The Crystal Structure of a Human PP2A Phosphatase Activator Reveals a Novel Fold and Highly Conserved Cleft Implicated in Protein-Protein Interactions

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Protein phosphatase 2A (PP2A) is a heterotrimeric Ser/Thr phosphatase that is involved in regulating a plethora of signaling pathways in the cell, making its regulation a critical part of the well being of the cell. For example, three of the non-catalytic PP2A subunits have been linked to carcinogenic events. Therefore, the molecular basis for the complicated protein-protein interaction pattern of PP2A and its regulators is of special interest. The PP2A phosphatase activator (PTPA) protein is highly conserved from humans to yeast. It is an activator of PP2A and has been shown to be essential for a fully functional PP2A, but its mechanism of activation is still not well defined. We have solved the crystal structure of human PTPA to 1.6 Å. It reveals a two-domain protein with a novel fold comprised of 13 α-helices. We have identified a highly conserved cleft as a potential region for interaction with peptide segments of other proteins. Binding studies with ATP and its analogs are not consistent with ATP being a cofactor/substrate for PTPA as had previously been proposed. The structure of PTPA can serve as a basis for structure-function studies directed at elucidating its mechanism as an activator of PP2A.

The heterotrimeric protein phosphatase 2A (PP2A)² is a major serine/threonine phosphatase constituted of a highly conserved 36-kDa catalytic subunit (PP2Ac), a regulatory subunit (PP2Ab), and a 65-kDa structural subunit (PP2Aa) that serves as a scaffold bringing the other two subunits together. The core enzyme is a dimer of PP2Ac and PP2Aa that forms a complex with one of several PP2Ab subunits, each belonging to one of three distinct structural families. PP2A is involved in the regulation of numerous processes in the cell such as DNA replication, translation, and transcription as well as RNA splicing, cell cycle progression, and development. It is highly regulated through the holoenzyme composition, post-translational covalent modifications, and through binding to several cellular and viral proteins (for reviews, see Refs. 1–7). PP2A is believed to be involved in cancer development. The β form of the PP2Aa is mutated in 15% of colon and lung tumors (8), and specific PP2Abs have recently been suggested to play a part in cellular transformations (9, 10).

PTPA is a PP2A-interacting protein once known as phosphotyrosyl phosphatase activator (PTPA) but now as protein phosphatase two A phosphatase activator. The reactivation of PP2Ac by PTPA in vitro was at first only identified as an increase in phosphotyrosyl phosphatase activity (11, 12) when in fact it appears to activate the phosphoserine/threonine-specific activity of PP2Ac from a poorly active and substrate unspecific metal-dependent state (13, 14). PTPA is an essential protein as revealed by its high evolutionary conservation as well as the lethality of the deletion of the two PTPA homologs in yeast, Rd1 and Rd2, in certain nutritional backgrounds (15). Rd1/2 also interact with other less abundant type 2A phosphatases (16, 17). Interestingly, the effect of deleting Rd1/2 is more severe than deletion of the yeast PP2Ac, most likely due to the fact that the different type 2A phosphatases in yeast can partly take over each others roles (18). However, they all interact with the PTPA subunits (16), an interaction that appears to be critical for the 2A phosphatase activity in the cell.

The exact role of PTPA in cells remains unclear although several proposals have been put forward. It has, for example, been suggested to be involved in the introduction metals to the active site of PP2Ac, potentially serving as a metal chaperone (14). PTPA was just recently suggested to have peptidyl prolyl cis/trans-isomerase (PPIase) activity, which acts specifically on Pro190 in human PP2Ac (19). This isomerase activity, seen in vitro, could potentially control the folding of PP2Ac and thereby its activity. Rd1 and Rd2 have been suggested to act directly as regulatory subunits as a part of a novel heterotrimERIC complex with PP2Ac, providing a complex with altered substrate specificity (17). These suggestions do not necessarily contradict each other, and the protein could, for example, be a...
third partner of a novel PP2A complex and at the same time catalyze the prolyl isomerization activity, bringing about a conformational change that makes metal binding more feasible. Nevertheless, the time is ripe for a conclusive assignment of the physiological function of PTPA, and the structure of the protein presented in the present study will help in defining appropriate experimental strategies directed toward this goal as well as providing a structural framework for understanding the function of this important protein.

