The X-ray Crystal Structures of Yersinia Tyrosine Phosphatase with Bound Tungstate and Nitrate

MECHANISTIC IMPLICATIONS*

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X-ray crystal structures of the Yersinia tyrosine phosphatase (PTPase) in complex with tungstate and nitrate have been solved to 2.4-Å resolution. Tetrahedral tungstate, WO₄²⁻, is a competitive inhibitor of the enzyme and is isosteric with the substrate and product of the catalyzed reaction. Planar nitrate, NO₃⁻, is isosteric with the PO₃ moieties of a phosphotransfer transition state. The crystal structures of the Yersinia PTPase with and without ligands, together with biochemical data, permit modeling of key steps along the reaction pathway. These energy-minimized models are consistent with a general acid-catalyzed, in-line displacement of the phosphate moiety to Cys⁴⁰³ on the enzyme, followed by attack by a nucleophilic water molecule to release orthophosphate. This nucleophilic water molecule is identified in the crystal structure of the nitrate complex. The active site structure of the PTPase is compared to alkaline phosphatase, which employs a similar phosphomonoester hydrolysis mechanism. Both enzymes must stabilize charges at the nucleophile, the PO₃ moiety of the transition state, and the leaving group. Both an associative (bond formation preceding bond cleavage) and dissociative (bond cleavage preceding bond formation) mechanism were modeled, but a dissociative-like mechanism is favored for steric and chemical reasons. Since nearly all of the 47 invariant or highly conserved residues of the PTPase domain are clustered at the active site, we suggest that the mechanism postulated for the alkaline phosphatase enzyme is applicable to all the PTPases.

Phosphorylation of tyrosine residues of intracellular or membrane proteins is a fundamental cellular signal for regulating cell growth, differentiation, and proliferation (1). The levels of phosphotyrosine in the cell are governed by the competing actions of protein tyrosine kinases and protein tyrosine phosphatases (PTPases; 1 EC 3.1.3.48).

A variety of receptor-like and nonreceptor-like PTPases is found in all eukaryotic cells. Presumably, this molecular diversity provides specific PTPases for different signal transduction pathways that can occur simultaneously in the cell. Although few PTPases have been assigned specific roles in signaling pathways, much is known about PTPase biochemistry, largely from studies of a homologous enzyme identified in Yersinia (2). The Yersinia PTPase, encoded on a virulence plasmid, is required for pathogenicity in this bacterium (3).

The PTPase reaction proceeds in two steps. First, the phosphate is transferred from tyrosine (R) to a functional group on the enzyme (I) and then the phosphoenzyme intermediate is hydrolyzed (II) (4), in a manner similar to the well studied alkaline phosphatase (5) (Reaction 1).

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\text{PTPase} \text{R} \to \text{PTPase} \text{RP} \to \text{PTPase} \text{R} + \text{P} \text{I}
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Mutagenesis and biochemical studies have identified several invariant residues in the PTPase signature sequence, (I/V)HXXGXXH(5/6)TG, important for phosphotyrosine hydrolysis. The phosphotyrosine is transferred to the thiol of Cys⁴⁰³ (4),² the pKₐ of which is lowered 2.7 pH units by His⁴⁰² (6) and 0.6 pH units by Thr⁴¹⁰ (7). Arg⁴⁰⁹ is critical for ligand binding and catalysis (8). Of invariant residues outside of the signature sequence, Asp⁴⁰⁶ was identified as a putative general acid and Glu⁴⁰⁷ was identified as a putative general base on the basis of mutagenesis and pH rate profile experiments (9).

The PTPases, unlike alkaline phosphatase, are very substrate-specific, selecting phosphotyrosines over the other phosphorylated residues (1). In addition, the Kₘ and kₗₐₜ are greatly improved for the PTPases when using a phosphotyrosine-containing peptide as opposed to phosphotyrosine by itself (10).

