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Counterion-mediated cluster formation by polyphosphoinositides

Yu-Hsiu Wang[a,d], David R. Slochower[c,d], Paul A. Janmey[b,d,e,*]

[a] Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, USA
[b] Department of Physics and Astronomy, University of Pennsylvania, Philadelphia, PA 19104, USA
[c] Graduate Group in Biochemistry and Molecular Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
[d] Institute for Medicine and Engineering, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
[e] Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

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ABSTRACT

Polyphosphoinositides (PPI) and in particular $PI(4,5)P_2$, are among the most highly charged molecules in cell membranes, are important in many cellular signaling pathways, and are frequently targeted by peripheral polybasic proteins for anchoring through electrostatic interactions. Such interactions between PIP_2 and proteins containing polybasic stretches depend on the physical state and the lateral distribution of PIP_2 within the inner leaflet of the cell's lipid bilayer. The physical and chemical properties of PIP_2 such as pH-dependent changes in headgroup ionization and area per molecule as determined by experiments together with molecular simulations that predict headgroup conformations at various ionization states have revealed the electrostatic properties and phase behavior of PIP_2-containing membranes. This review focuses on recent experimental and computational developments in defining the physical chemistry of PIP_2 and its interactions with counterions.

Ca^{2+}-induced changes in PIP_2 charge, conformation, and lateral structure within the membrane are documented by numerous experimental and computational studies. A simplified electrostatic model successfully predicts the Ca^{2+}-driven formation of PIP_2 clusters but cannot account for the different effects of Ca^{2+} and Mg^{2+} on PIP_2-containing membranes. A more recent computational study is able to see the difference between Ca^{2+} and Mg^{2+} binding to PIP_2 in the absence of a membrane and without cluster formation. Spectroscopic studies suggest that divalent cation- and multivalent polyamine-induced changes in the PIP_2 lateral distribution in model membrane are also different, and not simply related to the net charge of the counterion. Among these differences is the capacity of Ca^{2+} but not other polycations to induce nm scale clusters of PIP_2 in fluid membranes. Recent super resolution optical studies show that PIP_2 forms nanoclusters in the inner leaflet of a plasma membrane with a similar size distribution as those induced by Ca^{2+} in model membranes. The mechanisms by which PIP_2 forms nanoclusters and other structures inside a cell remain to be determined, but the unique electrostatic properties of PIP_2 and its interactions with multivalent counterions might have particular physiological relevance.

1. Introduction

The interface between the intracellular and extracellular environment, mediated by the cell's plasma membrane is a crucial site at which signals are generated by chemical stimuli, application of force, or formation of cell–cell and cell–matrix contacts. The signaling pathways often involve a class of multi-anionic phospholipids, polyphosphoinositides (PPIs), in the membrane lipid bilayers (Downes et al., 2005; Martin, 1998; Zhang et al., 2012) (Fig. 1).

A major challenge for understanding how PPIs function *in vivo* is the sheer number of PPI (usually $PI(4,5)P_2$) binding proteins that have been well characterized biochemically as specific and high affinity ligands for these lipids. (Catimel et al., 2008). The variety of PPI binding proteins and the different structures that bind these lipids suggest that the specificity and control within the cell might be attained by changing the physical state of the lipid within the membrane and not only its local or global concentration. An unresolved question is how PIP_2 distributes laterally within the plasma membrane and whether all PIP_2 molecules within a membrane are equally effective at binding their targets. The remaining critical issues include the relation of PIP_2 to the formation of cholesterol-dependent lipids rafts, and whether PIP_2 can self-associate to form clusters independent of or at least not requiring cholesterol. The first issue of whether PIP_2 is associated with cholesterol-dependent lipid rafts remains under debate. The primary evidence of PIP_2

* Corresponding author at: University of Pennsylvania, Department of Physiology, 1010 Vagelos Laboratories, 3340 Smith Walk, Philadelphia, PA 19104, USA.
Tel.: +1 215 573 7380; fax: +1 215 573 6815.
E-mail address: janmey@mail.med.upenn.edu (P.A. Janmey).