**EXPERIMENTAL PROCEDURES**

**Materials**—Tripeptide (VPH) and pentapeptide (EVPHE) were synthesized by Elin Biopharmaceuticals, Inc. Peptide LNEVPHEGPMCAL was synthesized by Bachem, Germany. All other chemicals were commercially available.

**Expression and Purification**—Residues 22–323 of the 323-residue-long human PTPA (hPTPA) protein were expressed in BL21(DE3) from the pET-based vector pNIC-Bsa4 as a N-terminal His6 fusion. A Tev protease site was present between the His tag and the protein. The presence and integrity of the gene in the vector were confirmed by sequencing. The protein was purified using a 1-ml HiTrap chelating HP column and a Superdex 75 gel filtration column (Amersham Biosciences). The >98% pure protein (as estimated from SDS-PAGE) was concentrated to 35.4 mg/ml in 20 mM HEPES, 300 mM NaCl, 10% glycerol, 0.5 mM TCEP, and a mass of 37.30 kDa was confirmed by mass spectrometry (high performance liquid chromatography-electrospray ionization-mass spectroscopy). The same methods were used for purifying the selenomethionine-labeled protein. The incorporation of seven selenomethionines was confirmed by mass spectrometry.

**Crystallization and Data Collection**—Hanging drop vapor diffusion was used to produce hPTPA crystals by making 2 µl of 1:1 protein:reservoir solution drops. Crystals of hPTPA were grown in 1.7–2.1 M (NH₄)₂SO₄, 0.2 M NaCl, and 0.1 M sodium cacodylate, pH 6.1–6.8 at 20 °C for 1 week. The crystals grew in clusters, and the size of a single crystal varied from 1.3 × 0.5 mm to 0.2 × 0.1 × 0.05 mm. Crystals of selenomethionine-labeled protein were obtained through seeding with native crystals. The drops to be seeded were made by mixing 1 l of reservoir solution. Data were collected on native crystals that grew in 1.9 M (NH₄)₂SO₄, 0.2 M NaCl, and 0.1 M sodium cacodylate, pH 6.3, and selenomethionine-labeled protein crystals that grew in 1.4 M (NH₄)₂SO₄, 0.2 M NaCl, and 0.1 M sodium cacodylate, pH 6.3. For data collection, the crystals were flash-frozen in liquid nitrogen after adding a cryosolution of 20% glycerol, 2.1 M (NH₄)₂SO₄, 0.3 M NaCl, and 0.1 M sodium cacodylate (pH of drop) directly to the drop. Soaks of native crystals were performed by adding the cryosolution with ligand or peptide to the crystallization drop. Soaks were performed in 8 mM ATP and AMP/PCP with 8 mM MgCl₂, 40 and 80 mM tripeptide (VPH), and 20 mM pentapeptide (EVPHE). The concentration of the pentapeptide was relatively low due to the severe effect the peptide had on pH. Co-crystallizations were performed by adding the peptide to the purified and concentrated hPTPA protein before crystallization to a final concentration of 5 mM. The native x-ray data were collected at station PXI at the Swiss Light Source in Zurich, Switzerland. The selenomethionine data were collected at Bessy-I in Berlin, Germany. All data were processed using the programs XDS and XSCALE (20). (Table 1).

**Phasing, Model Building, and Refinement**—The structure of hPTPA was solved by multiwavelength anomalous diffraction phasing using SOLVE (21), which located 5 out of 7 selenium sites in the asymmetric unit. RESOLVE (22) was used to carry out solvent flattening. ARP/wARP (23) was used to build the initial model and to place solvent molecules. Repeated rounds of manual model building using Coot (24) and refinement using Refmac (25) gave the final model consisting of residues 22–322 of hPTPA with seven residues of the Tev-linker along with four sulfates and one glycerol molecule. The two first residues of the N terminus were built in two conformations. The structure was refined to an R and an R_free of 0.153 and 0.180, respectively, and to a good stereo geometry. All residues of the model are within the most favored or additionally allowed regions of the Ramachandran plot. The coordinates and structure factors have been submitted to the PDB and have the accession code 2G62.

