Minireview

The structure of phosphatidylinositol transfer protein α reveals sites for phospholipid binding and membrane association with major implications for its function

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Abstract Elucidation of the three-dimensional structure of phosphatidylinositol transfer protein α (PI-TPα) void of phospholipid revealed a site of membrane association connected to a channel for phospholipid binding. Near the top of the channel specific binding sites for the phosphorylcholine and phosphorylinositol head groups were identified. The structure of this open form suggests a mechanism by which PI-TPα preferentially binds PI from a membrane interface. Modeling predicts that upon association of PI-TPα with the membrane the inositol moiety of bound PI is accessible from the medium. Upon release from the membrane PI-TPα adopts a closed structure with the phospholipid bound fully encapsulated. This structure provides new insights as to how PI-TPα may play a role in PI metabolism.

Key words: Phosphatidylinositol; Phosphatidylcholine; Lipid-binding site; Open and closed conformation

1. Introduction

Phosphatidylinositol transfer protein (PI-TP) occurs in two soluble isoforms (PI-TPα and PI-TPβ) which are found in all mammalian cells investigated to date [1]. Both proteins transfer in vitro phosphatidylinositol (PI) and, to a lesser extent, phosphatidylcholine (PC) between membranes. In addition, PI-TPβ is also able to transfer sphingomyelin (SM) [2]. Upon isolation from bovine brain two forms of PI-TPα were identified which showed different isoelectric points. This difference was related to the presence of either PI or PC in the lipid-binding site of the protein [3]. PI-TPα is highly conserved among mammalian species with a sequence identity of more than 99% [4,5]. The PI-TPβ isoform is very homologous to PI-TPα with an identity of 77% [6]. Localization studies using indirect immunofluorescence and microinjection of fluorescently labeled PI-TPs have shown that PI-TPα is mainly localized in the cytosol and the nucleus whereas PI-TPβ is predominantly associated with the Golgi complex [2,7].

In search of a function PI-TPα and PI-TPβ were extensively assayed in reconstitution studies. Both PI-TPs were able to stimulate the formation of constitutive secretory vesicles and immature granules in a cell-free system containing *trans*-Golgi membranes [8]. Furthermore, both proteins were able to restore GTPγS-stimulated protein secretion and phospholipase C (PLC)-mediated inositol lipid signaling in cytosol-depleted cells [9,10]. In contrast to this apparent similarity in function, these isoforms gave rise to remarkable differences in lipid metabolism when overexpressed in NIH3T3 cells. In cells with a two- to three-fold elevated level of PI-TPα a PI-specific phospholipase A_2 (PLA_2) was activated [11]. This activation was not observed in cells overexpressing PI-TPβ. Rather, in these cells it was shown that under conditions where plasma membrane SM was hydrolyzed to ceramide by exogenous sphingomyelinase, PI-TPβ was involved in maintaining the steady-state levels of SM [12]. Possibly in line with their effects on lipid metabolism, the PI-TPα overexpressors display an increased growth rate, whereas the growth rate of PI-TPβ overexpressors is decreased [11,12].

Another clue to the function of PI-TPα and PI-TPβ comes from studies in mice. As shown by Hamilton et al., the so-called mouse vibrator mutation results in an 80% decrease of PI-TPα levels in the brain, giving rise to severe neurodegeneration and juvenile death [13]. Interestingly, PI-TPα −/− mice develop to term but die soon after birth [14]. Efforts to generate a knock-out cell line lacking the alleles for PI-TPβ failed. This indicates that PI-TPβ is an essential protein in murine cells [14].

Another member of the PI-TP family is the retinal degeneration protein B (rdgB). This 170 kDa integral membrane protein contains a PI-TP-like domain at the amino terminus. It was first identified in *Drosophila*, where it is localized in the photoreceptors, antennae and specific regions of the brain. RdgB is thought to be essential for photoreceptor function [15]. The mammalian homolog of rdgB has also been identified [16]. The latest addition to the PI-TP family is the mammalian 38 kDa M-rdgBβ which contains the PI-TP-like do-

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Abbreviations: PI-TP, phosphatidylinositol transfer protein; PI, phosphatidylinositol; PC, phosphatidylcholine; PLC, phospholipase C; PLA_2 phospholipase A_2; PIP_2, phosphatidylinositol 4,5-bisphosphate

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main, but lacks the transmembrane domain [17]. In the following we discuss the 3D-structures of the open and closed form of mammalian PI-TPα and the implication of these forms for the mode of action of PI-TPα at the membrane interface.

