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MICROCALORIMETRIC STUDIES OF Ca^{2+} BINDING TO TRIPHOSPHOINOSITIDE AND PHOSPHATIDYLSERINE *

TOSHIHARU TAKIZAWA *

Department of Physics, Faculty of General Studies, Gunma University, Maebashi (Japan)

KOHEI HAYASHI

Department of Physiology, Faculty of Education, Ibaraki University, Mito (Japan)

SADATO YABUKI

Department of Physics, Faculty of General Studies, Gunma University, Maebashi (Japan)

YOSHIRO NAKATA

Department of Chemistry, Faculty of General Studies, Gunma University, Maebashi (Japan)

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ABSTRACT

The effects of Ca^{2+} ions on the physicochemical properties of an aqueous dispersion of phosphatidylinositol-4,5-bis(phosphate) (TPI) were investigated by microcalorimetry in comparison with the effects of Ca^{2+} ions on the properties of glycerylphosphoryl-inositoldiphosphate (GPIDP) and phosphatidylserine (PS). The mixing enthalpy per mole of the aqueous dispersion of TPI with the $CaCl_2$ solution was always positive in the whole range of $CaCl_2$ concentration, while those of GPIDP and PS were negative. A model to explain this unusual result is presented. The hydrocarbon chains of TPI molecules contribute positively to the mixing enthalpy, while those of PS make a negative contribution. The positive contribution of the hydrocarbon chains of TPI to the mixing enthalpy is attributed to the dehydration enthalpy of the hydrophobic hydration induced by Ca^{2+} ions. In the absence of Ca^{2+} ions, some spaces are likely to be produced between their hydrocarbon chains because of strong electrostatic repulsion between the large negative charges of the head groups of TPI and also because of the loose packing due to the highly unsaturated chain of the arachidonic acid of TPI. Then, the hydrophobic hydration in the interior of the TPI membrane would be made possible. The influence of pH on the mixing enthalpy of the aqueous dispersion of TPI with the $CaCl_2$ solution also agrees perfectly with this model.

INTRODUCTION

Triphosphoinositide (TPI), 1-(3-sn-phosphatidyl)-L-myo-3,4-bis(phosphate), is a hydrophilic phospholipid rich in the cell membrane of brain and

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kidney and has been considered to be involved in the change of membrane permeability accompanying nerve excitation [1,2].

Nowadays, many investigators regard TPI as the reservoir of triphosphoinositol, the head group of TPI, which acts as one of the second messenger molecules in the cell [3,4]. In this study the binding enthalpy of Ca^{2+} to TPI was measured in order to investigate the role of TPI in biomembranes in comparison with that of phosphatidylserine (PS).

MATERIALS AND METHODS

Mixtures of triphosphoinositide, diphosphoinositide, monophoshoinositide and phosphatidylserine were extracted from bovine brain by the method of Folch [5] and fractionated by diethylaminoethyl cellulose chromatography [6]. The preparations of the ammonium salts of the phospholipids were free of divalent cations. All lipids were purified by high performance thin-layer chromatography on silica gel plates.

The head groups of the phospholipids, glycerylphosphoryl-inositoldiphosphate (GPIDP) and glycerol-serine (GS), were prepared from pure TPI and PS by the deacylation method of Hawthorne and Huscher [7].

The Oyodenki and Tokyo-Riko twin-type heat conduction microcalorimeters were used to obtain the heats of mixing of aqueous dispersions of the phospholipids and $CaCl_2$ solutions.

The two solutions were kept separately in the two compartments of the sample cell, while the same volumes of distilled water and the $CaCl_2$ solution were held in the two compartments of the reference cell in order to compensate the slight amount of heat of dilution of the $CaCl_2$ solution. The solutions in the sample cell and reference cell were mixed by turning the cells upside down. The sensitivity of the calorimeter was higher than 0.16 μV μW^{-1}, which enabled us to detect the mixing enthalpies arising from a sample less than 1 mg in dry weight. Measurements of the heat of mixing were usually carried out at 21.5 °C using 0.88–1.1 mM aqueous dispersions of TPI and PS. The dispersions of TPI and PS were adjusted to a final pH of 7 by the addition of a dilute solution of NH_4OH to a fully dialysed acidic solution of TPI. TPI dispersions with different pH values were prepared by the addition of various amounts of dilute NH_4OH solution to the acidic solution of TPI.

