Chemistry and Physics of Lipids 182 (2014) 62–72

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Phosphatidylinositol-4,5-bisphosphate ionization in the presence of cholesterol, calcium or magnesium ions

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ARTICLE INFO

Article history:
Received 16 October 2013
Accepted 13 November 2013
Available online 2 December 2013

Keywords:
$PI(4,5)P_2$
Lipid electrostatics
Lipid domain formation
Lipid signaling
Model membranes
Solid-state ^{31}P NMR

ABSTRACT

Phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) is an important signaling lipid and plays a crucial role in a wide variety of cellular processes by interacting with protein targets and localizing proteins at the plasma membrane. These interactions are strongly influenced by the lateral distribution of $PI(4,5)P_2$ as well as its ionization state. The characterization of the $PI(4,5)P_2$ ionization state provides important information about how $PI(4,5)P_2$ interacts with other membrane resident or associated chemical species. In this study we have used solid-state MAS ^{31}P NMR to investigate the interactions of $PI(4,5)P_2$ with potential cluster promoting agents, divalent cations and cholesterol. Both Ca^{2+} and cholesterol were found previously to promote formation of local $PI(4,5)P_2$ clusters in vitro. The NMR approach allows us to probe independently the ionization state of $PI(4,5)P_2$ two phosphomonoester groups. We investigated mixed phosphatidylcholine (PC)/$PI(4,5)P_2$ multilamellar vesicles in the presence of micro and millimolar concentrations of Ca^{2+} and Mg^{2+}. We found that both cations lead to an increased downfield chemical shift of the $PI(4,5)P_2$ phosphomonoester peaks, indicating an increased ionization in the presence of the divalent cations. Ca^{2+} has a much larger effect on $PI(4,5)P_2$ as compared to Mg^{2+} at similar concentrations. Physiological concentrations of Ca^{2+} are significantly lower than those found for Mg^{2+} and the comparison of the $PI(4,5)P_2$ ionization in the presence of Ca^{2+} and Mg^{2+} at physiological concentrations resulted in similar charges of the phosphomonoester groups for both cations. $PI(4,5)P_2$ was also examined with vesicles containing cholesterol since cholesterol has been shown to promote $PI(4,5)P_2$ clustering. In the presence of 40 mol% cholesterol, the $PI(4,5)P_2$ phosphomonoester ^{31}P NMR peaks shifted slightly downfield, indicating a small increase in charge. Previously published data suggest that $PI(4,5)P_2$ is capable of forming an intra- and intermolecular hydrogen bond network, which leads to a reduction of the charge at the phosphomonoester groups through dissipation of the charge across the bilayer/water interface. We hypothesize that cholesterol participates in this intermolecular hydrogen bond network, resulting in a stabilization of $PI(4,5)P_2$ enriched domains due an increased spacing between the $PI(4,5)P_2$ headgroup. We also examined the cumulative effects of cholesterol combined with the divalent cations, phosphatidylethanolamine (PE), and phosphatidylinositol (PI), separately. The combination of cholesterol and divalent cations results in an additive effect on $PI(4,5)P_2$ ionization, while the effect of cholesterol on $PI(4,5)P_2$ ionization is reduced in the presence of PE or PI.

1. Introduction

Phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$] is an important signaling lipid in the cell plasma membrane. It is involved in diverse signaling processes including, cell growth, cytoskeletal remodeling, and transporter activity (Carney et al., 1985; Catimel et al., 2008; Suh and Hille, 2008). The many signaling roles of $PI(4,5)P_2$ are made possible by the rich functionality of its phosphorylated inositol headgroup that enables highly specific interactions with a broad range of proteins. This headgroup is highly negatively charged, but at the same time, has a high capacity for hydrogen-bond formation through the inositol hydroxyl groups and the two phosphomonoesters at the 4- and 5-positions of the inositol ring (Kooijman et al., 2009; Graber et al., 2012; Janmey, in this issue). $PI(4,5)P_2$ is a relatively minor component of the cell plasma membrane, comprising only 1% of the total lipids, while the total cumulative concentration of its protein ligands exceeds its concentration in the cell (Catimel et al., 2008). In addition the distribution and turnover of $PI(4,5)P_2$ in activated cells changes more than the total $PI(4,5)P_2$ levels (Varnai and Balla, 2006;

