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Structure–function relationships of phosphatidylinositol transfer proteins: involvement of phosphorylation sites

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Abstract

The mammalian low molecular weight phosphatidylinositol transfer proteins: PI-TPα and PI-TPβ are extremely well conserved and highly homologous. Surprisingly, the two proteins clearly show different cellular localizations and display contrary physiological functions. Phosphorylation of the proteins might be the regulatory factor to ensure the selective cellular functions. A major difference between PI-TPα and PI-TPβ is the capacity of PI-TPβ to bind and transfer sphingomyelin (SM) in vivo. This activity is correlated with phosphorylation of Ser262, which is only present in PI-TPβ. Structural aspects of phosphorylation sites of PI-TPs are analyzed in order to find an explanation for the functional data. We propose that phosphorylation of one serine residue (Ser165/166) in both PI-TPα and PI-TPβ is involved in the regulation of membrane binding of all PI-TPs and that phosphorylation of the unique Ser262 in PI-TPβ-like proteins ensures the right cellular localization of PI-TPβs that is necessary for the specific activity at the Golgi membrane.
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1. Introduction

Phosphatidylinositol transfer proteins (PI-TPs) belong to a family of highly conserved proteins that in vitro can catalyze the transfer of phosphatidylinositol (PI), phosphatidylcholine (PC) and sphingomyelin (SM) between membranes [1]. In mammalian tissues, at least two small molecular weight isoforms are identified: PI-TPα and PI-TPβ. The isoforms demonstrate a high homology (77%) and identity (91%) [2]. Information about possible cellular functions of these proteins has been obtained from experiments with reconstituted Golgi membrane systems, permeabilized cells, and from cells, tissues or animals in which the expression of the proteins has been manipulated.

Both proteins have been shown to stimulate the formation of secretory vesicles from isolated Golgi membranes as well as in permeabilized cells [3–5]. In permeabilized, cytosol-depleted HL60 cells both isoforms of PI-TP restored phospholipase C (PLC) mediated inositol lipid signaling [6,7]. However, other approaches emphasized that the PI-TP isoforms have separate cellular functions. Microinjection of fluorescently labeled PI-TPα and PI-TPβ into living cells as well as indirect immunofluorescence studies have shown that PI-TPα is localized in the nucleus and cytosol whereas PI-TPβ is associated with the Golgi system [8]. In intact cells, decreased expression of PI-TPα was shown to decrease the rate of proliferation and a lower rate of synthesis was observed for PC, lysoPC, SM, Choline and Glycerophosphocholine [9]. However, knock out of PI-TPα in mouse embryonic stem cells fails to compromise growth and has no significant consequence for bulk phospholipid metabolism. Moreover, the data showed that PI-TPα does not play an obvious role in any of the cellular activities where it has been reconstituted as an essential stimulatory factor. On the other

hand, knock out of PI-TPβ in mouse embryonic stem cells was shown to be a fatal deletion [10]. Increased expression of PI-TPα in mouse fibroblast cells lead to an increased rate of proliferation and an increased cell survival upon induction of apoptosis by UV-light or tumor necrosis factor α (TNFα). The cellular effects were attributed to a PI-TPα-dependent activation of a PI-specific phospholipase A_2 (PLA$_2$) since increased levels of lysoPI, Glycerophosphinositol, I(1)phosphate and I(2)phosphate were detected inside the cell [11]. In addition, highly mitogenic and anti-apoptotic arachidonic metabolites are secreted by the cells with increased expression of PI-TPα. These, as yet unidentified secreted factors demonstrate autocrine as well as paracrine activity and most probably belong to the family of endocannabinoids [12–14]. Increased expression of PI-TPβ in mouse fibroblast cells increased the duration of the cell cycle and made these cells highly susceptible towards induction of apoptosis by UV-light or TNFα [15]. Finally, studies with intact animals produced interesting data. Mice carrying the vibrator mutation showed early onset, progressive tremor and degeneration of neurons in the brain stem and spinal cord followed by juvenile death. An 80% decreased expression of PI-TPα is responsible for the aberrant pathological condition; the level of PI-TPβ is unchanged [16]. PI-TPα knock out mice show a normal prenatal development but soon after birth start to show many neurodegenerative symptoms. It was found to be impossible to establish PI-TPβ knock out mice [17].