2. The 3D-structures of PI-TPα

Recently, the crystal structures of rat PI-TPα carrying a PC molecule and of mouse PI-TPα lacking a phospholipid molecule were elucidated [18,19]. The sequences of mouse and rat PI-TPα are nearly identical with a single conservative replacement of Val167 for Ile167. The crystal structures of both PI-TPαs show some remarkable differences although the secondary structural elements are preserved (Fig. 1). The main structural feature is a concave β-sheet consisting of eight strands (1–8). Both structures further consist of seven α-helices (A–G) of which helices C–E are part of a relatively large loop between strands 6 and 8. This loop is referred to as the regulatory loop, which is postulated to be involved in the interaction of PI-TPα with lipid- and protein-modifying enzymes. In addition to the regulatory loop, three other functional regions can be identified. The main part of the structure is the lipid-binding core, which consists of the β-sheet and two helices (A and F) facing the interior of the β-sheet. A loop containing helix B acts as a lid to the lipid-binding core and is designated as the lipid exchange loop. Finally, the fourth region is the C-terminal domain (residues 233–271) that contains helix G. In PI-TPα containing PC the tail of this domain (residues 261–271) is part of the lid (Fig. 1, left panel). When the lid covers the lipid-binding cavity the structure is in a closed conformation. In apo-PI-TPα the lipid exchange loop has moved outward, resulting in an open conformation (Fig. 1, right panel). Together with this loop the C-terminal domain is dislodged, accompanied by a partial unfolding of helix G and a disordering of the C-terminal tail (residues 258–271). The yeast PI-TP Sec14 shows no sequence homology with the mammalian PI-TPα, yet it also contains a loop which acts as a lid to the phospholipid-binding core [20]. The outward movement of the lid is accompanied by a widening of the lipid-binding cavity and creates a large opening (350 Å^2) at the site of the lipid exchange loop. By flattening of the β-sheet (strands 2–4) a small opening (75 Å^2) is formed at the opposite side of the protein, resulting in the formation of a channel. The large opening exposes a hydrophobic surface, whereas the small opening is of hydrophilic nature and close to the site for lipid head-group binding.

3. The phospholipid-binding sites

Previous biochemical studies on the binding of PC and PI to PI-TPα provided evidence for the presence of separate binding sites for the phosphorylinositol and phosphorylcholine head group [3]. From competition binding experiments it was estimated that the protein has a 16-fold higher affinity for PI than for PC [21]. The structure of the closed form shows that the recognition site for the phosphorylcholine head group is fully encapsulated and buried inside the protein. This site consists of amino acid residues interacting with the phosphate moiety (Gln22, Thr97, Thr114 and Lys195) and residues interacting with the choline moiety (Glu86, Asn90 and Cys95) (Fig. 2). As mentioned in [18], modeling of the inositol moiety in this site is unsatisfactory and does not explain the polar head-group preference of PI-TPα. Comparison of the closed and open structures indicates that the phosphorylcholine-binding site remains intact. However, as a consequence of the formation of the lipid-binding channel, modeling studies predict the induction of a separate binding site for the phosphorylinositol head group about 4 Å away from the phosphorylcholine-binding site towards the small channel opening [19]. In the open form, the putative inositol-binding site consists of Glu86 and Asn90 and the displaced residues Thr59 and Lys61, whereas the interaction with Cys95 is lost (Fig. 2). Due to the shift of the lipid-binding site the amino acid residues interacting with the phosphate moiety are limited to Gln22 and Thr97. Lys61 and Lys195 are the only positively charged residues directed towards the lipid cavity, but neither make contact with the phosphate moiety of phosphorylinositol. It appears that Thr59 plays a crucial role in the binding of the inositol head group as a mutation of this residue resulted