Our laboratory recently reported the x-ray crystal structure of the unliganded Yersinia PTPase to 2.5-Å resolution and the complex with tungstate, WO₄²⁻, to 2.6-Å resolution (11). We have also solved the structure of a Cys⁴⁰³ → Ser mutant complexed with sulfate, SO₄²⁻ (12). The PTPase is an α+β protein with an eight-stranded, mixed β-sheet. The central feature of the structure is a strand-loop-helix motif where the loop contains the PTPase signature sequence, including the Cys⁴⁰³ nucleophile. Oxygen ions bind within this loop, which we have

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² The abbreviations used are: PTPase, protein tyrosine phosphatase; PTPase-WO₄, the crystal structure of the Yersinia PTPase in complex with tungstate; PTPase-NO₃, the crystal structure of the nitrate complex; PTPase-SO₄, the crystal structure of the Cys⁴⁰³ → Ser mutant of the Yersinia PTPase in complex with sulfate; WAT, a hypothetical nucleophilic water molecule used in modeling the reaction.

³ Residues are numbered as in the Yersinia PTPase protein sequence throughout the text.
termed the phosphate binding or P-loop. Three of the anion oxygens are coordinated by main chain amides of the P-loop while the fourth, apical, anion oxygen (O-4) points away from the P-loop. Asp$^{356}$, the putative general acid, is located on a movable loop, termed the WpD-loop (for the conserved Trp-Pro-Asp sequence). Upon binding of an anion, the carbonyl group of Asp$^{356}$ shifts 8 Å toward the active site and this apical oxygen (12).

To better understand the roles of the conserved residues and the specificity of this enzyme, we have refined the tungstate complex structure to a higher resolution, 2.4 Å. Tungstate, a tight competitive inhibitor ($K_I = 61 \mu M$ at pH 7.0; Ref. 8), is tetrahedral, like the phosphate moieties of the substrate (phos-photorysine) and product (orthophosphate). One of the oxygens of the orthophosphate product is derived from a water molecule. Thus, in any crystal structure of the PTPase in complex with a tetrahedral oxyanion, one of the oxygens of the ligand will be analogous to the water-derived oxygen of the orthophosphate product. In the hopes of observing the nucleophilic water molecule prior to attack, crystals were grown in the presence of nitrate, NO$_3^-$, a trigonal planar anion. Nitrate is also iso-

Energy-minimized models of reaction intermediates that are consistent with the known biochemistry were derived from these crystal structures. These models highlight residues that are likely to be of key importance in the hydrolysis. The models also place certain limits on the nature of the reaction mechanism.

**EXPERIMENTAL PROCEDURES**

**Structure Solution—**The catalytic domain (residues 163–468) of Yop51, a PTPase from Yersinia enterocolitica, was expressed and purified as described previously (13). Plate-like crystals of the PTPase-WO$_4^-$ complex (space group $P2_12_12_1$, $a = 56.3 \AA$, $b = 49.8 \AA$, $c = 100.6 \AA$; typical size $0.8 \times 0.2 \times 0.02 \text{mm}^3$) were grown by vapor diffusion by mixing equal volumes of a PTPase solution (20 mg/ml) containing 1 mg/mL sodium tungstate, with precipitant (27% (w/v) polyethylene glycol 4000, 200 mg/mL Li$_2$SO$_4$, 10% $\beta$-mercaptoethanol, 0.1% diethylpyrocarbonate, and 100 mM Tris-HCl, pH 8.5). The crystals diffract to at least 2.0 Å, and 9875 reflections (89% completeness) between 7.0 and 2.4 Å were recorded from 4 crystals (Table I). Isomorphous crystals of the PTPase-NO$_3^-$ complex ($P2_12_12_1$, $a = 56.7 \AA$, $b = 49.7 \AA$, $c = 99.5 \AA$; size $0.8 \times 0.4 \times 0.06 \text{mm}^3$) were grown by mixing equal volumes of PTPase solution (23 mg/ml) with precipitant (27% (w/v) polyethylene glycol 1500, 10% 2-methyl-2,4-pentanedioiol, 0.1% $\beta$-mercaptoethanol, 220 mg/mL sodium nitrate, and 10 mM imidazole, pH 7.2). For the PTPase-NO$_3^-$ complex, 9205 reflections (84% completeness) between 7.0 and 2.4 Å were collected from a single crystal (Table I).