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0009-3084/$ – see front matter © 2014 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.chemphyslip.2013.11.004

Varnai et al., 2006). In this context the question arises how PI(4,5)P_2 mediated signaling events are fine tuned in space and time. Local PI(4,5)P_2 accumulation has been demonstrated to occur in vivo (Franca-Koh et al., 2007; James et al., 2008; Johnson et al., 2008; Gao et al., 2011). This accumulation may play a large role in the control of PI(4,5)P_2 signaling, and there are many cellular components that have been suggested to promote this clustering of PI(4,5)P_2, including cationic proteins such as MARCKS (Wang et al., 2003; Kwiatkowska, 2010), divalent cations (Wang et al., 2012), cholesterol (Dasgupta et al., 2009; Gericke, in this issue), and phosphatidylinositol (PI) (Graber et al., 2012).

Calcium (Ca^{2+}) has been identified as an important factor in many PI(4,5)P_2 signaling events. Ca^{2+} is thought to promote local accumulation, or clustering, of PI(4,5)P_2 by shielding its negative charge which shifts the balance between repulsive charge interactions (the PI(4,5)P_2 headgroup charge is about −4 at physiological pH) and attractive forces like intermolecular hydrogen bond formation toward a net force that is attractive. Ca^{2+} is also important for mediating C2 domain binding to PI(4,5)P_2 (Evans et al., 2004; Knight and Stahelin, in this issue). Ca^{2+} has been suggested to alleviate MARCKS based sequestering of PI(4,5)P_2 by forming a complex with Calmodulin and binding to the MARCKS protein (McLaughlin and Murray, 2005). PI(4,5)P_2 and Ca^{2+} are also linked through the classical PLC signaling pathway, whereby PLC cleaves the headgroup of PI(4,5)P_2 to form Ins(1,4,5)P_3, which causes the release of Ca^{2+} from the endoplasmic reticulum. This Ca^{2+} release may have a strong impact on PI(4,5)P_2 signaling, raising the possibility that it acts as a feedback loop. While Ca^{2+} has been shown to have an important role in PI(4,5)P_2 signaling, magnesium (Mg^{2+}) shares calcium's divalent charge and is found in much higher concentrations in the cytosol. It is therefore important to consider the effect of Mg^{2+} on PI(4,5)P_2 as well. Previous work has shown that the binding constant of both Ca^{2+} and Mg^{2+} for PI(4,5)P_2 are similar but that only Ca^{2+} is able to induce local PI(4,5)P_2 clustering (Wang et al., 2012).

Cholesterol is a major component of the cell membrane, playing an important role in the formation of so-called 'raft' domains. Cholesterol is also of relevance in PI(4,5)P_2 signaling since several PI(4,5)P_2 signaling events were found to be affected by cholesterol levels (Elhyany et al., 2004; Cinar et al., 2007; Lasserre et al., 2008; Chun et al., 2010; Murray and Tamm, 2011; Koushik et al., 2013). Based upon the cholesterol dependence of several PI(4,5)P_2 mediated signaling events, some have suggested that PI(4,5)P_2 partitions into so-called lipid rafts, while others have challenged this notion (van Rheenen et al., 2005). The acyl chain composition of PI(4,5)P_2 is stearoyl-arachidonoyl and therefore, PI(4,5)P_2 is unlikely to partition into ordered lipid domains. For outer leaflet raft compositions (enriched in sphingolipid and cholesterol) it has been found that raft resident PI(4,5)P_2 binding proteins or peptides are required for PI(4,5)P_2 partitioning into lipid rafts (Tong et al., 2008). Despite this, in vitro experiments have shown cholesterol to promote PI(4,5)P_2 cluster formation (Dasgupta et al., 2009; Gericke, in this issue). The nature of the interaction that leads to cholesterol induced PI(4,5)P_2 cluster formation is only beginning to emerge.

