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Phosphatidylinositol-4,5-bisphosphate ionization and domain formation in the presence of lipids with hydrogen bond donor capabilities

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ABSTRACT

Phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) is an important lipidic signaling molecule that is involved in a broad range of cellular processes. Its interaction with proteins and its lateral distribution are governed by the ionization state of the phosphomonoester groups and its ability to form intra- and intermolecular hydrogen bonds. In this study we have investigated the ionization state of $PI(4,5)P_2$ in ternary lipid vesicle systems that contain in addition to $PI(4,5)P_2$ and phosphatidylcholine (PC) either phosphatidylethanolamine (PE), phosphatidylserine (PS) or phosphatidylinositol (PI). In the presence of PE we find an increased ionization of $PI(4,5)P_2$, which can be attributed to increased deprotonation due to hydrogen bond formation between PE and the $PI(4,5)P_2$ phosphomonoester groups. However, the effect of PE on $PI(4,5)P_2$ ionization is significantly smaller than it had been found previously for phosphatidic acid in the presence of PE (Kooijman et al., 2005). The reduced impact of PE on $PI(4,5)P_2$ ionization can be attributed to competing intramolecular hydrogen bond formation between the phosphomonoester groups and neighboring hydroxyl groups. It is noteworthy that the presence of PE affects more strongly the ionization of the 5-phosphate group than that of the 4-phosphate, suggesting that the interaction of PE with the 5-phosphate is stronger. In $PI(4,5)P_2$/PS/PC lipid vesicles, the presence of PS was expected to yield an increased protonation of the $PI(4,5)P_2$ phosphomonoester groups due to a decreased interfacial pH as a result of the increased negative interfacial charge. However, the effect of PS on $PI(4,5)P_2$ ionization is only minor, potentially suggesting that PS and $PI(4,5)P_2$ are demixed. The $PI(4,5)P_2$/PI/PC vesicle system was characterized by a surprising mixing behavior that has potentially far reaching consequences: fluorescence microscopy measurements of giant unilammellar vesicles composed of $PI(4,5)P_2$/PI/PC at physiological concentrations show that PI and $PI(4,5)P_2$ form macroscopic, fluid phase domains in contact with a fluid PC rich phase (fluid/fluid demixing). Despite the fact that PI and $PI(4,5)P_2$ co-localize, the effect of PI on $PI(4,5)P_2$ ionization behavior is only noticeable above pH 7. Apparently two opposing effects lead to the observed behavior: Due to the presence of the anionic PI, the interfacial pH drops, which is expected to lead to an enhanced protonation of the $PI(4,5)P_2$ phosphomonoester groups. In turn, hydrogen bond formation between PI and $PI(4,5)P_2$ would lead to the opposite, i.e. increased deprotonation of the phosphomonoester group. Apparently these two effects compensate each other for pH values smaller than about 7, while for higher pH values the increased interfacial pH in the presence of PI has a stronger impact than PI/$PI(4,5)P_2$ hydrogen bond formation. The cooperative formation of PI/$PI(4,5)P_2$ mixed domains has potentially important ramifications for the spatial organization of phosphoinositide mediated signaling events.

1. Introduction

Phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) is an important signaling molecule that mediates a broad range of physiological processes through interactions with distinct protein targets (Gamper and Shapiro, 2007). The diverse physiological behavior of $PI(4,5)P_2$ is rooted in the rich functionality of its headgroup, which is negatively charged and at the same time can engage in hydrogen bond formation through its phosphate and hydroxyl

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groups. PI(4,5)P_2 is a minority component in the inner leaflet of the plasma membrane with a global concentration of about 1% (McLaughlin and Murray, 2005). However, the cumulative concentration of PI(4,5)P_2 protein ligands exceeds the cellular PI(4,5)P_2 concentration (Catimel et al., 2008) and during activation of cells, PI(4,5)P_2 distribution and turnover changes much more than total PI(4,5)P_2 levels (Varnai and Balla, 2006; Varnai et al., 2006). Local accumulation of PI(4,5)P_2 has been shown in vivo (Franca-Koh et al., 2007; James et al., 2008) and factors like the interaction with cationic peptides like MARCKS (Wang et al., 2003) or bivalent cations (Wang et al., 2012) are likely contributors to such accumulation. However, the exact mechanisms that lead to the local accumulation of PI(4,5)P_2 remains elusive. Electrostatic interactions with proteins are strongly affected by the overall charge of the PI(4,5)P_2 headgroup, which depends on the ionization states of the two phosphomonoester groups. Furthermore, the mutual PI(4,5)P_2 interaction as well as the interaction with other anionic lipids is equally affected by the overall charge of the lipid.