Optical density measurements were carried out using the Hitachi spectrophotometer type 101 at 450 nm.

RESULTS

The mixing enthalpies of the aqueous dispersions of the neutral ammonium salts of TPI and GPIDP with $CaCl_2$ solutions are shown as a function of $CaCl_2$ concentration in Fig. 1. The abscissa represents the final $CaCl_2$ concentration after mixing, and the ordinate represents the mixing enthalpies per mole of TPI and GPIDP. The mixing enthalpy of TPI is always positive. The initial large increase is followed by a dramatic decrease in the mixing enthalpy owing to an increase in the Ca^{2+} concentration. Consequently, a sharp maximum is observed in the mixing enthalpy curve. To clarify the origin of the abnormal mixing enthalpy curve of the aqueous dispersion of TPI with the $CaCl_2$ solution, the mixing enthalpies of the aqueous solutions of GPIDP-NH_4, the head group of TPI-NH_4, with the $CaCl_2$ solutions were also measured. In this case, the mixing enthalpy is negative in the low concentration range of $CaCl_2$, but becomes positive at a concentration higher than 5 mM. Subtracting the mixing enthalpies for GPIDP-NH_4 from those for TPI-NH_4, we obtain the positive enthalpies given by the broken curve, which may be interpreted as the contribution of the hydrocarbon chains to the mixing enthalpy of the aqueous dispersion of TPI with the $CaCl_2$ solution.

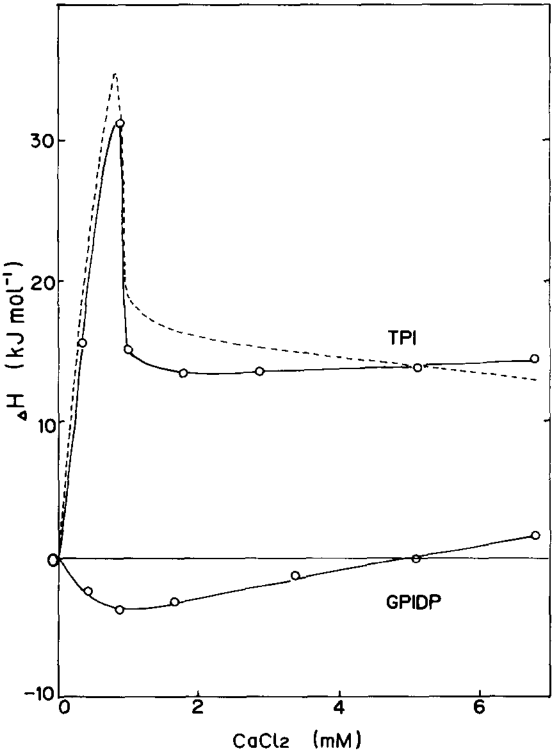

Fig. 1. Mixing enthalpies of aqueous dispersions of TPI and GPIDP solutions with $CaCl_2$ solutions. The broken curve represents the subtraction of the mixing enthalpy for GPIDP-NH_4 from that for TPI-NH_4.

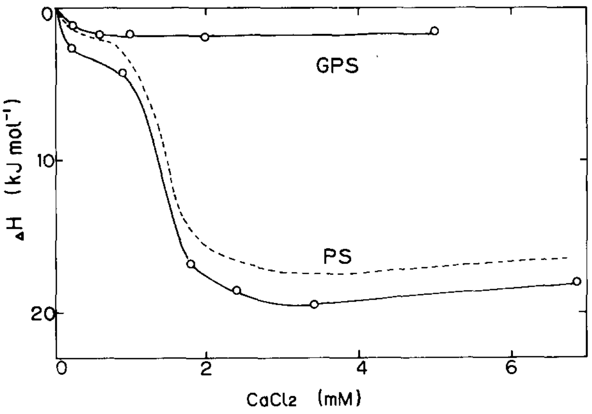

Fig. 2. Mixing enthalpies of the aqueous dispersions of PS and GS with $CaCl_2$ solutions. The broken curve represents the subtraction of the mixing enthalpy for GS from that for PS.