Electrostatics are obviously important for protein–PI(4,5)P_2 interactions due to PI(4,5)P_2's high negative charge. In the plasma membrane under physiological conditions (but not bound to proteins) the charge of PI(4,5)P_2 is −4 (Kooijman et al., 2009; Janmey, in this issue). In the cellular environment, many factors can affect the ionization of PI(4,5)P_2 and may subtly alter PI(4,5)P_2's organization in the membrane. We have previously determined the charge of PI(4,5)P_2 and other polyphosphoinositides in membrane model systems and have investigated the impact of interactions with other membrane phospholipids using solid state magic angle spinning (MAS) ^{31}P NMR spectroscopy (Kooijman et al., 2009; Graber et al., 2012). For the dissociation of the second proton from the respective phosphomonoester groups of PI(4,5)P_2, a bimodal titration curve was observed, which is due to intramolecular hydrogen-bond formation and sharing of the last remaining proton between the 4- and 5-phosphates. This is contrasted by the "normal titration curves" (single sigmoidal curve, from 1 to 2 negative charges) of PI(3,5)P_2 where the phosphates are separated by a hydroxyl group at the 4-position of the inositol ring. The presence of hydrogen-bond capable phospholipids like phosphatidylethanolamine (PE) or phosphatidylinositol (PI) was found to increase deprotonation of the PI(4,5)P_2 headgroup, where the effect on charge was largest for PE. This increased deprotonation is due to the stabilization of the negative charge on the respective phosphomonoester group through the formation of direct or water mediated hydrogen-bonds between the phosphomonoester groups and the hydrogen-bond donor (e.g., PE headgroup), also termed the electrostatic-hydrogen-bond switch mechanism (Kooijman et al., 2005, 2007). ^{31}P NMR is uniquely able to monitor independently the ionization state of the respective phosphomonoester groups based upon the observed chemical shifts (CS). In this study, we have utilized MAS ^{31}P NMR spectroscopy to investigate the interaction of PI(4,5)P_2 with Ca^{2+}, Mg^{2+}, and cholesterol, which allows us to characterize the effect of these chemical species on the ionization state of the phosphoinositide headgroup.

2. Materials and methods

2.1. Materials

1,2-Dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE), L-α-phosphatidylinositol-4,5-bisphosphate (brain, porcine-triammonium salt) [brain PI(4,5)P_2], and cholesterol (ovine, wool), were purchased from Avanti Polar Lipids (Birmingham, AL) and used as received. Acyl chain composition of brain PI(4,5)P_2 is primarily stearoyl and arachidonoyl, which is the predominant acyl-chain composition in many natural biomembranes. Plant phosphatidylinositol (PI) was obtained from Matreya (Pleasant Gap, PA) and used as received. Primary acyl chain composition of the plant PI was palmitoyl and linoleoyl. Lipid chemical structures are shown in Fig. 1. Lipid was purchased in powder form and dissolved in a 2:1 by volume mixture of chloroform and methanol, or for the more polar lipids such as PI(4,5)P_2, in a 20:9:1 mixture of chloroform, methanol and water before making lipid films. The concentration of the resulting lipid stock solutions was generally tested via phosphate assay (Rouser et al., 1970), except for PI(4,5)P_2. Phospholipid stock solutions were tested for purity via thin layer chromatography using a 65:25:4 (chloroform, methanol, water) running solvent. Lipids were deemed pure if only a single spot was observed, and no additional band in the front appeared (indicative of acyl-chain hydrolysis). Ca^{2+} and Mg^{2+} ionophore A23187 was obtained from Sigma–Aldrich (St. Louis, MO) and dissolved in ethanol. MilliQ water had a resistivity of at least 18.2 MΩ. $CaCl_2$ and $MgCl_2$ were obtained as anhydrous powder from Sigma–Aldrich (St. Louis, MO), and dissolved in MilliQ water to 100 mM $CaCl_2$ and $MgCl_2$ solutions. A 10 mM stock of $CaCl_2$ was used to prepare the 0.1 mM Ca^{2+} containing samples.

2.2. NMR sample preparation

Lipid films for NMR experiments were formed by mixing lipid stocks in appropriate volumes to achieve the desired lipid composition. An additional 400–600 μL of chloroform was added to the lipid (in solvent) mixtures according to Kooijman et al. (2009). Typically each lipid film contained 0.2 μmol of brain PI(4,5)P_2. Lipid solutions were placed in custom made borosilicate test tubes