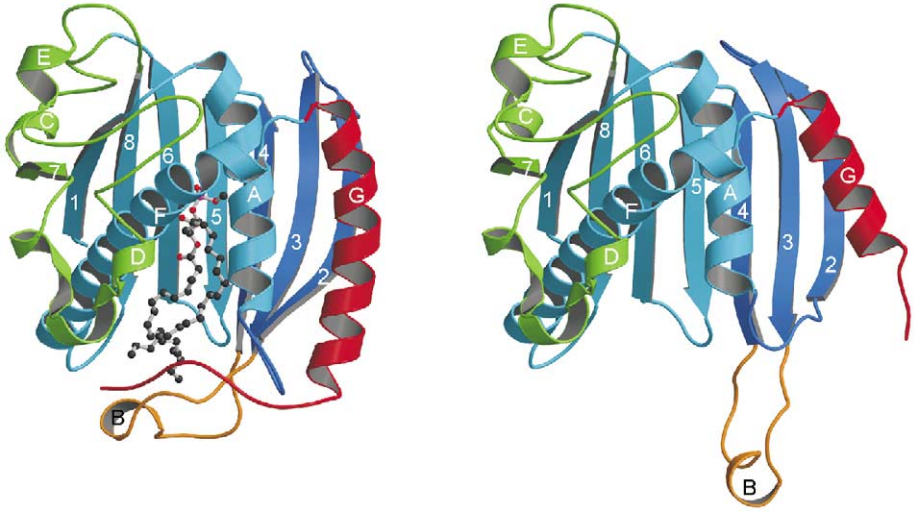

Fig. 1. A ribbon diagram of the closed (left panel) and open conformation (right panel) of PI-TPα from rat and mouse, respectively. The structures contain four functional regions, i.e. the lipid-binding core (blue) with the flexible part of the β-strand (dark blue), the regulatory loop (green), the C-terminal region (red) and the lipid exchange loop (orange). The lipid molecule bound to the closed form is PC.

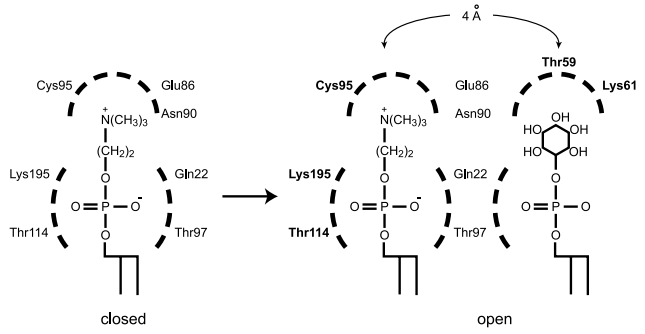

Fig. 2. The PC head group-binding site in the closed form of PI-TPα is extended to separate binding sites for the PC and PI head group in the open form. The PI head group-binding site is predicted from modeling [36]. As indicated by the amino acid residues involved in the binding, the major part of the PC head group-binding site is intact in the open form and 4 Å apart from the PI head group-binding site. In binding phospholipids only one of the two sites is occupied.

in the abolishment of PI-transfer, whereas the transfer of PC was not affected [22]. The distinct preference of PI-TPα for PI over PC can be explained by the formation of hydrogen bonds between Glu86, Asn90, Thr59 and Lys61 of the inositol-binding site and the inositol hydroxyls. This hydrogen bond formation does not occur with the choline moiety. In agreement with this, electrospray time-of-flight mass spectrometry analysis of the PI-TPα-lipid complex has shown that PI is much stronger bound than PC [23].

By measuring the binding and transfer of molecular species of PI and PC it was inferred that PI-TPα has separate binding sites for the *sn*-1 and *sn*-2 acyl chains, with each site having a distinct preference for a particular fatty acyl chain length. Similarly, it was concluded that the acyl chains of PI are probably accommodated in the same binding sites as the acyl chains of PC [24]. In addition, time-resolved fluorescence spectroscopy revealed that the *sn*-2 fatty acyl chains of both phospholipids are completely immobilized in the lipid-binding site [3]. The structural elucidation of PC in the lipid cavity of the PC–PI-TPα complex has confirmed that the *sn*-1 and *sn*-2 acyl chains are accommodated in different sites [18].

4. Membrane association

The open structure of PI-TPα is taken to represent the membrane-associated form, where the lipid-exchange loop acts as a membrane anchor [19]. In support of this interaction with the membrane, the injection of PI-TPα under a phospholipid monolayer spread at the air-water interface, gives rise to a slight increase in surface pressure [25]. In addition, association of PI-TPα with membrane vesicles renders the protein susceptible to proteolytic cleavage by trypsin at the C-terminal residues Arg253 and Arg259 as a result of dislodging and partially unfolding of the C-terminal region [26]. It is to be noted that C-terminally truncated PI-TPαs demonstrated an enhanced membrane affinity and a more relaxed structure when compared to full-length PI-TPα[27]. This suggests that in the absence of the C-terminal tail the open membrane-bound conformation is favored. Under these conditions truncated PI-TPαs in which 18–20 amino acids were deleted from the C-terminus, were inactive [27,28]. Upon interaction with the membrane PI-TPα undergoes a structural rearrangement so as to allow contact and interaction of the aromatic residues Tyr103, Trp203 and Trp204, and positively charged residues Lys105, Lys202 and Lys209 of the structurally invariant part of the lipid-binding core with the phospholipid interface. In addition, Lys68, Phe72 and Arg74 of the lipid exchange loop, and Lys153 and possibly Lys156 of the regulatory loop may also contribute to the membrane binding (Fig. 3A). Apart from the Trp203–Trp204 pair in the putative membrane association site, it was reported that two adjacent tryptophan residues are also part of a core bilayer insertion unit (TA-PAS-1 microdomain) of the cAMP-specific phosphodiesterase