The mixing enthalpies of the aqueous dispersions of PS and GS with $CaCl_2$ solutions are shown in Fig. 2. Both mixing enthalpies are negative and the contribution of the hydrocarbon chains to the mixing enthalpy (the broken curve) is always negative.

The mixing enthalpies of the aqueous dispersions of TPI with $CaCl_2$ solutions depend strongly on the pH value. Figure 3 shows the mixing enthalpies as a function of pH at a $CaCl_2$ concentration of 1 mM. This concentration is a little higher than that which gives the maximum mixing enthalpy at neutral pH as shown in Fig. 1. The mixing enthalpies at a lower

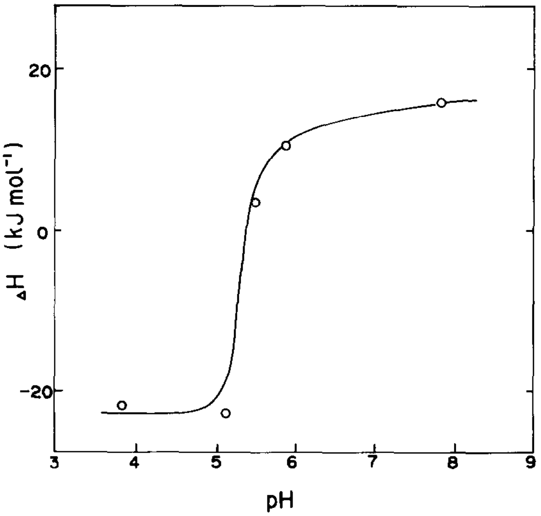

Fig. 3. Effect of pH on the mixing enthalpy of the aqueous dispersion of TPI with the $CaCl_2$ solution. The measurements were performed at 1 mM $CaCl_2$ concentration.

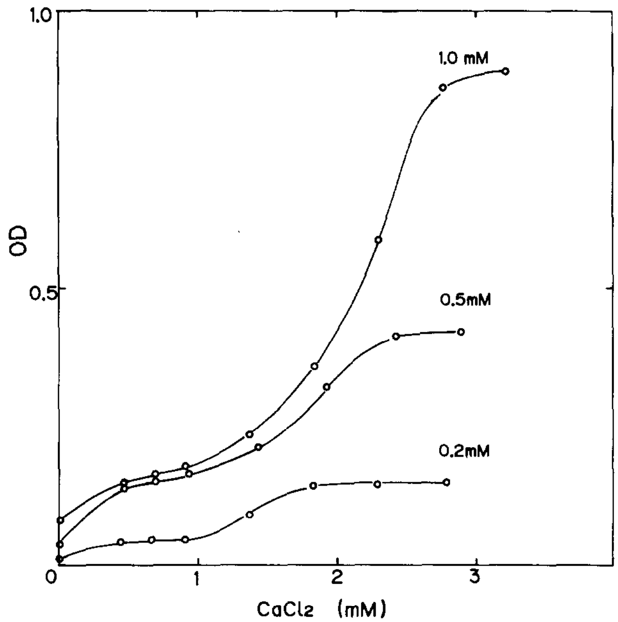

Fig. 4. Optical density change in the aqueous dispersion of TPI induced by the addition of a $CaCl_2$ solution.

pH are negative and an abrupt change in the sign is observed at a pH between 5 and 5.5.

