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SHP family protein tyrosine phosphatases adopt canonical active-site conformations in the apo and phosphate-bound states

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Abstract

Protein tyrosine phosphatase (PTP) catalytic domains undergo a series of conformational changes in order to mediate dephosphorylation of their tyrosine phosphorylated substrates. An important conformational change occurs in the Tryptophan-Proline-Aspartic acid (WPD) loop, which contains the conserved catalytic aspartate. Upon substrate binding, the WPD loop transitions from the 'open' to the 'closed' state, thus allowing optimal positioning of the catalytic aspartate for substrate dephosphorylation. The dynamics of WPD loop conformational changes have previously been studied for PTP1B, HePTP, and the bacterial phosphatase YopH, however, have not yet been comprehensively studied for the non-receptor tyrosine phosphatase SHP-1 (PTPN6). However, there is no characterization of the extent of WPD loop movement between open and fully closed states. To structurally describe the changes in WPD loop conformation, we have determined the 1.4 Å crystal structure of the catalytic domain of SHP-1 in the Apo state and the 1.8 Å crystal structure of the SHP-1 catalytic domain in complex with a phosphate ion. We provide structural analysis for the WPD loop closed state of SHP phosphatases and the conformational changes that occur upon WPD loop closure.

Keywords

protein tyrosine phosphatase; WPD loop; conformational changes; cytokine signaling

INTRODUCTION

The regulation of phosphorylation events is critical for normal signal transduction and cellular function. Protein tyrosine kinases and protein tyrosine phosphatases (PTPs) maintain the balance of cellular phosphotyrosine levels by catalyzing the addition or removal, respectively, of phosphate groups within their phosphorylated substrates. The Src Homology-2 (SH2) domain containing family of phosphatases (SHP) are cytoplasmic PTPs that play regulatory roles in cytokine and growth factor signaling, as well as cell adhesion and phagolysosome maturation [1–3]. The domain architecture of SHP family phosphatases consist of two tandem N-terminal SH2 domains, which act as both autoinhibitory and targeting domains, followed by the catalytic domain and a C-terminal tail [4]. In vertebrates, the SHP family is comprised of two phosphatases: SHP-1 and SHP-2. SHP-1 is predominantly expressed in hematopoietic cells and mainly acts as a down-regulator of signal transduction. Conversely, SHP-2 is ubiquitously expressed and has been mostly associated with upregulation of signaling events [5].

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The use of small molecule aryl phosphate analogs has proved useful for the elucidation of the mechanism of tyrosine dephosphorylation by PTPs [6]. PTP catalyzed dephosphorylation is a cysteine dependent reaction and consists of the formation of a covalent phospho-enzyme intermediate that is subsequently hydrolyzed by water [6–8]. Within the catalytic domain, the PTP signature motif (H/V)CX₅R(S/T) harbors the catalytic cysteine and is responsible for the recognition of the phosphotyrosine phosphoryl group. In addition to cysteine, the conserved aspartic acid and glutamine residues within the WPD loop motif (WPD_XGXP) and Q loop motif (Q_{TXX}QYXF), respectively, play key roles in substrate dephosphorylation [9] (Figure 1).

The WPD loop is a dynamic motif and undergoes conformational changes upon substrate binding, allowing for optimal positioning of the conserved aspartate for catalysis [10]. Prior to substrate binding to the PTP signature motif, the WPD loop is in the denoted “open” state, where it is oriented away from the catalytic pocket. Upon substrate binding, the WPD loop moves toward the catalytic pocket, bringing the catalytic aspartic acid in close proximity to the substrate. This conformation is termed the WPD loop “closed” state.

The dephosphorylation reaction is initiated by the nucleophilic attack of the catalytic cysteine to the phosphotyrosine phosphorus atom. At this step, the conserved aspartate residue facilitates the release of the leaving group (i.e. the tyrosine residue) by donating a proton and stabilizing its charge. A phospho-enzyme intermediate is generated as the phosphoryl group is transferred from the substrate to the catalytic cysteine. The conserved aspartate then behaves as a base and activates a water molecule by abstracting a proton. The activated water molecule acts as a nucleophile and hydrolyzes the phospho-enzyme intermediate, resulting in the liberation of inorganic phosphate and enzyme regeneration [6, 8, 11].