**Thermal Stability Shift Assay**—The thermal stability shift assay was run on an iCycler from Bio-Rad in a DNA- and RNase-free 96-well PCR plate. The total volume in each well was 25 µl with 10 µg of protein, 5 mM TCEP, SYPRO orange (Molecular Probes, Eugene, OR) diluted 5000X and 10 mM peptide or 1 mM ATP or ATP analog. Fluorescence was measured from 20 to 89.6 °C, and the thermal stability shift was determined as described in Ref. 26.

**RESULTS**

A construct of residues 22–323 of hPTPA was produced in *Escherichia coli* as a N-terminal His6 fusion with a Tev cleavage site between the His tag and the protein. It was crystallized in 1.9 M (NH₄)₂SO₄ at pH 6.3, and the structure was determined at 1.6 Å resolution and refined to good stereo geometry. The structure of hPTPA constitutes a novel fold as the DALI fold recognition server (27) was not able to detect any structures with an overall fold similar to that of hPTPA.

The fold of the structure is shown in Fig. 1A. It is a two-domain protein where the core of the larger domain is constituted by five long helices (helices 3–7 (see Fig. 1C for helix numbering)) forming a helix bundle and the smaller domain is formed by four shorter helices (helices 9–12). The two domains are connected by helix 8. The C- and N-terminal helices (helices 1, 2, and 13) cover one phase of the protein. Helices 7 and 8 are...
The Novel Fold of PTPA

To explain the structural basis for the observed ATP/Mg\(^{2+}\)-dependent PPlase activity of PTPA reported by Jordens et al. (19), we performed binding studies with potential ligands to PTPA in solution and in the crystal phase. Crystal soaks of native hPTPA crystals with 8 mM ATP and the ATP analog AMPPCP did not reveal any significant density implying poor binding in the crystal. As Pro\(^{190}\) of PP2Ac had been assigned as the scissile proline for the recently suggested PPlase activity of PTPA (19) peptides corresponding to this region were designed. Trimer and pentamer peptides corresponding to Val\(^{189}\)-His\(^{191}\) and Glu\(^{188}\)-Glu\(^{192}\) of PP2Ac, respectively, were soaked into the crystal. A high concentration of the trimeric peptide was used (40 and 80 mM) and a moderate concentration of the pentamer (20 mM). Cocrystallization with a 5 mM concentration of the peptide corresponding to Leu\(^{188}\}-Leu\(^{196}\) of PP2Ac was also performed. None of the peptide-treated crystals revealed a bound peptide. However, a new crystal form of PTPA was obtained in the cocrystallization attempts. This new crystal form belongs to space group P2\(_1\), and has two molecules in the asymmetric unit while the native crystal form belongs to space group P2\(_1\);2\(_1\);2\(_1\), with one molecule in the asymmetric unit.

Interestingly, the Tev-linker still interacts with a neighboring molecule in the P2\(_1\) crystal packing obtained from cocrystallization experiments but with an altered conformation. It is possible that the change of space group is induced by competition with the peptide binding in solution, but in the refined structure of the cocrystallized protein, no obvious electron density for a bound peptide was seen.

To shed further light on the interactions of PTPA with its potential substrates, a thermal stability shift assay was performed using the thermofluor methodology (26). No detectable interaction was observed using ATP or its analogs AMPPCP, AMPPNP, AMPCPP, or ATP\(_{pyS}\) (results not shown). Unfortunately, the binding studies using the peptides did not behave normally in the thermofluor experiment, probably due to interactions of the fluoro-probe with the peptide, excluding conclusive interpretation of the data.

**DISCUSSION**

hPTPA has a novel two-domain fold constituted by 13 α-helices. A highly conserved cleft (cleft 2 in Fig. 2) is present in the protein where a peptide from the Tev-linker region of a neighboring molecule in the crystal binds. The high degree of conservation of this cleft along with the binding of the Tev-linker

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**FIGURE 1.** A, a schematic representation of the structure of hPTPA colored from N (blue) to C (red) terminus. A narrow cleft (cleft 1) is present between the greenish-yellow (helix 7) and the orange (helix 11) helices. B, a topology diagram of the structure, where the helices represented by circles have the same coloring as in A. C, i, the secondary structure elements; ii, hydrophobicity (pink, hydrophobic; gray, intermediate; cyan, hydrophilic); iii, accessibility (blue, accessible; cyan, intermediate; white, buried); and iv, intermolecular participation (in red if shortest distance between interacting residues is less than 3.2 Å) of the residues in hPTPA. Part C of this figure was made using the ESPRIT utility (35).

the basis of a very conserved loop that is not visible in the current model. All three loops, A—C, shown in Fig. 2, connecting helix pairs 3/4, 5/6, and 7/8, respectively, are conserved and are all located on the same side of the protein as is the less conserved loop D connecting helix pair 11/12.