Figure 4 shows the optical density change in an aqueous dispersion of TPI induced by the addition of a $CaCl_2$ solution. The concentrations of TPI used were 0.2, 0.5 and 1.0 mM. In each case, the optical density increases in two steps. After the initial rapid increase, the optical density remains in the plateau region with a Ca^{2+} concentration from 0.4 to about 1 mM, and this step is followed by an abrupt increase, indicating the coagulation of the dispersions of TPI.

DISCUSSION

The reason why the mixing enthalpies were not plotted vs. the molar ratio of $CaCl_2$ to TPI but vs. the $CaCl_2$ concentration is as follows. The concentration range of $CaCl_2$ corresponding to the first plateau region of the optical density change appears nearly constant, irrespective of the TPI concentration (Fig. 4). It is to be noted that the plateau of the optical density and the maximum mixing enthalpy are observed in nearly the same concentration range of $CaCl_2$. Our recent study by quasi-elastic light scattering spectrophotometry has made it clear that TPI is dispersed as particles greater than 20 nm in diameter, although the structure of the particle is as yet unknown [8,9]. These facts imply that it is not the molar ratio of $CaCl_2$ but the concentration of $CaCl_2$ itself which is the essential factor in the binding process of Ca^{2+} to a dispersed particle of TPI. The ionic equilibrium of Ca^{2+} ions between the free state and the non-specifically bound state to the surface of a TPI particle is considered to be determined mainly by the surface charges on the particles. This is the reason why the physical properties of the TPI dispersions changed sensitively depending on the ionic strength of the Ca^{2+} ions.

It is remarkable that positive mixing enthalpies of aqueous dispersions of TPI with $CaCl_2$ solutions and a maximum in the molar enthalpies are observed (Fig. 1). The hydrocarbon chains in TPI make a large positive contribution to the mixing enthalpy, while the hydrocarbon chains in PS make the opposite contribution to the mixing enthalpy (Fig. 2). A possible explanation of the positive enthalpy changes is as follows. A dispersed particle of TPI may contain a number of water molecules even in the internal hydrophobic regions because of intermolecular repulsion due to the highly negative charges of the head group (inositol-triphosphate) exposed on the surface of the particle and the loose packing due to the steric hindrance of the highly unsaturated chain of the arachidonic acid [10]. When the Ca^{2+} concentration is low, Ca^{2+} ions may non-specifically neutralize the negative charges on the surface of the particle, which may enable the hydrocarbon chains to approach more closely, and penetrating water molecules may be excluded from the internal hydrophobic regions, resulting in a positive enthalpy. After dehydration of the interior of the membrane structure in the particle is achieved, Ca^{2+} ions promote coagulation of the dispersed particles, resulting in a decrease in enthalpy, as in the case of PS. These two kinds of reaction, with opposite contributions to the mixing enthalpy, produce the maxima in the curves (Fig. 1).

The surface charges of the dispersed particles of TPI could be diminished by binding not only Ca^{2+} ions but also hydrogen ions. A negative mixing enthalpy should be observed also at a low pH and was in fact observed as shown in Fig. 3, because the dehydration process has already been achieved by the hydrogen ions. This is positive evidence for the possible specific action of Ca^{2+} ions on the dispersed particles of TPI. At a low pH, Ca^{2+} ions are inclined to be bound to the dispersed particles of TPI to make larger aggregates. On the other hand, at a high pH, the Ca^{2+} ions adsorbed onto the surface of the dispersed particles sweep the water molecules out into the bulk water phase. This process is endothermic and hence a positive mixing enthalpy should be observed. The existence of water molecules which have penetrated into the hydrophobic region in the lipid bilayer was also suggested by Blume in a study of the partial specific heat capacity of various kinds of phospholipids [11].

Kai and Hawthorne proposed the possible control of membrane permeability of nerve cells by TPI [1]. They supposed that TPI membranes are permeable to water and monovalent cations when the ionic part of TPI is free from Ca^{2+} ions but that they become impermeable when Ca^{2+} ions bind to the surface layer of TPI membranes. Our experimental results seem to support their model.

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