Diffraction intensities were collected on a SDMX multiwire area detector system mounted on a Rigaku RU-200 rotating anode generator (50 kV, 100 mA), integrated (14), and scaled (15). The PTPase-WO$_4^-$ amplitudes (and those from a mercury derivative) were used to phase data from isomorphous crystals of the Yersinia PTPase Cys$_{403}$ → Ser mutant containing bound sulfate, PTPase-SO$_4^-$ (11). After much of the Cys$_{403}$ → Ser structure had been traced, a tetrahedral tungstate ion was fit into a difference electron density map and the model (residues 186–468) was further refined with X-PLOR (16) against the PTPase-WO$_4^-$ amplitude data. The first 23 residues of the polypeptide chain are not visible and are presumed disordered. Force field interactions between the tungstate and Cys$_{403}$ were turned off to allow close approach of these two groups if necessary. An overall anisotropic B-factor was applied. One residue (Cys$_{356}$) has been modeled with two alternate conformations. The PTPase-WO$_4^-$ structure currently has a crystallographic R-factor of 17.9% for all non-zero reflections between 7 and 2.4 Å (Table I). One hundred forty-three crystallographically observed reflections (89% completeness) between 7 and 2.4 Å were recorded from 7 to 2.4 Å.

**Atomic Coordinates for PTPase-WO$_4^-$ and PTPase-NO$_3^-$** have been submitted to the Brookhaven Protein Data Bank (17) (entry codes 1YTW and 1YTN, respectively). The coordinates for the unliganded enzyme and for PTPase-SO$_4^-$ are from Protein Data Bank entries 1YPT (11) and 1YTS (12), respectively.

**Resulting Reaction Intermediates—**All charge, bond-length, and angle parameters for the ligands were derived from reported small molecule structures (18–24) or published quantum mechanical calculations (25), or were extrapolated from existing amino acid parameters (26) (Table II).

An atomic model for the substrate complex was constructed starting with the PTPase-WO$_4^-$ protein atom coordinates. The phosphohexapeptide Asp-Ala-Asp-Glu-Tyr(P)-Leu (10) was modeled initially in an extended conformation. The phosphate moiety, assumed deprotonated of a Ramachandran $\phi$-$\psi$ plot.

**Anion Binding**

Nitrates bind within the P-loop (Fig. 1) and effect the closed conformation of the Asp$_{356}^-$-containing WpD-loop, as do tung-
state (11) and sulfate (12), supporting the hypothesis that oxyanion interactions with Arg 409 precipitate the closing of the WpD-loop (12). The orientation of the oxyanion is the same in each oxyanion-bound crystal structure and results in up to 11 hydrogen bonds between the P-loop and the anion oxygens, along with hydrogen bonds to crystallographically observed activesite water molecules (Table IV). Three anion oxygens are coordinated by five of the seven amide nitrogens from the P-loop and also by the side chain of Arg 409. Arg 409 is in turn coordinated in a bidentate salt-bridge with Glu 290, the putative general base (9). The apical anion oxygen, O-4, in the PTPase-SO 4 and PTPase-WO 4 complexes points away from the nucleophilic cysteine and the P-loop, and, in PTPase-WO 4, is coordinated by an invariant glutamine residue, Gln 446.

The anion in PTPase-SO 4 is 0.6 Å deeper in the active site than in PTPase-WO 4, primarily because the Ser 403 hydroxyl in PTPase-SO 4 is smaller than the Cys 403 thiolate in PTPase-WO 4. This permits Asp 356 to shift farther into the active site in the PTPase-SO 4 crystal structure. Asp 356 in PTPase-NO 3 occupies a position intermediate to those seen in PTPase-WO 4 and PTPase-SO 4.