Structural analysis of phosphatase domain structures in both the WPD loop open and closed states have yielded descriptions of WPD loop conformational changes in PTP1B [12, 13], HePTP [14], and bacterial phosphatase YopH [15]. However, for the SHP family of phosphatases, there is currently not a structural description of the WPD loop transition from the ‘open’ to ‘closed’ state. Here we present a structural description of the open and closed states of the WPD loop in SHP-1 phosphatase. We have determined structures of the catalytic domain of SHP-1 in the Apo state to 1.4 Å resolution, as well as in complex with a phosphate ion, an analog of the phosphotyrosine phosphoryl group, to 1.8 Å resolution. Our studies provide the first analysis of the WPD loop closed state in SHP-1 protein tyrosine phosphatase.

MATERIALS AND METHODS

Protein Expression and Purification

The catalytic domain (residues 243–530) of human SHP-1 was subcloned into a modified pET-32 expression vector and expressed in Rosetta (DE3) *Escherichia coli*. SHP-1 was expressed as an N-terminal hexahistidine (6xHis) fusion protein by overnight induction with 0.5 mM isopropyl 1-thio-β-D-galactopyranoside at 16°C. Cell pellets were resuspended in lysis buffer (20 mM Tris pH 8.5, 200 mM NaCl, 1.5 mg/mL lysozyme) supplemented with protease inhibitors, and lysed by repetitive freeze/thaw cycles and sonication. Lysate was cleared by centrifugation at 20,000 rpm for 1 hour at 4°C. The affinity tag was excised by overnight incubation at 4°C with TEV protease. In order to remove remaining 6xHis-SHP-1 and 6xHis-TEV, a second Ni-NTA affinity chromatography step was performed. Untagged SHP-1 was further purified by anion exchange chromatography using a Resource Q column (GE). 6xHis-tagged SHP-1 was purified over a Ni-NTA FF affinity column, followed by

anion exchange chromatography using a Resource Q column (GE). SHP-1-C453S was generated using the QuikChange Lightning Site-Directed Mutagenesis Kit (Stratagene).

Peptide preparation—A phosphopeptide corresponding to the JAK1 activation loop, an SHP-1 physiological substrate, with sequence K¹⁰³²EpYpYTV was synthesized, HPLC purified and lyophilized by Tufts University Core Facility. The peptide was resuspended in 100 mM Tris pH 8.0, 100 mM NaCl buffer.

Crystallization—For the two crystal forms described in this study, sparse matrix and grid screens (Qiagen and Hampton Research) were set with a Matrix Hydra-II eDrop crystallization robot (Thermo Scientific) [16] in order to identify initial crystallization conditions. Crystal quality was optimized by micro-seeding [17]. SHP-1_{APO}: Purified SHP-1 wild-type was buffer exchanged to 20 mM Tris pH 8.0, 50 mM NaCl, 1 mM TCEP and concentrated to 10 mg/mL. Initial crystals were obtained in 0.1 M HEPES pH 7.5, 1.4 tri-sodium citrate. Crystals were then optimized and improved to diffraction-quality using a precipitant of 0.1 M HEPES pH 7.5, 1.0 M tri-sodium citrate, 2–4% 1-propanol by the hanging drop method at room temperature. SHP-1_{PO4}: Purified 6xHis SHP-1-C453S was buffer exchanged to 20 mM Tris pH 8.0, 50 mM NaCl, 1 mM TCEP and concentrated to 15 mg/mL. 6xHis SHP-1-C453S was incubated on ice for ~1 hour with JAK1 activation loop peptide at a 1:3 protein:peptide ratio prior to crystallization. Initial crystals were obtained in 0.1 M HEPES pH 7.5, 20 mM MgCl₂, 22% sodium polyacrylate 5100. Crystals were then optimized and improved to diffraction-quality using a precipitant of 0.1 M HEPES 7.5, 20 mM MgCl₂, 28% sodium polyacrylate 5100 by the hanging drop method at room temperature.