Two notable clefts are found in the structure. Cleft 1 is narrow and formed in between the two domains (Fig. 1A), while the larger cleft 2 is in the large domain lined by the most C-terminal helix 13 (Fig. 2). Both these clefts constitute potential candidates for active sites/protein-protein interaction surfaces. Binding site analysis by the structure-function relationship server Profunc (28) finds cleft 2 to be the most likely surface on the protein to participate in interaction with other molecules. Electron density for a glycerol molecule (used as a cryoprotectant) and several water molecules were found in the central region of cleft 2. The conserved loops B–D all face cleft 2, and conserved loop A is situated above it (Fig. 2). That is, cleft 2 is highly conserved (Fig. 3).

The last seven residues of the Tev-linker region can be seen in the present model. The backbone and side chains of the first and second visible residues of the N terminus were built in two conformations. A peptide of the N-terminal Tev-linker from a neighboring molecule in the crystal lattice interacts with one end of cleft 2 where a phenylalanine enters into the conserved hydrophobic pocket formed by residues Ile\(^{278}\), Leu\(^{291}\), Met\(^{294}\), Val\(^{287}\), and Val\(^{281}\) (Fig. 3).
The authors do not show that Pro190, which they assign as a trimer and a pentamer peptide corresponding to Val189–in fact that this residue is isomerized. We attempted to soak the proline isomerized by PTPA, is crucial for this binding or function relationships has also proven somewhat difficult for structural information. The definition of detailed structure-challenging to explain the PPIase activity of PTPA using the type of PPIase. Unfortunately, this also means that it is more

The PTPA structure is not similar to the structure of any of the three types of PPIases known today: cyclophilins, FKBP, or parvulins (for a PPIase review, see Ref. 29), supporting the claim of Jordens et al. (19) that PTPA is a new type of PPIase. Unfortunately, this also means that it is more challenging to explain the PPIase activity of PTPA using the structural information. The definition of detailed structure-function relationships has also proven somewhat difficult for the established PPIases (29). The NMR data of Jordens et al. (19) gives strong support for binding of a synthetic peptide corresponding to residues Leu186–Leu198 of PP2Ac to PTPA. The authors do not show that Pro190, which they assign as the proline isomerized by PTPA, is crucial for this binding or in fact that this residue is isomerized. We attempted to soak a trimer and a pentamer peptide corresponding to Val189–His191 and Glu188–Glu192 of PP2Ac, respectively, into the crystals of native hPTPA but were unable to detect any significant electron density indicative of binding. Cocrystallization with the peptide, corresponding to Leu186–Leu198 of PP2Ac used by Jordens et al. (19) was also unsuccessful even though a new crystal form of the protein was obtained. The lack of binding could potentially be explained by poor accessibility in the crystal lattice or by direct competition with the Tev-linker peptide for the binding site in cleft 2. Alternatively, it might be that the kinetics of binding in the experiments of Jordan et al. (19) only allows for transient interactions of reaction components in the PPIase reaction.

The activity of PTPA has been reported to be ATP/Mg2+-dependent in vitro (13, 19), but it is not clear what role ATP has for this activity. ATP and Mg2+ can on their own activate phosphotyrosyl phosphatase function of PP2A (14). The recently observed PPIase activity was stimulated by ATP/Mg2+, indicating a direct interaction of ATP/Mg2+ with PTPA. However, ATP in excess of Mg2+ is inhibitory (19) raising the possibility that magnesium ions might play a dominant role. The lack of ATP binding in the crystal, as well as in the thermal stability shift assay is intriguing, indicating that ATP might not be a PTPA ligand. Furthermore, nucleotide-binding proteins crystallized at high concentrations of ammonium sulfate usually have sulfate ions bound in the nucleotide binding pocket, where they mimic the physicochemical properties of the phosphate groups of nucleotides. hPTPA is crystallized in 1.9 m ammonium sulfate, but the crystal binding sites of the four SO42− molecules that are present in the structure do not have the characteristics of a ATP binding site and the residues that coordinate them are generally not conserved. Together our data make it unlikely that the involvement of ATP in PTPA activity observed in vitro is due to direct ATP binding to PTPA. Furthermore, Profunc, the protein function prediction server (28) was not able to detect any ligand binding motifs in the structure, even though a model where the missing loop had been modeled in was used.