Little is known about the molecular mechanisms and regulatory processes that are involved in the described physiological events. It is not clear whether actual lipid transfer is involved in the physiological function of PI-TPα or PI-TPβ or how the binding/exchange of PI/PC/SM is established or regulated. Single or multiple phosphorylation of the two PI-TP isoforms has been described and it has been shown that this phosphorylation is a regulatory factor for to the specific cellular localization, the lipid ligands bound and several of the cellular functions of the PI-TPs investigated [15,18,19].

The crystal structure of PI-TPα without a phospholipid (the apo-form) as well as bound to a PC or a PI molecule have been described [20–22]. The structure of the apo-form was different from the PI- or PC-bound forms although the secondary structural elements are preserved. However, the overall fold of the PI- and PC-bound forms is highly similar, in contrast to previous suggestions [22]. In all current structures the main structural feature is a concave β-sheet consisting of eight strands (1–8) and seven α-helices (A–G). The part of the structure forming the lipid-binding site consists of the β-sheet en two α-helices, A and F facing the interior of the β-sheet. In the apo-structure, the lipid-binding core is open; when a lipid is bound, helix G and loop B are moved over the lipid-binding core. The apo- and PI/PC-bound forms are shown in Fig. 1.

One of the putative membrane surface interaction sites of the molecule consists of a loop comprising two tryptophan residues, Trp203 and Trp204. The PI-TPα double mutant W203A/W204A is able to bind PI or PC but lacks PI/PC transfer activity and is unable to reconstitute PLC activity. The lipid head group cavity reveals a set of amino acids that can form H-bonds with inositol. The Choline head group is less well H-bonded explaining the poor affinity for PC relative to PI [20].

The crystal structure of PI-TPβ has not yet been elucidated but because of the very high homology between PI-TPα and PI-TPβ, a good model structure for PI-TPβ can be derived from that of the α-species. Closer inspection of structural details reveals that only two amino acids are different in the phospholipid binding region: Ile84 and Phe225 in
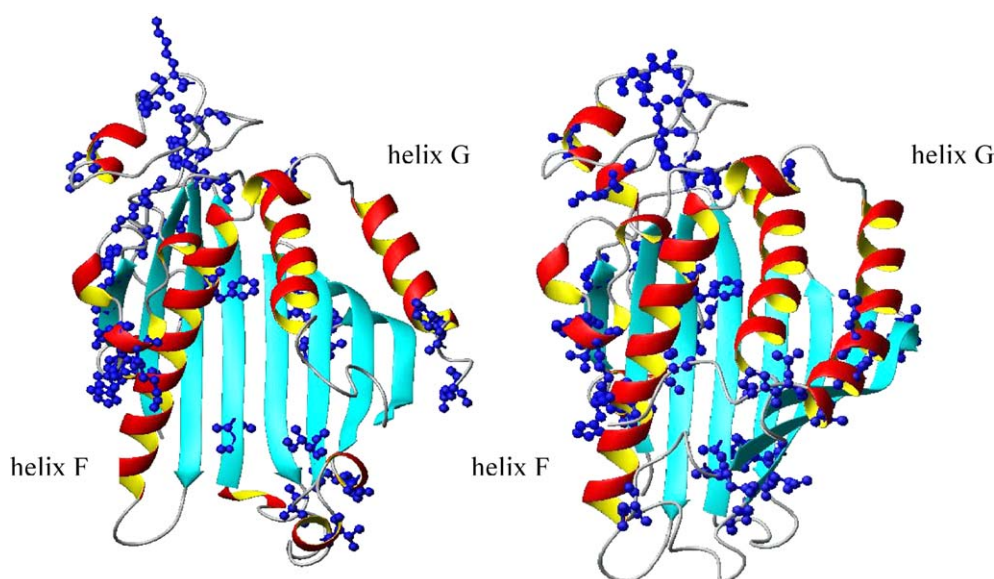

Fig. 1. The structures of apo- (left) and PI- or PC-bound PITPα (right) are shown indicating structural changes induced by phospholipid binding. Amino acid side chains are shown only for those 24 residues that are functionally different for the PI-TPα and PI-TPβ species. Most of these residues are not close to the phospholipid-binding site.