Crystallography—X-ray diffraction data were collected on beamlines X25 (for SHP-1_{APO}) and X6A (for SHP1:PO₄) of the National Synchrotron Light Source (NSLS) (Table 1). All data were processed using HKL2000 [18]. Initial phases were obtained for each structure by molecular replacement using Phaser [19] with the SHP-1 phosphatase domain (PDB ID: 1FPR) [20] as the search model. Each structure was then autobuilt using ARP/wARP [21] to mitigate model bias. Standard refinement and model building were conducted using Phenix [22] and COOT [23]. The final model was validated using MolProbity [24]. Structures have been deposited to the PDB with accession codes: 4HJP and 4HJQ for SHP-1_{APO} and SHP-1_{PO4}, respectively.

Hydrogen bond analysis—Active site hydrogen bonds (Table 2) were calculated with CCP4 Molecular Graphics [25], using the default parameters: minimum donor-acceptor distance = 2.00 Å, maximum donor-acceptor distance = 3.90 Å, maximum hydrogen atom acceptor distance = 2.50 Å, minimum neighbor-donor-acceptor angle = 90.00°, donor-acceptor-neighbor angle = 90.00°, minimum hydrogen atom-acceptor-neighbor angle = 90.00°, and minimum donor-hydrogen atom-acceptor angle = 90.00°.

RESULTS AND DISCUSSION

Crystal structure of the catalytic domain of SHP-1 in the Apo state

We determined the crystal structures of the catalytic domain of SHP-1 in the Apo state (denoted SHP-1_{APO}) and in complex with a phosphate ion to 1.4 Å and 1.8 Å resolution, respectively (Table 1). It had been previously reported that the WPD loop in Apo SHP-1 adopts an atypical half-open/half-closed conformation, where the catalytic aspartic acid, D419, occupies the catalytic pocket [26]. In contrast, our SHP-1_{APO} structure reveals the WPD loop in an open conformation with an accessible catalytic pocket (Figure 2A). Interestingly, in this crystal structure the carbonyl oxygen atom of D419 makes contacts

with symmetry-related molecules suggesting that these interactions stabilize this WPD loop open conformation (**Figure S1**).

Structural description of the SHP-1 WPD loop closed state

The structure of SHP-1 in complex with a phosphate ion (denoted SHP-1_{PO4}) contains two chains in the asymmetric unit. These chains are highly similar with root-mean-square deviations (r.m.s.d.) between them of 0.54 Å over 281 Cα (Figure 2B). Each chain contains one phosphate ion bound at the catalytic pocket. In the SHP-1_{PO4} structure, the WPD loop in the SHP-1_{PO4} structure is displaced 3.8 Å away from its position in the SHP-1_{APO} (open state) structure, as determined from the difference of the distance between residues H420 and G456 in the WPD loop open and closed states [27] (Figure 2C). This conformation of the WPD loop corresponds to the WPD loop closed state and is representative of a substrate bound conformation of the enzyme. Although the WPD loop is a conformationally dynamic region of the PTP fold, in both SHP-1_{APO} and SHP-1_{PO4} structures we observe strong electron density for the WPD loop (Figures 2D–E). Previously published co-crystal structures of SHP phosphatases with tungstate, sulfate, and phosphorylated substrate show the WPD loop in the open state [20, 26, 28]. The SHP-1_{PO4} structure, then, provides the first description of the WPD loop closed state for the SHP family of phosphatases.

N-terminal α2' helix conformation

Upon comparison of the SHP-1_{APO} and SHP-1_{PO4} structures, we noticed two distinct conformations of the N-terminal α2' helix (Figure 2C). In the SHP-1_{APO} structure the α2' helix is extended away from the catalytic domain and participates in crystal contacts with symmetry mates (Figure 3A), whereas the α2' helix in SHP-1_{PO4} packs against the α6 helix and the α5-α6 loop of the catalytic domain (Figure 3B). The conformation of the α2' helix we observe in the SHP-1_{PO4} structure is also observed in the full length structures of SHP phosphatases [28–30], suggesting that this is a biologically relevant orientation. We therefore infer that the apparent conformational flexibility of the α2' helix arises from crystallization of this shorter SHP-1 catalytic domain construct.