Seven different splice variants of hPTPA have been identified, but only two of those have been detected in tissue: PTPAα, the most abundant one presented here, and PTPAβ. PTPAβ has a 35-residue-long insertion (30) between helices 2 and 3. This insertion appears feasible from a structural perspective and could possibly be involved in changing the protein-protein interaction pattern of PTPA.

The yeast PTPA homologs Rrd1 and Rrd2 interact with the conserved Tap42 protein (α4 in humans) and with the type 2A phosphatases (16, 17). Tap42 also interacts with type 2A phosphatases, and this complex is a crucial part in mediating the signal through the TOR pathway, a nutrient-responsive signaling pathway (31–33). Fellner et al. (14) have shown that Rrd2 is necessary for the full and correct
activity of PP2A in yeast and Tap42 seems necessary for correct function of Sit4 (a type 2A phosphatase called PP6 in humans) and PpH21/2 (yeast PP2Ac). Neither Zheng and Jiang (17) nor Van Hoof et al. (16) find it likely that Rrd1/2 carries out its function through a traditional PP2A dimer or a trimer. Recently, Zheng and Jiang (17) suggested that Rrd1 or Rrd2 were a third partner in the Tap42-type 2A phosphatase complex. They propose that the heterotrimeric complex of Tap42-Rrd2-Pph21/2 administers the PP2A activity absent in Rrd1/2-depleted cells and therefore claim that it is a novel heterotrimeric PP2A complex with a distinct substrate specificity.

Therefore, PTPA is likely to form a number of biologically important protein-protein interactions, and the structure of PTPA can now serve as a basis for work directed at defining these interactions. The most obvious candidate protein-protein interaction surface is the highly conserved cleft 2 (Fig. 2). It is possible that this cleft could constitute the binding site for PP2Ac and/or α4. The interaction formed by the Tev-peptide segment with PTPA might model a real peptide interaction in a complex containing PP2Ac and would then constitute the first experimentally derived model for an interaction in a PP2A complex. The structure does not provide any clues to how the protein could catalyze the proposed PPlase reaction, or alternatively, serve as a metal chaperone. However, several fully conserved residues are present in cleft 2 as well as in the disordered loop C located in cleft 2, which should be interesting candidates for site-directed mutagenesis studies directed at defining, or alternatively, discarding, these activities.

In summary, we have described the unique fold of hPTPA where conserved residues are clustered in cleft 2, which along with the peptide binding supports the notion that this cleft constitutes a major surface for interaction with other protein components. The structural data together with binding studies indicate that ATP might not be a cofactor/substrate as previously proposed. Together, this data should provide an excellent starting point for more detailed studies of the structure-function relationship of PTPA.