PITPα are replaced by a Phe and Leu in the β-species, respectively. Only minor differences in substrate preference are to be expected from such subtle differences between the two forms—based on the properties of the substrate-binding region only.

In this paper we will review the data on phosphorylation of PI-TPα and PI-TPβ and the in vitro and in vivo consequences of selective phosphorylation. Based on these data we will propose a model for the relation between phosphorylation, protein structure and function as well as the regulation of activation and cellular localization by phosphorylation.

2. Phosphorylation of PI-TPα and PI-TPβ in vitro and in vivo

The specific capacity of PI-TPs to bind and transfer PI and PC between membranes formed the basis of the hypothesis that the proteins are involved in net transfer of PI (and exchange with PC) from the site of phospholipid synthesis, the Golgi network, to the plasma membrane. In the plasma membrane, PI is phosphorylated to phosphatidylinositol-4-phosphate (PIP) by a PI-kinase and then to phosphatidylinositol-3,4-bisphosphate (PIP$_2$) by a PIP-kinase. Subsequently, upon stimulation of the cells PIP$_2$ is degraded by PLC into inositol-3,4,5-trisphosphate (IP$_3$) and diacylglycerol (DAG). Two aspects of this metabolic pathway are of interest in the context of PI-TPs: the depletion of the PI pool in the plasma membrane by the stimulation of PIP$_2$-degradation and the generation of DAG that activates protein kinase C [23]. Upon stimulation of PI-metabolism in Swiss Mouse 3T3 cells, the cellular localization of PI-TP, studied by indirect immunofluorescence, was changed. In contrast to what was expected, PI-TP did not move to the plasma membrane but it relocalized from the cytosol to the Golgi membranes. The Golgi localization was confirmed by double labeling with specific Golgi markers [24,25]. A comparable redistribution of PI-TP was observed when the cells were incubated with a direct activator of protein kinase C (replacing DAG): phorbol, 12-myristate, 13-acetate (PMA). These results suggest the involvement of phosphorylation of PI-TP in the cellular relocalization. Metabolic labeling of mouse fibroblast cells with ^{32}P-anorganic phosphate enabled the detection of phospho-PI-TPα but failed to show phospho-PI-TPβ because of the very low cellular concentration of PI-TPβ [15,18,25]. Using cells with increased expression of PI-TPβ and the use of very sensitive detection methods also allowed observation of the in vivo phosphorylation of PI-TPβ [19].

The amino acid sequences of mouse PI-TPα and PI-TPβ were analyzed for the presence of consensus sequences as putative phosphorylation sites that are specific for protein kinase C. In PI-TPα one highly conserved serine residue can be phosphorylated by protein kinase C: Ser166. In PI-TPβ the same serine residue, S165, is a protein kinase C-dependent phosphorylation site. A second serine residue in PI-TPβ that is phosphorylated by protein kinase C is Ser262.

An additional putative protein kinase C-dependent phosphorylation site is Thr59 but PI-TPα or PI-TPβ are not phosphorylated on Thr59 when protein kinase C is stimulated by PMA or DAG nor upon in vitro phosphorylation by purified protein kinase C [18,19,25].

Thr59 is very well conserved in the many members of the PI-TP family including the human Protein Tyrosine kinase 2 N-terminal domain-interacting receptors (Nir2 and Nir3). The Nirs are membrane-bound proteins containing an N-terminal PI-TP-like domain [26]. Nirs are the human homologues of the *Drosophila* retinal degeneration B (RdgB) protein that is suggested to be involved in the fly photo transduction cascade in which the PI-TP domain is critical for its function [27–29]. A specific mutation of Thr59 in this domain induces a dominant retinal degeneration phenotype [27]. Nir2 is not an integral, membrane-spanning protein but is associated to Golgi/endoplasmatic reticulum membranes. A T59E mutation in Nir2 changed the cellular localization to spherical cytosolic lipid droplets [30]. It is suggested that phosphorylation of Thr59 and relocalization of Nir2 is stimulated by incubation with oleic acid. Since stimulation of protein kinase C by PMA had no apparent effect on Nir2 localization it was suggested that a phorbol ester-insensitive isoform of protein kinase C or some other Ser/Thr kinase induces threonine phosphorylation of Nir2 in response to oleic acid treatment [30].