Concerted movement of PTP signature motif R459 and WPD loop W417 upon loop closure

WPD loop movement is critical for the effective dephosphorylation of substrates [8, 12, 31, 32]. In the WPD loop open state found in the SHP-1_{APO} structure, the conserved R459 from the PTP signature motif is in an extended conformation and makes a salt bridge with the highly conserved E355 in the E loop (β5-β6 loop), a contact previously described as important for maintaining the E loop's stability [14]. The aliphatic portions of R459 also make van der Waals contacts with the conserved WPD loop tryptophan, W417 (Figure 4A). These interactions are well conserved among other PTPs [12, 14, 33, 34].

Upon phosphate ion binding, as observed in the SHP-1_{PO4} structure, the side chain of R459 rotates towards the catalytic pocket. In this altered conformation R459 hydrogen-bonds to E355, the backbone carbonyl of W417 and two of the phosphate ion oxygen atoms (Figures 4B–C). R459 still maintains van der Waals contacts with the indole ring of W417. This conformational change is coupled with W417 positioning into a hydrophobic pocket (Figures 4D–F) defined by residues Y414, V429, I463, F507, P423, T462, and the aliphatic portion of R459 [6, 12, 35]. We note that I463 changes conformation upon WPD loop closure, potentially to accommodate W417 in the closed state. Positioning of the conserved tryptophan residue into this hydrophobic pocket is important for the transition of the WPD loop into a catalytically competent conformation. Accordingly, mutation of this residue in PTP1B and YopH results in lower phosphatase activity due to impaired WPD loop closure [12, 36]. Also, it is worth noting that in the SHP-1 structures in complex with phosphorylated substrate peptide [20] or with sulfate [28], R459 remains in the extended

conformation and does not contact the carbonyl oxygen of W417. This contrasts with the conformation of R459 in our SHP-1_{PO4} structure and provides a possible explanation as to why the WPD loop maintains an open conformation in these substrate-bound SHP-1 structures. Thus, the R459/W417 interaction plays an important role in facilitating and stabilizing the WPD loop closed conformation.

Stabilization of WPD loop closed state

In addition to the interactions between R459 and W417, other contacts contribute to maintaining the WPD loop in the closed conformation (Figures 5A–C and Table 2). E loop residue E355, which makes two hydrogen bonds with R459 in the open state (Figure 5A), maintains a single contact with R459 and also makes hydrogen bonds with the backbone amide of D419 in the closed state. In addition, residue K360 in the E loop makes a salt bridge with the side chain of D419 (Figure 5C). This interaction is conserved in other PTPs in the WPD loop closed state [14].

The WPD loop closed state is also stabilized by the residue following the catalytic aspartic acid [26]. In PTP1B, F122 stabilizes the enzyme/substrate intermediate by making contacts with the phenyl ring in the substrate phosphotyrosine (Figure 5B) [34]. YopH, contains a glutamine residue at this position (Q357), which is expected to stabilize the closed conformation by making contacts with the phosphoryl moiety of phosphotyrosine [37]. In contrast, in SHP-1, the residue following D419 is a histidine, H420. In the closed state we observe H420 to make hydrogen bonds with the conserved Q500, which orients the catalytic water in the second step of the reaction (Figure 5C). These interactions may be important for the proper orientation of the catalytically important Q500, which in the open state points away from the catalytic pocket.

SHP-1_{PO4} resembles the second transition step of tyrosine dephosphorylation

The mechanism of tyrosine dephosphorylation takes place in two steps. In the first step, a covalent phospho-cysteine intermediate is formed upon nucleophilic attack by the cysteine thiolate group. The catalytic aspartate (D419) acts as an acid at this stage, by donating a proton to the leaving group. The second step involves the hydrolysis of this covalent intermediate. In this step of the reaction, the aspartate acts as a base by abstracting a proton from a water molecule [6–8]. In the SHP-1_{PO4} structure, the WPD loop is in the closed conformation allowing the conserved D419 to contact the active site water, also coordinated by Q500 and Q504. The oxyanion does not engage in contacts with the catalytic aspartate, but instead is coordinated by hydrogen bonding with the PTP signature motif backbone and by the side chain of S453 and R459 (Figure 5C). The SHP-1_{PO4} structure then, thus resembles the second stage of tyrosine dephosphorylation.

