by the presence of cylindrical structures (fig. 2).

IV. Heat content

Studies on the heat content of lipid mesophases can reveal in which conformation lipid molecules are packed in the bilayers. In fully hydrated 1,2 diacylphosphatidylcholines it was found that at the transition temperature there was an enthalpy change of 0.5 kcal/CH₂ group. This allows for a much greater freedom of chain motions than in anhydrous lipid systems [6, 7]. In a similar way we have tried to analyse the contribution of the polar headgroup to the mobility of the hydrocarbon chains. It has been demonstrated recently [1–3] that divalent cations considerably raise the transition temperature of negatively charged phospholipids. Upon measuring the differential scanning calorimetry peaks we found that the heat content was also affected by divalent ions. Didodecanoylphosphatidylglycerol in 100 mM NaCl has an enthalpy change of 4.5 kcal/mol which is similar to that of didodecanoyl phosphatidylcholine (4.3 kcal/mol). However, in the presence of Ca²⁺ or Mg²⁺ (Ca²⁺ or Mg²⁺ : phosphatidylglycerol < 1 : 2) values for the enthalpy change of the acidic phospholipid increased to 5.0–5.5 kcal/mol.

It might be concluded that in the liposomal system, which is present when di-C12-phosphatidylglycerol is dispersed in a low concentration of Ca²⁺ or Mg²⁺ (Ca²⁺ or Mg²⁺ : phosphatidylglycerol < 1 : 2) this heat value corresponds with a close packing in the apolar moiety because of a change in the polar part. Similar conclusions were reached from monolayer experiments, which showed that the lipid molecules became closely packed on a Ca²⁺ or Mg²⁺ containing subphase [4]. It is also of interest to see that a mixture like di-C12-lysylphosphatidylglycerol–di-C12-phosphatidylglycerol (1/1) which on monolayers appears to be packed very closely (58 Å²/mol) [5], has an increased heat content (5.0 kcal/mol).

When the phospholipid was precipitated at a high concentration (Ca²⁺ or Mg²⁺ : phosphatidylglycerol > 1 : 2) both the thermotropic behaviour and the ultrastructural morphology profoundly changed. The cylinders produced at this concentration and representing the stable gel form appeared to melt with an extraordinary high enthalpy change of about 10 kcal/mol. We suggest that in this system by the interaction with Ca²⁺ or Mg²⁺, phosphatidylglycerol molecules are organized in a lattice in which the mobility of the hydrocarbon chains is strongly limited.

The remarkably high amount of energy which is required for the transition of the cylindrical gel to the liquid crystalline liposomal system reflects the occurrence of two intimately connected processes, both the melting of the hydrocarbon chains and the disruption of the crystalline lattice (cylinders).

Our study indicates that the ionic environment strongly influences the molecular packing in phosphatidylglycerol bilayers. This is evident not only from the upward shift of the transition temperature and an increase of the heat content but also from a decrease of the area/molecule as measured by the monolayers technique and the generation of cylindrical structures visualised with freeze-etch electron microscopy.

Furthermore, we found that in the presence of divalent ions substantial supercooling was recorded, which in the case of Mg²⁺ ions, led to the formation of a

metastable gel phase.* In anhydrous phosphatidyl ethanolamines such phenomena of metastability and supercooling have been recognized by a variety of techniques including differential thermal analysis and X-ray diffraction [8].

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* Recently a similar calorimetric behaviour is found with di-C14 phosphatidylglycerol (Dr. P. van Dijk, personal communication).