**Coordination of the Nucleophile**

Although five of the seven amide hydrogens are directed toward the bound anion, the two remaining P-loop amides point to the nucleophilic Cys 403 (Table V). The amide nitrogens of Gly 406 and Thr 410 and the conserved side chain hydroxyl of Thr 410 make hydrogen bonds with the nucleophile. The amide nitrogen of Arg 404 is within hydrogen bonding distance of both the bound anion and the nucleophile.

**Active Site Water Molecules**

Several water molecules have been identified in the active site (Table VI). WAT1 is observed in all three anion complexes and has an average crystallographic temperature factor of 16 Å 2, WAT1 is buried under the ligand and is well coordinated.
with four tetrahedrally positioned hydrogen bonds. In PTPase-NO₃, Asp₃⁵⁶ approaches within 3.1 Å of WAT1. A second, poorly ordered water molecule, WAT2, sits above the oxyanion in the active site. In PTPase-SO₄, WAT2 is 3.0 Å from Gln₄⁴⁶; however, in the other two structures WAT2 makes good hydrogen bonds only with other water molecules.

A unique active site water molecule is present in PTPase-NO₃ (Fig. 1). Nitrate lacks the apical oxygen present in tungstate and sulfate. Instead, in PTPase-NO₃ WAT3 sits 4.2 Å directly above the nitrate, perfectly in line with the sulfur of the nucleophile, Cys⁴⁰₃ (O–N–S angle of 168°, O–S distance of 7.3 Å). WAT3 is well ordered (B = 17 Å²) and is coordinated principally by the side chains of Gln⁴⁴⁶ and the Gln³⁵⁷. Although Gln⁴⁴⁶ is invariant, Gln³⁵⁷ is found only in the Yersinia PTPase sequence.

### Second Anion Binding Site

A second anion binding site, far from the active site, contains a sulfate in the PTPase-WO₄ and PTPase-SO₄ complex structures, coordinated by the side chains of Arg⁴⁰⁹ and Ser³⁸⁸, and the main chain nitrogen of Ser³⁸⁹ (none of these residues is...
Gln446 and Gln357 define the other side of the phosphotyrosine were generated of the midpoint of the transfer of the PO₃ through a trigonal bipyramidal state (Fig. 3) displacement mechanism, in which the phosphorous passes against the hydrophobic face of Phe229 (Fig. 3) lining the active site. Specifically, the phosphotyrosine ring of the phosphotyrosine side chain is well defined by residues energy minimization, as described above (Fig. 2). The position into the active site pocket and nearby grooves was subjected to the active site binding pocket with the substrate present. Asp356, Ile232, and Arg409 and Glu290 are both slightly solvent-accessible, while Asp356 is well exposed to bulk solvent. Stabilization of the Thiolate—Cys403 must be ionized to participate in the phosphotransfer reaction, and its apparent pKₐ is 4.7 (6). The crystal structure suggests that the pKₐ of Cys403 is lowered by hydrogen bonds to specific P-loop nitrogens and Thr410 and by electrostatic interactions with His402 (Table V) (11). His402 is on the opposite face of a β-strand from Cys403. Although the N1 of His402 is 6.5 Å from the Sγ of Cys403, the histidine is buried deep within the protein, so that its electrostatic effect is not mitigated by bulk solvent. The other nearby charged residues that might modulate the pKₐ of Cys403 are Arg409 (4.3 Å to the Nε), Asp356 (6.9 Å to the Oδ) and Glu290 (7.3 Å to the Oε). Arg409 and Glu290 are both slightly solvent-accessible, while Asp356 is well exposed to bulk solvent.

Stabilization of the PO₃ Moiety—The PO₃ moiety in the models maintains the hydrogen bonds exhibited by the three lower oxygens (O-1, O-2, and O-3) of the oxyanions (Table IV). Arg409 makes bidentate hydrogen bonds to the PO₃ moiety.

Stabilization of the Leaving Group—During the molecular modeling, Asp356 moved to within 3.0 Å of the leaving group, in a position where it could act as a general acid, donating a hydrogen to the leaving group.