Ser165/Ser166 and Thr59 are highly conserved through the large family of PI-TP-like proteins but Ser262 is only present in a few low molecular weight PI-TPβ-like proteins (Table 1).

2.1. In vitro phosphorylation of PI-TPα: kinetic analysis

The first PI-TP that was purified to homogeneity from bovine brain had a molecular weight of 35 kDa [31,32]. This protein was later denoted PI-TPα. When this protein was first isolated from bovine brain cytosol two forms of PI-TPα were detected: PI-TPαI and PI-TPαII. These two forms could be separated because of a difference in isoelectric point, which was caused by the charge difference of the phospholipid species bound to the PI-TPα. PI-TPαI has an isoelectric point of 5.5 (as determined under non-denaturing conditions) and was shown to have the negatively charged PI molecule in the lipid binding site of the protein, while PI-TPαII has an isoelectric point of 5.7 and was found to contain the zwitte-

Table 1
Members of the PI-TPβ-family containing the Serine262 residue

PI-TPβ rat	MRKKG S VRGTS
PI-TPβ mouse	MRKKG S VRGTS
PI-TPβ human	MRKRG S VRGTS
PI-TPn-prov protein *Xenopus laevis*	MRQKG T VRGMS
zgc:55645 *Danio rerio* Table 3)	MRSQG S VRGMK
m-RdgB2	KMAQF S EEGPS

R: canonical consensus sequence for all known 10 isoforms of protein kinase C. K, R: canonical consensus sequence for conventional protein kinases C and for novel and a-typical protein kinases C.

rionic PC molecule [33]. The cellular concentration of PI-TPαI is about eightfold higher as compared to the concentration of PI-TPαII. Since the affinity of PI-TPα for PC is about 16-fold lower than the affinity for PI, the relative amounts of the two charge isomers reflect the accessible pools of PI and PC in the cell [33,34].

Recombinant murine PI-TPα and PI-TPα isolated from bovine brain were substrates for rat brain protein kinase C and were phosphorylated in a Ca^{2+} and a phosphatidylserine (PS) dependent manner in vitro [25,35]. Under optimal conditions, the calculated stoichiometry of the phosphorylation was 0.2 mol of phosphate/mol of PI-TPα.

The charge isomers isolated from bovine brain were phosphorylated by protein kinase C at different rates. The estimated V_{max} values for PI-TPαI and PI-TPαII were 2.0 and 6.0 nmol/min, respectively. The K_m values for both isomers were about equal, i.e. 0.7 μM (Table 2, [18]). Phosphorylation of charge isomers of recombinant mouse PI-TPα confirmed that the PC-containing isomer was the better substrate. Phospho-amino acid analysis of in vitro and in vivo ^{32}P-labeled PI-TPαs showed that serine was the major site of phosphorylation. In addition, phosphorylated PI-TPαII contained a trace of phospho-threonine [18]. Replacement of Ser166 with an alanine residue confirmed that this serine residue is the site of phosphorylation. This mutation completely abolished PC and PI-transfer activity. This was also observed when Ser166 was replaced with an aspartic acid residue implying that the mere presence of a negatively charged amino acid residue is not sufficient for regulating the function of PI-TPα (Table 3).

Another putative protein kinase C-dependent phosphorylation site, Thr59, has been shown to play a crucial role in the activity/function of PI-TPs. Phosphorylation of threonine residues has not been observed either in in vivo or in vitro phosphorylated PI-TPα. However, the trace of phospho-

Table 2
K_m and V_{max} values of the protein kinase C-dependent phosphorylation of S166/165 and S262 in PI-TPα and PI-TPβ

	K_m (μM)	V_{max} (nmol/min)
Bovine brain PI-TPαI	0.65	2.0
Bovine brain PI-TPαII	0.72	6.0
Rec. PI-TPβ	0.06	21.7
Rec. PI-TPβS165A	0.06	24.6
Rec. PI-TPβS262A	0.30	5.2

Table 3
Lipid transfer activity of mutant PI-TPα and PI-TPβ proteins in vitro

Mutation	Lipid transfer activity
PI-TPα	
S152A	+
S166A	−
S166D	−
PI-TPβ	
S165A	−
S262A	+
S165A, S262A	−

threonine that was detected in phosphorylated PI-TPαII could represent Thr59 [18]. Phosphorylation of Thr59 by another threonine/serine kinase cannot be excluded [30].