CONCLUSIONS

In this study we describe the conformational changes that occur within the WPD loop of SHP-1 upon binding of a phosphate ion, an analog of the phosphotyrosine phosphoryl group. Previous studies have proposed that SHP-1 adopts a WPD loop open conformation upon substrate binding [20]. The WPD loop in these structures, however, may have been stabilized in an open conformation due to interactions with symmetry mates in the crystal. Here, we have determined the crystal structures of the catalytic domain of SHP-1 in both the WPD-loop open and WPD loop closed states. Our WPD loop open state structure (SHP-1_{AP0}) captures a different conformation of the open state than previously published [26] that is similar to open conformations of PTP1B and YopH [38, 39]. The dynamic nature of this critical regulatory loop allows it to acquire different conformations, indeed, molecular dynamics simulations for the YopH WPD loop indicate that it oscillates between

the open and closed state with a frequency of ~3.8 ns [40]. The WPD loop open state reported here corresponds to an SHP-1 conformation that is competent for substrate binding.

Our studies for the first time present a structural description of WPD loop transition from the open to the closed state for an SHP family phosphatase. Previously, SHP-1 structures in complex with oxyanions such as sulfate and tungstate ions show that the WPD loop remains in the open state [14, 26]. Here we describe an SHP-1 structure in which phosphate ion stabilizes the WPD loop closed state. We find that the conformation of the SHP-1 closed state and the interactions that stabilize it are similar to those in other PTPs and resembles the second transition state of the dephosphorylation reaction [34]. We also identified an interaction within the WPD loop H420 and the catalytic Q500 that might be important for orientation of Q500 as well as for stabilizing the WPD loop closed state. A similar interaction is conserved in the HePTP closed state between residues H237 and Q314 [14, 41], but not in PTP1B or YopH because these proteins contain phenylalanine (F122) and glutamine (Q357) residues, respectively, at the site corresponding to H420. We expect the mechanism of WPD loop closure in SHP-2 to be similar to that of SHP-1 as the residues that mediate and stabilize the transition are conserved in both SHP family members. These structures therefore define the extent of conformational movement that can occur in the WPD loop for the SHP family members.

Our analysis also highlights the importance of phosphate ion binding at the active site in WPD loop closure. While the WPD loop oscillates between the open and closed states [40], the presence of a phosphate ion at the active site stabilizes the WPD loop closed state by inducing conformational changes in key residues, concomitantly altering the local hydrogen bonding network (Table 2). Notably, phosphate ion binding alters the orientation of the conserved R459 in the PTP motif, which allows for its interaction with residue W417 in the WPD loop, thus assisting its positioning into the hydrophobic pocket formed by residues Y414, V429, I463, F507, P423, T462, and the aliphatic portion of R459. Accommodation of W417 into this hydrophobic pocket is critical to the stabilization of the WPD loop closed state and, consequently, to phosphatase activity [12, 36].

Finally, protein tyrosine phosphatases involved in the negative regulation of immune cell response are emerging as targets for cancer therapy, generating a significant interest in the development of PTP specific inhibitors. Sodium stibogluconate (SSG), an SHP-1 specific inhibitor [42], has shown anti-tumor activity by enhancing the activation of the immune response induced by interferon alpha (IFN α). Phase I clinical trials for SSG in combination with IFN α as treatment of advanced malignancies show that this combination therapy indeed increases immune response in patients with IFN α responsive cancers [43, 44]. A more potent SHP-1 targeted inhibitor, tyrosine phosphatase inhibitor-1, in combination with IFN α , has also been shown to inhibit tumor growth in melanoma and colon cancer mouse models [45], becoming a promising lead for cancer treatment. SHP-1 is therefore an attractive target for improving cancer immunotherapy and our Apo and phosphate-bound structures will aid *in silico* structure-based drug discovery by providing guide-points for the extent of conformational changes that occur in the catalytic pocket of SHP-1.