Model of the First Phosphotransfer

The geometry of the PTPase active site dictates an in-line displacement mechanism, in which the phosphorous passes through a trigonal bipyramidal state (Fig. 3B). Two models were generated of the midpoint of the transfer of the PO₃ moiety from oxygen of the phosphotyrosine to the Cys403 sulfur of the PTPase, one reflecting an associative reaction and one for a dissociative reaction. In an associative reaction, the bond from the incoming nucleophile is formed prior to bond breakage to the leaving group. In a dissociative reaction, the bond to the leaving group is broken before the bond to the nucleophile is made. The different pathways are distinguished by the bond orders to the phosphorus, which are reflected in the bond lengths and the location of the three negative charges (Ref. 29 and Table VII).

Of special interest is the interaction of the protein and substrate at three key places: the attacking nucleophile, the PO₃ group, and the leaving group:

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Model of the Second Phosphotransfer

The first phosphotransfer generates a phosphocysteine intermediate (Fig. 3C), which is then hydrolyzed (Fig. 3D) to form free orthophosphate. In modeling the configuration of the active site just before hydrolysis of the phosphocysteine, one water molecule was restrained to be at a van der Waals distance from the phosphorus atom. Although Asp356 donated its proton in Step I, Asp356 was modeled as protonated at this step since it is on the surface of the enzyme, presumably in equilibrium with bulk solvent.

After energy minimization, the restrained nucleophilic water molecule, WATNuc, is coordinated by the phosphocysteine oxygen in addition to the side chains of Glu346 and Asp356 (Fig. 3C). WAT1 is the only water molecule that can fit in the active site binding pocket with the substrate present. Asp356, modeled as protonated, moved to within hydrogen bonding distance (2.7 Å) of the ester oxygen during energy minimization.

A phosphoserine-containing peptide modeled in this pocket renders the phosphate group 7 Å away from the nucleophile. Bringing the phosphoserine into contact with the nucleophile requires severe distortion of the geometry of the phosphoserine and steric clashes with the side chain of Phe229 and the other residues lining this pocket.

Model of the Michaelis Complex

A phosphotyrosine-containing hexapeptide manually docked into the active site pocket and nearby grooves was subjected to energy minimization, as described above (Fig. 2). The position of the phosphotyrosine side chain is well defined by residues lining the active site. Specifically, the phosphotyrosine ring packs against the hydrophobic face of Phe229 (Fig. 3A). Ile32 and Ile443 also contribute to a hydrophobic binding site, while Glu446 and Gln357 define the other side of the phosphotyrosine pocket. This modeled peptide-Yersinia PTPase complex is consistent with the recently reported crystal structure of substrate bound to the human PTPase, PTP1B (Ref. 28, and see “Discussion”). WAT1 is the only water molecule that can fit in the active site binding pocket with the substrate present. Asp356, modeled as protonated, moved to within hydrogen bonding distance (2.7 Å) of the ester oxygen during energy minimization.

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After energy minimization, the restrained nucleophilic water molecule, WATNuc, is coordinated by the phosphocysteine oxygen in addition to the side chains of Glu346 and Asp356 (Fig.
3C). It also forms hydrogen bonds with water molecules in the positions identified in the crystal structures as WAT1 and WAT2. WAT\textsuperscript{Nuc} is in position to take part in a second in-line displacement reaction and is just 0.6 Å from the apical oxygen (O-4) of tungstate in PTPase-WO\textsubscript{4} and 2.2 Å from the position seen for WAT3 in the PTPase-NO\textsubscript{3} structure. We postulate that WAT3 is analogous to WAT\textsuperscript{Nuc}, but WAT3 cannot move closer to the nitrate without colliding with the nitrogen, which is not available for hydrogen bonding (Fig. 1).