2.2. In vivo phosphorylation of PI-TPα

When PI-TP was immune-precipitated from cells that were labeled with ^{32}P-inorganic phosphate, an increase in radioactivity in PI-TP from PMA-stimulated cells was observed leading to the conclusion that in stimulated cells, PI-TP is phosphorylated and redistributed in the cell [25]. Phospho-amino acid analysis of the in vivo phosphorylated PI-TP indicated exclusive phosphorylation on serine residues [18]. A complication in this conclusion is that at the time the existence of a highly homologous isoform of PI-TP was not yet known. Therefore, it is unclear whether the immune-precipitated, phosphorylated protein represents phospho-PI-TPα, phospho-PI-TPβ or a mixture of both. However, the 2-D-phosphopeptide map of in vivo phosphorylated PI-TP closely resembles the 2-D-phosphopeptide map of in vitro phosphorylated PI-TPα [19].

Upon PMA-stimulation of NIH3T3 mouse fibroblast cells transfected with cDNA encoding myc-tagged PI-TPα, PI-TPα relocalization from the cytosol and nucleus to the Golgi membranes occurs [18]. In cells transfected with mutated PI-TPα: myc-PI-TPα(S166A) or myc-PI-TPα(S166D), the myc-tagged mutant proteins were localized in the nucleus and the cytosol comparable to the myc-tagged wild type PI-TPα. However, the mutant proteins did not relocalize upon stimulation by PMA suggesting that phosphorylation of S166 is required for relocalization to the Golgi membranes. Mouse fibroblast cells transfected with the mutant PI-TPαS166A demonstrated characteristics comparable to those of the wild type fibroblast cells and different from those of the fibroblast cells with increased expression of PI-TPα (unpublished results, [11,18]) indicating that the mutant PI-TPα failed to function as PI-TPα in the cell. Whether this is due to lack of phospholipid binding/transfer activity or to the missing phosphorylation site is yet unknown.

2.3. In vitro phosphorylation of PI-TPβ

Unexpectedly it was found that PI-TPβ purified to homogeneity from bovine brain could not be phosphorylated by protein kinase C in vitro despite the presence of the same putative phosphorylation sites present in PI-TPα and even a few more (Table 1, [19,25]). On the other hand, mouse recombinant PI-TPβ was shown to be an excellent substrate for protein kinase C in vitro. The V_{max} and K_m values as shown in Table 2 confirmed this. The explanation was found when it was shown that in vivo PI-TPβ is already phosphorylated to a high level preventing additional phosphorylation of the native protein in vitro. PI-TPβ was exclusively phosphorylated on serine residues and mutation studies showed that Ser165 (comparable to Ser166 in PI-TPα) is a minor phosphorylation site while Ser262 is a major phosphoryla-

tion site [19]. Mutant PI-TPβ with Ser165 replaced by an alanine residue lost the capacity to transfer PI and PC in vitro as was shown for PI-TPα. However, mutation of Ser262 had no effect on lipid transfer activities (Table 3).

2.4. In vivo phosphorylation of PI-TPβ

In wild type mouse fibroblast cells, the level of PI-TPβ is too low to enable dependable studies on phosphorylation or cellular localization [15]. Mouse fibroblast cells with a 10–15-fold increased expression of PI-TPβ (SPIβ cells) showed significantly different physiological properties when compared to the wild type mouse fibroblast cells [15,36]:

- The cell cycle duration increased from 21 to 34–36 h.
- The SM content of the plasma membrane was replenished more efficiently and rapidly upon degradation by exogenous SMase.
- SPIβ cells were extremely sensitive to apoptosis induced by UV-light or TNFα.