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Figure 1. Crystal structure of the PTP catalytic domain

The crystal structure of the PTP catalytic domain is shown to illustrate conserved catalytic motifs. These have been highlighted and are indicated by arrows. The catalytic cysteine is indicated by a yellow sphere within the PTP Signature Motif. SHP-1_{PO4} (PDB ID: 4HJQ) is shown.

Figure 2. Crystal structure of the SHP-1 catalytic domain in WPD loop open and WPD loop closed conformations

A) Structure of Apo SHP-1 (SHP-1_{APO}) is in the WPD loop open conformation. **B)** Structure of phosphate bound SHP-1 (SHP-1_{PO4}) in the WPD loop closed conformation. **C)** Superposition of the SHP-1_{APO} and SHP-1_{PO4} structures. The distance of WPD loop displacement between open and closed states is indicated. **D)** The $2F_{\text{obs}} - F_{\text{calc}}$ map for the WPD loop in the open conformation contoured at $\sigma = 1.0$ is shown. **E)** The $2F_{\text{obs}} - F_{\text{calc}}$ map for the WPD loop in the closed conformation contoured at $\sigma = 1.0$ is shown.

Figure 3. N-terminal $\alpha 2'$ helix conformation in SHP-1_{APO} structure

A) The conformation of the N-terminal $\alpha 2'$ helix in SHP-1_{APO} is stabilized by crystal contacts with symmetry mates. Symmetry mates are displayed in different colors (orange and gray). **B)** Superposition of autoinhibited full-length SHP-1 (2B3O, green), non-inhibited full-length SHP-1 (3PS5, cyan), autoinhibited full-length SHP-2 (2SHP, magenta), SHP-1_{PO4} (4HJQ, light blue), and SHP-1_{APO} (4HJP, orange). Inset shows a close-up view of helix $\alpha 1'$ and helix $\alpha 2'$.

Figure 4. Concerted movement of Trp417 and Arg459 during WPD loop closure
View of the catalytic pocket shows residues W417 and R459 and their interactions in the **A)** WPD loop open and **B)** WPD loop closed state. **C)** Stereoview of the superposition of the open and closed state is shown. Hydrogen bonds in the open state are colored orange. **D–F)** Residues surrounding W417 are represented as spheres to highlight the hydrophobic pocket in the **D)** WPD loop open state and **E)** WPD loop closed state. **F)** Top view of the superposition of WPD loop open (orange) and closed (blue) states shows W417 movement towards the hydrophobic pocket. Residues making up the hydrophobic pocket are labeled.

Figure 5. SHP-1_{pO4} resembles the second step of the dephosphorylation reaction

View of the catalytic pocket of SHP-1 and PTP1B at different steps of the dephosphorylation reaction. **A)** SHP-1_{APO} represents the open state, prior to substrate binding. **B)** PTP1B in complex with substrate peptide [34] (PDB ID: 3I7Z) captures the first transition state. WPD loop is in the closed state and D121 is interacting with the phenolic oxygen in Y1016. Q262 is not oriented towards the catalytic pocket. **C)** SHP-1_{pO4} structure resembles the second transition state as the WPD loop is in the closed conformation and both D419 and Q500 interact with the catalytic water. Residues discussed in the text are labeled.

TABLE 1

Data collection and refinement statistics.