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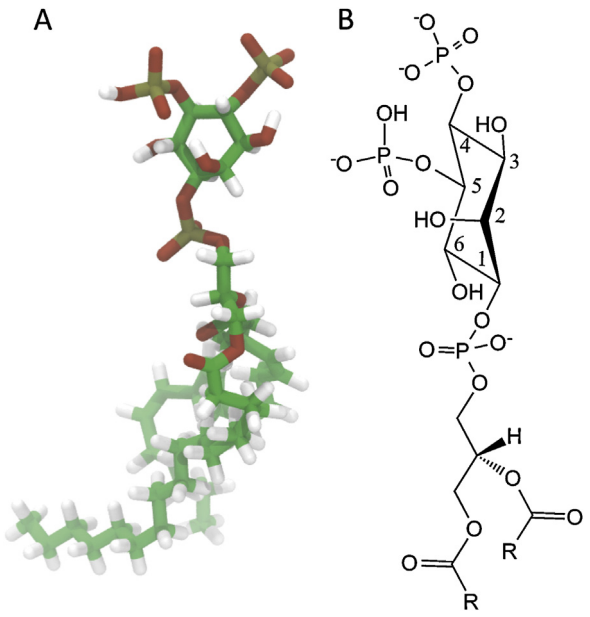

Fig. 1. (A) MD simulated structure and the corresponding (B) chemical structure of PI(4,5)P2.

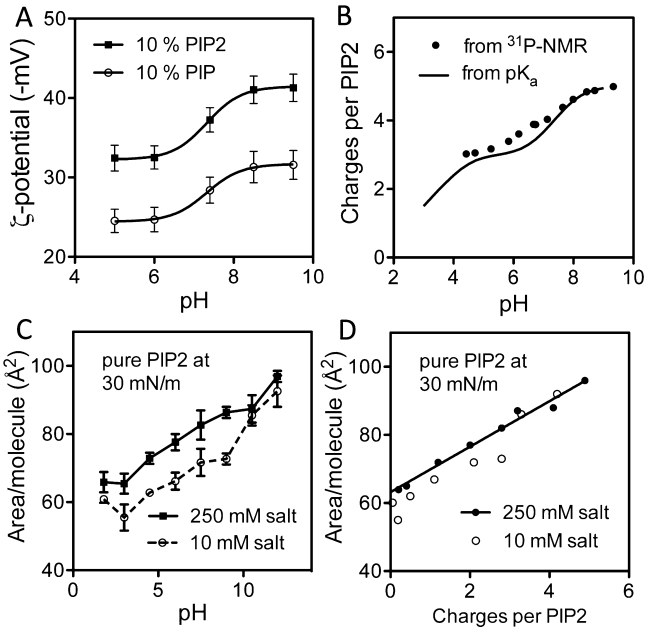

Fig. 2. pH-dependent change in PIP_2 ionization and area per molecule. (A) Zeta potential of MLVs containing 10 mol% PIP_2 or PIP measured at different pH values. (B) PIP_2 charges are calculated base on ^{31}P NMR measurements (Kooijman et al., 2009) of five pK_as at varying pH values (Levental et al., 2008b). (C) pH-dependent change and (D) Charges-dependent change of area per molecule at 30 mN/m using pure PIP_2 monolayer at different salinities. Image (A) is adapted from Colloids and Surfaces B: Biointerfaces, 2010, 79, 210–218, copyright 2010 and images (C) and (D) are adapted from Biophysical Journal 2008, 95, 1199–1205, copyright 2008, with permission from Elsevier.