In a previous study (Kooijman et al., 2009), we have found by using solid state magic angle spinning (MAS) ^{31}P-NMR that the ionization states of phosphatidylinositol bisphosphates and phosphatidylinositol(3,4,5)trisphosphate are strongly affected by intra- and intermolecular hydrogen bond formation. We have found that hydroxyl groups vicinal to the respective phosphomonoester group engage in hydrogen bond formation that leads to an increased deprotonation (higher charge) of the respective group. For PI(4,5)P_2 and phosphatidylinositol-3,4-bisphosphate (PI(3,4)P_2) we found that for pH > 7 the last remaining proton is shared between the two phosphate groups. In contrast, such a sharing of the last proton is not possible for isolated phosphate groups as they are found in phosphatidylinositol-3,5-bisphosphate (PI(3,5)P_2). As a result, the charge for PI(3,5)P_2 is higher than for PI(3,4)P_2 and PI(4,5)P_2. For PI(3,4,5)P_3 we observed that the ionization of the three phosphmonoester groups increased monotonically for pH values < 7, while around pH 7 the charge of the 4-phosphate drops. This can be attributed to the fact that the 4-phosphate binds more strongly the last remaining proton, while the 3- and 5-phosphates are stabilized in a more deprotonated state via hydrogen bond formation with neighboring hydroxyl groups. This is an ionization behavior that had been observed previously for inositol trisphosphates with neighboring phosphate groups like inositol-1,4,5,6-tetrakisphosphate (Guedat et al., 1997; Schlewer et al., 1998). In addition to intramolecular hydrogen bond formation between the hydroxyl and phosphomonoester groups as well as between vicinal phosphomonoester groups, we found strong evidence for intermolecular hydrogen bond formation between neighboring phosphoinositide molecules. We rationalized this surprising behavior with the formation of an intricate inter- and intramolecular hydrogen bond network that leads to a dissipation of the negative charge of the phosphoinositide headgroup and therefore, reduced repulsive forces

In the previous work described above, we investigated the ionization behavior of PI(4,5)P_2 in mixed vesicles with phosphatidylcholine (PC). PC functions in this case as a "matrix lipid" that allows for the fabrication of vesicles (PI(4,5)P_2 by itself does not form stable vesicles). It is assumed that the zwitterionic PC lipid does not affect the ionization properties of PI(4,5)P_2.

This study expands on our earlier work by examining the effect of inner leaflet membrane lipids on the ionization behavior of PI(4,5)P_2. Phosphatidylethanolamine (PE) is a zwitterionic phospholipid that is expected to engage in hydrogen bond formation with PI(4,5)P_2, leading to enhanced deprotonation of the phosphomonoester groups as it was observed for phosphatidic acid (PA) (Kooijman et al., 2005). Phosphatidylserine (PS) is, at pH 7, an anionic lipid that can also engage to some extent in hydrogen bond formation. As a result, two opposing effects might act on the PI(4,5)P_2 headgroup: The negative charge of the PS headgroup will lead to a reduction of the interfacial pH and hence an increased protonation of the PI(4,5)P_2 headgroup. If hydrogen bond formation occurs between PS and PI(4,5)P_2, this is expected to lead to an increased deprotonation of the PI(4,5)P_2 phosphomonoester groups. The overall PI(4,5)P_2 charge will be determined by the balance between these two opposing effects. This holds even more for phosphatidylinositol (PI), which is also an anionic lipid that is capable of engaging in hydrogen bond formation with PI(4,5)P_2. What sets PI apart from PS is the richer hydrogen bond capability of the PI headgroup in comparison to PS. For either of these anionic lipids, hydrogen bond formation will only occur if the attractive PS/PI(4,5)P_2 or PI/PI(4,5)P_2 force due to hydrogen bond formation is stronger than the repulsive force due to negative charges on the headgroups.

2. Materials and methods

2.1. Materials

1,2-Dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), 1-palmitoyl, 2-oleoyl-sn-glycerophosphatidylcholine (POPC), 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE), 1,2-dioleoyl-sn-glycero-3-phosphatidylserine (DOPS), L-α-phosphatidylinositol 4,5-bisphosphate (brain, porcine-triammonium salt) [brain PI(4,5)P2], L-α-phosphatidylinositol (liver, bovine), and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (ammonium salt) (RhB DOPE) were purchased from Avanti Polar Lipids (Birmingham, AL). The acyl chain composition of liver PI and brain PI(4,5)P_2 is primarily stearoyl/arachidonoyl, the acyl chain combination predominantly found in nature. The phosphatidylinositol lipids were dissolved in a 20:9:1 by volume mixture of chloroform, methanol and water. Other lipids were dissolved in a 2:1 mixture of chloroform and methanol. The resulting, gravimetrically prepared, lipid stock solutions were checked by phosphate assay (according to Rouser et al., 1970) to verify or correct the concentration. HPLC grade water was purchased from Fisher Scientific.

2.2. Sample preparation

NMR samples were prepared from dry lipid films. Lipid films were prepared by mixing appropriate volumes of lipid stock solution, along with 400–600 μl chloroform to increase the total volume according to Kooijman et al. (2009). Briefly; 0.2 μmol of brain PI(4,5)P_2 was used in each sample and appropriate amounts of additional lipids were added to prepare films containing either 5 or 2 mol% brain PI(4,5)P_2 as indicated in the results. The lipid solutions were placed in specially made borosilicate glass tubes (15 mm test tube size) and dried using a rotary evaporator. The resulting lipid films were placed in an inert N_2 atmosphere and dried overnight at 40–50 °C in a high vacuum. Generally 15–18 lipid films were prepared for each lipid composition to prepare a single titration curve.

Lipid films were then hydrated with 2 mL of buffer. The following buffers were used for the indicated pH ranges; 20 mM citric acid, 30 mM MES for pH 4–6.5, 50 mM HEPES for pH 6.5–8.5, and 50 mM glycine for pH 8.5–10. Buffers also contained 100 mM NaCl and 2 mM EDTA to complex divalent cations. Samples were vortexed thoroughly to disperse the lipid film in the solution. The lipid dispersions were freeze–thawed twice to remove metastable states (Kooijman et al., 2009). The freeze–thaw procedure consisted of quickly freezing the lipid suspension in a mixture of ethanol and dry ice, and then thawing in warm water, occasionally vortexing the