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REFERENCES
1. Schonthal, A. H. (2001) Cancer Lett. 170, 1–13
2. Janssens, V., and Goris, J. (2001) Biochem. J. 353, 417–439
3. Klumpp, S., and Kriegstein, J. (2002) Curr. Opin. Pharmacol. 2, 458–462
4. Janssens, V., Goris, J., and Van Hoof, C. (2005) Curr. Opin. Genet. Dev. 15, 34–41
5. Sontag, E. (2001) Cell. Signal. 13, 7–16
6. Gallego, M., and Virshup, D. M. (2005) Curr. Opin. Cell Biol. 17, 197–202
7. Arroyo, J. D., and Hahn, W. C. (2005) Oncogene 24, 7746–7755
8. Wang, S. S., Esplin, E. D., Li, J. L., Huang, L. Y., Gazdar, A., Minna, J., and Evans, G. A. (1998) Science 282, 284–287
9. Arnold, H. K., and Sears, R. C. (2006) Mol. Cell Biol. 26, 2832–2844
10. Chen, W., Possemato, R., Campbell, K. T., Plattner, C. A., Pallas, D. C., and Hahn, W. C. (2004) Cancer Cell 5, 127–136
11. Cayla, X., Goris, J., Hermann, J., Hendrix, P., Ozon, R., and Merlevede, W. (1990) Biochemistry 29, 658–667
12. Van Hoof, C., Cayla, X., Bosch, M., Merlevede, W., and Goris, J. (1994) Eur. J. Biochem. 226, 899–907
13. Longin, S., Jordens, J., Martens, E., Stevens, I., Janssens, V., Rondelez, E., De Baere, I., Derua, R., Waefkens, E., Goris, J., and Van Hoof, C. (2004) Biochem. J. 380, 111–119
14. Fellner, T., Lackner, D. H., Hombauer, H., Piribauer, P., Mudrak, I., Zaragoza, K., Juno, C., and Ogris, E. (2003) Genes Dev. 17, 2138–2150
15. Rempola, B., Kaniak, A., Migalski, A., Rytk, J., Slonimski, P. P., and di Rago, J. P. (2000) Mol. Gen. Genet. 262, 1081–1092
16. Van Hoof, C., Martens, E., Longin, S., Jordens, J., Stevens, I., Janssens, V., and Goris, J. (2005) Biochem. J. 386, 93–102
17. Zheng, Y., and Jiang, Y. (2005) Mol. Biol. Cell 16, 2119–2127
18. Van Hoof, C., Janssens, V., De Baere, I., de Witte, J. H., Winderickx, I., Dumortier, F., Thevelein, J. M., Merlevede, W., and Goris, J. (2000) J. Mol. Biol. 302, 103–119
19. Jordens, J., Janssens, V., Longin, S., Stevens, I., Martens, E., Bultynck, G., Engelbergs, Y., Lescrinier, E., Waefkens, E., Goris, J., and Van Hoof, C. (2006) J. Biol. Chem. 281, 6349–6357
20. Kabsch, W. (1993) J. Appl. Crystallogr. 26, 795–800
21. Terwilliger, T. C., and Berendzen, J. (1999) Acta Crystallogr. Sect. D Biol. Crystallogr. 55, 849–861
22. Terwilliger, T. C. (2002) Acta Crystallogr. Sect. D Biol. Crystallogr. 58, 1937–1940
23. Perekis, A., Morris, R., and Lamzin, V. S. (1999) Nat. Struct. Biol. 6, 458–463
24. Emsley, P., and Cowtan, K. (2004) Acta Crystallogr. Sect. D Biol. Cry- stallogr. 60, 2126–2132
25. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Acta Crystallogr. Sect. D Biol. Crystallogr. 53, 240–255
26. Pantoliano, M. W., Petrella, E. C., Kwasnoski, J. D., Lobanov, V. S., Myslik, J., Graf, E., Carver, T., Asel, E., Springer, B. A., Lane, P., and Salemme, F. R. (2001) J. Mol. Biol. 310, 429–440
27. Holm, L., and Sander, C. (1993) J. Mol. Biol. 233, 123–138
28. Laskowski, R. A., Watson, J. D., and Thornton, J. M. (2005) J. Mol. Biol. 351, 614–626
29. Fanghanel, J., and Fischer, G. (2004) Front. Biosci. 9, 3453–3478
30. Janssens, V., Van Hoof, C., Martens, E., De Baere, I., Merlevede, W., and Goris, J. (2000) Eur. J. Biochem. 267, 4406–4413
31. Di Como, C., and Arndt, K. (1996) Genes Dev. 10, 1904–1916
32. Beck, T., and Hall, M. N. (1999) Nature 402, 689–692
33. Inoki, K., Ouyang, H., Li, Y., and Guan, K. L. (2005) Microbiol. Mol. Biol. Rev. 69, 79–100
34. Wang, H., Wang, X., and Jiang, Y. (2003) Mol. Biol. Cell 14, 4342–4351
35. Gouet, P., Robert, X., and Courcelle, E. (2003) Nucleic Acids Res. 31, 3320–3323