Crystal PDB ID	SHP-1 _{APO} 4HJP	SHP-1 _{PO4} 4HJQ
Data collection		
Space group	<i>P</i> 2 ₁ 2 2 ₁	<i>P</i> 1
Cell		
a, b, c (Å)	45.5, 57.4, 110.8	45.6, 57.8, 77.3
α, β, γ (°)	90.0, 90.0, 90.0	102.4, 94.7, 110.0
X-ray Source	NSLS X25	NSLS X6A
Wavelength (Å)	1.1	0.9801
Resolution (Å) ^a	50.0-1.4 (1.45-1.40)	30-1.80 (1.86-1.80)
Total reflections	342191	124997
Unique reflections	57659	63496
Completeness (%) ^a	99.4 (98.2)	96.6 (95.4)
<i>R</i> _{merge} (%) ^a	5.0 (75.6)	5.3 (37.3)
<i><I/σ(I)></i> ^a	29.8 (2.2)	13.1 (2.2)
Multiplicity ^a	5.9 (5.6)	2.0 (2.0)
Wilson <i>B</i> -factor (Å ²)	20.2	17.0
Refinement statistics		
Resolution (Å) ^a	42.0-1.40 (1.42-1.40)	28.9 - 1.80 (1.83-1.80)
<i>R</i> factor (%) ^a	16.4 (47.5)	16.5 (28.0)
Free <i>R</i> factor (%) ^a	18.6 (52.3)	20.2 (34.1)
Free <i>R</i> reflections (%) ^a	5.1 (5.1)	5.1 (5.2)
No of free <i>R</i> reflections	2910	3216
Molecules in asymmetric unit	1	2
Residue range built	A/243-523	A/243-523
	-	B/243-523
Ions built	-	A/PO ₄
	-	B/PO ₄
No. of non-H atoms	2694	5334
No. of water molecules	280	682
Model quality		
R.m.s.d. bond lengths (Å)	0.01	0.007
R.m.s.d. bond angles (Å)	1.345	1.021
Mean <i>B</i> -factors (Å ²)		
Overall	29.1	23.2
Protein atoms		

Crystal PDB ID	SHP-1 _{APO} 4HJP	SHP-1 _{PO4} 4HJQ
Chain A/Chain B	23.2	24.0/22.4
PO ₄	-	11.5
Water	35	35
Ramachandran plot (%)		
Favored/Allowed/Disallowed	97.7/2.0/0.3	97.8/1.9/0.4

^a values in parentheses indicate values for the high resolution shell

Table 2

SHP-1 active site hydrogen bonds in the WPD loop open and closed states

SHP-1 _{apo} – WPD loop open				SHP-1 _{po4} –WPD loop closed			
Residue	Atom	Distance (Å)	Atom	Residue	Atom	Distance (Å)	Atom
T351	OG1	2.84	CD	E355	T351	OG1	CD
R352	N	3.00	CD	E355	R352	N	CD
E355	N	3.08	C	R358	E355	N	C
R358	N	3.04	C	E355	R358	N	C
W417	NE1	2.94	C	G421	W417	NE1	C
H420	ND1	3.43	CG	D419	D419	N	CD
G421	N	3.38	C	P418	H420	ND1	CD
G456	N	3.34	C	C453	G421	N	C
	N	3.29	CB:A ^a	C453	S453 ^b	OG	CB
I457	N	3.86	CB:A	C453	OG	OG	P
R459	N	3.77	CB:B	C453	S454	N	P
	NH1	2.93	C	T350	A455	N	P
	NH1	3.00	CD	E355	G456	N	P
	NH2	3.28	C	P418	I457	N	P
	NH2	2.92	CD	E355	G458	N	P
T460	N	3.70	CB:A	C453	R459	N	P
	N	3.27	CB:B	C453	NE	NE	P
G461	N	3.28	C	G458	NH1	NH1	C
	N	3.14	C	I457	NH1	NH1	C
V499	N	2.93	C	I457	NH2	NH2	P
Q500	N	2.83	CD	Q504	T460	N	CB
T501	N	3.01	CD	Q504	G461	N	C
A503	N	3.52	CB	T501	V499	N	C
Q504	N	3.18	C	T501	Q500	N	CD
	N	3.06	CB	T501	T501	N	CD

SHP-1 _{APO} – WPD loop open				SHP-1 _{PO4} –WPD loop closed			
Residue	Atom	Distance (Å)	Atom	Residue	Atom	Distance (Å)	Residue
				A503	N	3.40	T501
				Q504	N	3.10	T501
					N	3.06	T501
				NE2		3.07	G421

Hydrogen bonds that are broken and formed upon WPD loop closure are highlighted in red and green, respectively.

^a Atoms labeled CB:A and CB:B correspond to CB in alternate conformations.

^b SHP-1PO4 contains a C453S mutation.