In vivo phosphorylation of PI-TPβ could be studied by 2-D-gel electrophoresis followed by western blotting. PI-TPβ is detected as a pH 6.5 form, phospho-PI-TPβ as a pH 6.2 form. In SPIβ cells more than 85% of PI-TPβ is phosphorylated. In cells transfected with a mutant PI-TPβ, S262A, (SPIβS262A cells) no phospho-PI-TPβ could be detected. Incubation of SPIβ cells with a protein kinase C inhibitor, GF109203X, resulted in a shift from the phospho-PI-TPβ pH 6.2 form to the PI-TPβ pH 6.5 form [19]. In SPIβS262A cells the mutant PI-TPβ does not colocalize with the Golgi membranes as native PI-TPβ does [8] indicating a direct relationship between phosphorylation and cellular localization of PI-TPβ. This was confirmed by the finding that incubation of SPIβ cells with the protein kinase C inhibitor GF109203X prevented the association of PI-TPβ with the Golgi membranes. The link between phosphorylation, localization and cellular function was given by the finding that SPIβS262A cells fail to show the characteristics of SPIβ cells. The cell cycle duration, the sensitivity to induction of apoptosis by UV-light or TNFα and the replenishment of the degraded SM-pool in the plasma membrane in SPIβS262A cells are comparable to those in wild type cells [15,19]. Since mutant PI-TPβ(S262A) demonstrates the same in vitro lipid transfer activity as PI-TPβ, it was concluded that the phosphorylation-dependent association of PI-TPβ with the Golgi membranes is essential for its cellular function. This indicates that Ser262 in members of the PI-TPβ family is necessary to direct the protein towards the Golgi membranes upon phosphorylation and that this event is essential for the correct functioning of the protein in the cell.

A psi BLAST analysis of proteins showing homology with PI-TPα and PI-TPβ indicated that Ser262 is only present in a few members of the PI-TP family (Table 1): in PI-TPβ from rat, mouse and human [2,10,37]. In zebrafish, a hypothetical protein derived from DNA sequences showed a high homology with PI-TPβ including the Ser262 with a protein kinase C consensus sequence [38]. In *Xenopus* a

protein, PI-TPn was identified demonstrating a homology with PI-TPβ. In PI-TPn a threonine residue is located on the 262 position instead of a serine residue but the surrounding amino acids form a consensus site for protein kinase C [39]. Murine mRdgB2 (a high molecular weight, transmembrane member of the PI-TP family) contains a Ser262 but lacks the consensus properties [40].

3. Structural aspects of phosphorylation of PI-TPα and PI-TPβ

The crystal structure of PI-TPα presents us the location of the putative protein kinase C-dependent phosphorylation sites in PI-TPα and PI-TPβ, Ser165/Ser166, Ser262 and Thr59 [20–22]. As is shown in Fig. 2 a striking observation is the invariant position of Ser166 upon phospholipid binding [20]. The γ-OH of Ser166 is an unlikely target for phosphorylation in this orientation because it is not solvent accessible. Ser165/166 is found in a small pocket between the 165 and 172 loop and the remainder of the protein.

Several highly conserved residues anchor this loop including Lys168, Arg171, Asp234 and D238.

Based on these structural aspects it was concluded that for Ser166 to be phosphorylated, major conformational changes must occur such as may happen when PI-TPα is perturbed by interaction with either a membrane or a protein [20,41].

Fig. 3 shows the positions of Ser166 and another candidate for phosphorylation, Thr59, relative to a bound PI molecule.
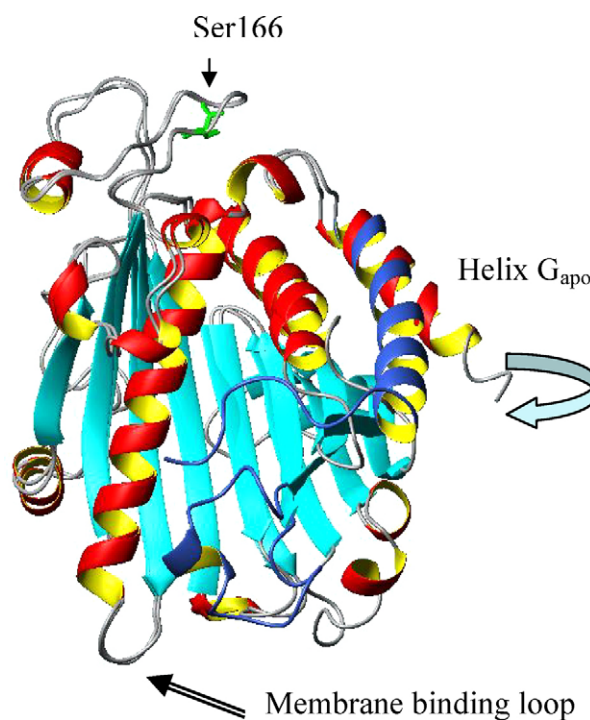