enrichment in lipid microdomains is based on the observation that PIP_2 is enriched in detergent insoluble fractions of the plasma membrane (Hope and Pike, 1996; Klopfenstein et al., 2002; Pike and Casey, 1996) and is delocalized when cholesterol is depleted from the cell membrane (Liu et al., 1998; Pike and Miller, 1998). This hypothesis is supported by a later observation that PIP_2 forms clusters in the presence of cholesterol alone (Dasgupta et al., 2009). Paradoxically, fluorescent PIP_2 is found to be excluded from the liquid-ordered phase of a model membrane in the presence of cholesterol (Levental et al., 2009). Several other reports have cast doubt on the results of cyclodextrin-mediated cholesterol depletion experiments, as the cholesterol depletion by itself is found to change the structural and physical properties of the membrane (Kwik et al., 2003; van Rheenen et al., 2005). As the first issue is beyond the scope of this review and has been widely addressed in the literature, this review focuses on the latter mechanism in which PIP_2 or other PPI's form nanoscale, dynamic clusters as they interact with divalent and multivalent counterions. Both experimental and computational studies are beginning to reveal how nanodomains enriched in PPIs might form in mixed lipid membranes.

2. Physical chemical characterization of PIP_2 and other polyphosphoinositides

A lipid fraction isolated from brain and enriched in phospholipids containing inositol was isolated at least as far back as 1946 and found to be composed of a large amount of diphosphoinositide, the phospholipid now called phosphatidylinositol phosphate (Folch, 1946, 1949). This fraction was later found to contain three inositol lipid species that differed in phosphate content and from which triphosphoinositide (now called phosphatidylinositol bisphosphate) could be isolated (Dawson and Dittmer, 1961; Dittmer and Dawson, 1961; Grado and Ballou, 1961). The possible isomers of triphosphoinositides were an early subject of interest, even before the three different species produced in mammalian cells, $PI(4,5)P_2$, $PI(3,4)P_2$ and $PI(3,5)P_2$, were identified, and in most early studies, these lipids are referred to generically as TPI or PIP_2. The potential importance of phosphoinositides was suggested by the finding that unlike other phospholipids that were thought to be mainly structural and that were relatively stable after isolation from the cell, the amount of PIP_2 that was isolated from cells and tissues depended very strongly on preparation details, and the isolated lipids were rapidly degraded or modified by enzymes, often in manner that depended on divalent cations (Akhtar and Abdel-Latif, 1978; Best et al., 1982; Grove et al., 1981).

2.1. Anionic charge of PIP_2

Polyphosphoinositides are among the most highly charged molecules in the cell membrane and have often been assumed to be uniformly distributed in the plasma membrane due to the electrostatic repulsion between their highly negatively charged head groups. These negative charges arise from deprotonated phosphomonoester and phosphodiester groups, some of which have pKa's within a biologically relevant range. The ionization state of PIP_2 affects its area within the membrane and its interaction with proteins and other ligands, and has therefore been the subject of many studies. The pH-dependent change in the net charge of PPIs has been estimated from the zeta potential of PPI-containing multilamellar vesicles as shown in Fig. 2A (Ohki et al., 2010). Similar electrophoretic mobility measurements of PIP_2 vesicles in the presence of 100 mM KCl suggest that the charge of PIP_2 is approximately −3 at pH 7.0 (McLaughlin et al., 2002; Toner et al., 1988; Wang et al., 2002), and that both a proton and a potassium ion are likely bound to PIP_2 under physiologically realistic conditions. The pH-dependent change in PIP_2 ionization has also been investigated by ^{31}P NMR using 5 mol% PIP_2 in phosphatidylcholine lipid multilamellar suspensions (Kooijman et al., 2009). The overall charge of $PI(4,5)P_2$, calculated from the degree of protonation on the 4- and 5-phosphate as detected from the chemical shifts in ^{31}P NMR spectra, is approximately −4.0 in a buffer containing 100 mM NaCl, 2 mM EDTA and 50 mM Tris at pH7.0 (Fig. 2B). Alternatively, the charge per PIP_2 can be calculated from the five pKa values as summarized