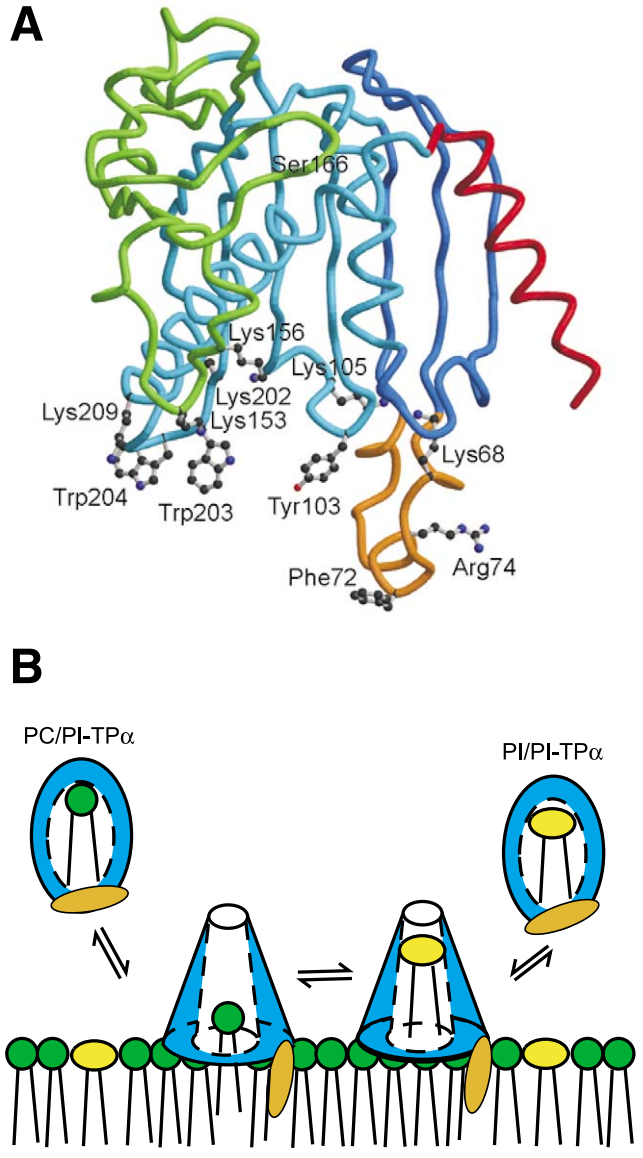

Fig. 3. A: Position of the amino acid residues involved in the association of PI-TPα with the membrane interface. B: Schematic representation of the conformational changes when PI-TPα (blue shape) binds to the membrane and when it is released into the medium. Water-soluble PC–PI-TPα and PI–PI-TPα have the closed form and PI-TPα at the interface the open form. The lipid exchange loop (orange shape), which closes the lipid cavity of the water-soluble forms, is inserted into the membrane when PI-TPα is associated with the membrane interface. At the interface PC (green shape) is inserted into the membrane in exchange for PI (yellow shape) that is bound to the protein

PDE4A1 [29]. In support of positively charged amino acid residues being involved in membrane association, van Paridon et al. have shown that binding of PI-TPα to phospholipid vesicles increases when the membranes are more negatively charged [30].

PI-TPα has a specific protein kinase C-dependent phosphorylation site (i.e. Ser166) which is present in the regulatory loop. To mimic phosphorylated serine, Ser166 was replaced with an aspartic acid residue yielding a protein which lacked phospholipid transfer activity [31]. This inactivation may result from Asp166 or phosphorylated Ser166 inducing structural rearrangements in the regulatory loop such that Lys153 and Lys156 present in this loop fail to interact with the membrane interface. Phosphorylation of Ser166 is a prerequisite for PI-TPα to be relocalized from the nucleus and cytosol to the Golgi complex [31]. This specific relocalization implies that upon phosphorylation PI-TPα most likely associates with a specific docking protein on the Golgi.