Fig. 2. Overlay of apo- and PI-bound PITPα species highlighting conformational changes induced by phospholipid binding, but also the invariant location of residue Ser166 and the membrane-binding loop containing Trp203 and Trp204. Changed conformations in loops and helices are colored blue in the PI-bound form only.
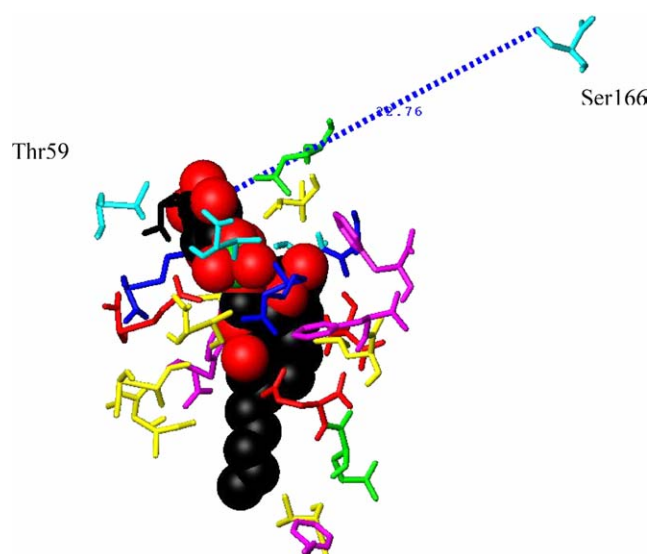
Fig. 3. Bound PI molecule surrounded by residues involved in substrate binding for PI-TPα. In addition the location of Ser166 is shown relative to the substrate binding site.

The head group of PI is at a distance of about 23 Å away from Ser166, which is in line with the lack of a reorientation of Ser166 upon PI binding. In contrast, Thr59 is directly involved in substrate recognition. Phosphorylation of Thr59 would certainly interfere with PI recognition (see more details below). The third residue mentioned, i.e. Ser262, is only present in PI-TPβ. This residue is most likely accessible to solvent, and therefore, easily phosphorylated. However, the lack of a detailed model, particularly for PI-TPβ with bound substrate prevents more definite predictions to be made.

We propose the following model that will allow for a conformational change leading to phosphorylation of Ser166 after binding of PI-TP to a membrane: Helix F may slightly reorient when the loop at the N-terminal side of this helix (involving Trp203 and Trp204) becomes embedded in the membrane. Ser166 is located at the C-terminal end of helix F above the helical axis at a distance of about 6 Å. This reorientation could trigger a slight conformational change leading to exposure of the γ-OH function of Ser166 allowing for this residue to become phosphorylated by protein kinase C.

This would predict that only membrane-bound PI-TPα is easily phosphorylated at Ser166. What about the presence or absence of bound substrate influencing these events? In vitro phosphorylation of PI-TPα showed that PI-TPαII was a better substrate for protein kinase C than PI-TPαI. The interactions of either lipid substrate, PI or PC, with the flanking helix F may modulate the forces that are needed to expose Ser166. The ligand in PI-TPα can either facilitate or inhibit this process explaining the different kinetics of phosphorylation of PI-TPαI and PI-TPαII [18]. In vitro phosphorylation of PI-TPα showed that the surface activity properties of the phosphorylated proteins change dramatically: the phosphorylated proteins bind quantitatively to the reaction vessels

that are used for the phosphorylation reaction (plastic or glass) and could only be removed by detergents. The inability to recover native phosphorylated PI-TPα prevented the comparison of the lipid transfer activities of the phosphorylated and non-phosphorylated PI-TPαI and PI-TPαII (unpublished data G.T. Snoek).

Protein kinase C-dependent phosphorylation of Thr59 in PI-TPα from bovine brain or recombinant mouse PI-TPα has not been detected although a low level of phospho-threonine was found in phosphorylated PI-TPαII (the PC-carrying isomer) from bovine brain [18]. Replacement of Thr59 in rat PI-TPα by Ser, Gln, Val, Ile, Asn, Asp or Glu abolished PI-transfer activity but did not affect PC transfer activity [42]. This can be explained by the proposed models for PI-TPα where the inositol headgroup interacts directly with Thr59 whereas the choline headgroup does not [20,21]. Replacement of His60 by Q has no effect on either PI- or PC transfer activity. Thr59 is highly conserved among the family of PI-TPs, and therefore, may be of importance in the regulation of the protein activity. It is not yet known whether structural aspects or phosphorylation are involved in the regulatory role of Thr59 in the activity of PI-TP. In a high molecular weight member of the PI-TP family, Nir2, Thr59 can be phosphorylated suggesting the possibility that this residue can become solvent-accessible at some point during the lipid transfer cycle but that a different protein kinase is involved [30].

The lipid-binding site in the PI-TPβ model is virtually identical to the lipid-binding site in PI-TPα. Mutation of Ser165 in PI-TPβ completely abolished phospholipid transfer activity, as was found also for PI-TPα [18,19]. Since in PI-TPβ, Ser165 is also phosphorylated by protein kinase C, we propose that regulation of the specificity and activity of PI-TPβ is also controlled by phosphorylation of Ser165 comparable to PI-TPα.

A special feature of PI-TPβ is that it contains a second protein kinase C-dependent phosphorylation site: Ser262 that can be phosphorylated to a much higher level than S165 [19]. Mutation or phosphorylation of Ser262 has no effect on the lipid transfer activity in vitro. Phosphorylation of PI-TPβ on Ser262 was shown to be responsible for its cellular localization at the Golgi membranes. Microinjection of fluorescently labeled PI-TPβ isolated from bovine brain immediately localized to the Golgi membranes because, as was shown later, native PI-TPβ is already phosphorylated to a very high level [8,19].

4. Conclusions and discussion

As in vivo phosphorylation of Ser166 is strongly correlated with the binding to a membrane surface, the phosphorylation and dephosphorylation cycle of Ser166 could regulate the specificity and the rate of lipid binding and/or transfer by PI-TPα and/or PI-TPβ.

Stimulation of phosphorylation of Ser166 in PI-TPα mediates a translocation of PI-TPα to the Golgi membranes and

vesicle-like structures. However, since the cellular functions of PI-TPα and PI-TPβ have been shown to be clearly distinct, it must be concluded that Golgi-association of PI-TPα serves a different purpose than Golgi association of PI-TPβ. It is not known yet whether the Golgi-association of PI-TPα is necessary for its cellular function. Expression of PI-TPαS166A in mouse fibroblast cells has no effect on growth characteristics in contrast to an increased expression of PI-TPα. Whether this lack of effect is caused by the absence of the phosphorylation site in PI-TPαS166A or by the biological inactivity of the expressed protein remains to be established.

Phosphorylation of Ser262 is restricted to PI-TPβ-like proteins and its major function seems to be the regulation of the cellular localization at the Golgi membranes. This localization nevertheless is essential for the adequate cellular functioning of PI-TPβ. Expression of PI-TPβS262A in mouse fibroblast has no effect on growth characteristics of the cells in contrast to increased expression of PI-TPβ. This difference can be contributed to the absence of the phosphorylation site in PI-TPβS262A since the protein is biologically active (in vitro).

Major differences between PI-TPα and PI-TPβ are the capacity of PI-TPβ to bind and transfer SM and the presence of Ser262 in PI-TPβ regulating its essential Golgi localization. Thus, phosphorylation/dephosphorylation of one conserved serine residue (Ser165/166) in both PI-TPα and PI-TPβ seems to be involved in regulation of membrane binding of all PI-TPs and phosphorylation of the unique Ser262 in PI-TPβ-like proteins seems to ensure the right cellular localization of these proteins that is necessary specific activity at the Golgi membrane.

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