As shown by Yoder et al. [18], PI-TPα carrying a PC molecule has a closed conformation which can be characterized as the phospholipid transport intermediate (Fig. 3B). Upon binding to the membrane, the structure relaxes into the open conformation, which, as proposed by Schouten et al. [19], most probably corresponds to the membrane-associated state. This relaxation exposes the lipid-binding channel to the membrane interface and presents separate binding sites for the phosphorylcholine and phosphorylinositol head groups located close to the hydrophilic opening at the far end of the channel. Given the additional stability by hydrogen bonding, PI-TPα will preferentially bind a PI molecule in exchange for a PC molecule. Extraction of either phospholipid from the membrane interface may be facilitated by a low dielectric constant in the channel, which would reduce the bilayer stability at the site of interaction. Subsequent closing of the channel by the lipid exchange loop and refolding of the C-terminus will dissociate the phospholipid–PI-TPα complex from the membrane. It remains to be established whether PI-TPα carrying PI has a closed conformation similar to that of PI-TPα carrying PC. If so, one would predict subtle structural rearrangements around the binding site for the phosphorylinositol head group, which is more bulky than the phosphorylcholine head group. These subtle changes may offer an explanation for the observation that PKC phosphorylates Ser166 in PI/PI-TPα at a lower rate than in PC/PI-TPα [31]. It could be that Ser166 in PC/PI-TPα is better accessible for the PKC or that PKC has a higher affinity.

5. Implications of the structure for PI-TPα functioning

Although the exact function of PI-TPα is still a matter of debate, it is generally accepted that this protein is involved in the presentation of PI to various inositol lipid-metabolizing enzymes, yielding metabolites that are essential for lipid signaling. Cunningham et al. have shown that PI-TPα is necessary to reconstitute GTPγS-mediated PLCβ signaling in permeabilized HL60 cells [32]. Subsequent reconstitution studies using other cell lines showed that it was also able to stimulate PLCγ and δ [33,34]. In these studies it was proposed that PI-TPα promotes the synthesis of phosphatidylinositol 4,5-bisphosphate (PIP_2), which upon hydrolysis by PLC gives rise to increased levels of inositol phosphates [32]. In this PLC-signaling reaction PI-TPα may provide PI to PI 4-kinase and PI 4,5-kinase for PIP_2 to be synthesized at the plasma membrane. It has also been proposed that PI bound to PI-TP is directly presented to the kinases for sequential phosphorylation [32,33]. In this model PIP_2 remains bound to PI-TP for it to be presented directly to PLC. In a similar way, PI-TP may also play a role in delivering PI to PI 3-kinases [35]. In the case of PI being bound to PI-TPα at the membrane interface, the inositol moiety is in front of the hydrophilic opening of the lipid-binding channel. From modeling [36] it followed that the hydroxyl groups at positions 3, 4 and 5 are most likely accessible from the medium. Given the 75 $Å^2$ size of the small opening it is unlikely that the PI-kinases can directly phosphorylate the bound PI. Another possibility remains that the interaction of PI-kinases with PI-TPα at the interface gives rise to a structural change so that PI can switch from the lipid-binding site to the active site of the kinase. Phosphorylation of PI also occurs in the nucleus. Since PI is synthesized in the endoplasmic reticulum, it could be possible that PI-TPα is also required for supplying PI to the nuclear kinases [37]. Given that PC is synthesized in the nucleus [38], PI-TPα-mediated delivery of PI into the nucleus could then be coupled to a transfer of PC in the opposite direction. This proposed role is in line with the cytosolic and nuclear localization of PI-TPα and presumes a continuous flux of PI-TPα between these two compartments [7].

Overexpression of PI-TPα in NIH3T3 fibroblast cells had a profound effect on PI metabolism. Equilibrium labeling of these cells with *myo*-[^{3}H]inositol demonstrated that, compared with non-transfected cells, the overexpressors had increased levels of glycerophosphoinositol, inositol 1-phosphate, inositol 2-phosphate and lysoPI. These results indicated that in NIH3T3 cells PI-TPα activated a PI-specific PLA_2 rather than a PLC [11]. Similar to the PI-kinases, it is feasible that PLA_2 interacts with PI-TPα, resulting in a transfer of PI to the active site of PLA_2. On the other hand, it is well possible that PI-TPα takes PI to those membranes where the PI-specific PLA_2 is present.

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