For the transfer of the phosphate moiety from the Cys\textsuperscript{403} to WAT\textsuperscript{Nuc}, both an associative and a dissociative pathway were modeled (Table VII), although the dissociative-like mechanism is depicted in Fig. 3D. As in Step I, charges on the thiol and PO\textsubscript{3} group are balanced by specific hydrogen bonds and ionic interactions (Tables IV and V).

**DISCUSSION**

Analysis of Modeled Substrate Binding—The hexapeptide Asp-Ala-Asp-Glu-Tyr(P)-Leu is an effective substrate for the Yersinia PTPase, with a $k_{\text{cat}}$ of 1381 s\textsuperscript{-1} and a $K_{\text{m}}$ of 100 μM (10). Although generated independently, the modeled peptide-PTPase coordinates used here are similar to those recently
In solution, displacements of phosphomonoesters proceed by associative approaches close to hydrogen bond distance to the ester oxygen of the scissile bond, and Arg<sup>409</sup> of PTP1B, which Arg<sup>409</sup> here, forms a bidentate hydrogen bond with two of the phosphate oxygens. Differences between this model and the PTP1B crystal structure are due to either limitations in the modeling or the extensive sequence differences between the Yersinia PTPase and PTP1B (only 15% sequence identity for the residues in the Yersinia crystal structure). Crystallographic studies of this hexapeptide bound to the Yersinia PTPase confirm the general interactions outlined above.<sup>3</sup>

Associative Versus Dissociative—Although only an associative and a dissociative mechanism were modeled, these two schemes can be viewed as two extremes on a continuum of possible pathways. Between the associative and dissociative pathways would be a "half-bond" mechanism in which the transition state stabilization for an enzyme to select this pathway over a dissociative one. Indeed, the most direct evidence to date suggests that enzyme-catalyzed phosphomonester hydrolysis is more dissociative-like (29, 34, 35). Alkaline phosphatase and hexokinase both show an inverse<sup>18</sup>O-secondary isotope effect, indicating an increase in bond order to the equatorial oxygens, consistent with a dissociative-like mechanism (see Table VII).

A phosphorionate esterase can catalyze a dissociative reaction by balancing three negative charges: at the leaving group, the PO<sub>3</sub> group, and the attacking nucleophile (36, 37). In the analogous alkaline phosphatase catalyzed reaction, these charges are coordinated with Zn<sup>2+</sup> ions for the serine nucleophile and the leaving group and an arginine for the PO<sub>3</sub> moiety (Fig. 4) (38). As shown in the model, the Yersinia PTPase could use specific charged and uncharged groups on the enzyme to stabilize these three negative charges at places structurally analogous to the arginine and Zn<sup>2+</sup> in alkaline phosphatase.

The use of an arginine to ligate the phosphate oxygens would seem to favor an associative pathway, since the dissociative pathway requires a transient increase in charge on the phosphate oxygen. However, in the case of the Yersinia PTPase at least, the charge on Arg<sup>409</sup> appears to be insufficient for catalytic activity. Changing Arg<sup>409</sup> to alanine reduces <i>k</i><sub>cat</sub> by 10,000, and increases <i>K</i><sub>m</sub> over an order of magnitude (8). The positive charge alone is sufficient for binding, since the <i>K</i><sub>m</sub> of an Arg<sup>409</sup> → Lys mutant is restored to its wild type value. However, the <i>k</i><sub>cat</sub> of the Arg<sup>409</sup> → Lys mutant is nearly identical to that for the Arg<sup>409</sup> → Ala mutant. Thus, the geometry of the guanidinium group must be essential for turnover.

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3 J. A. Stuckey and M. A. Saper, manuscript in preparation.
A requirement for a planar guanidinium group is consistent with a dissociative-like transition state. Since the O–P bonds in metaphosphate have more double-bond character (5/3 each) than in orthophosphate (4/3 each), the planar geometry of the metaphosphate would be expected to show at least an order of magnitude decrease in rate, unless it has a pK_a of at least 7.0. The apparent pK_a of Asp^{356} is about 5.1 from the pH dependence of k_cat and k_cat/K_m (9, 27).

If Asp^{356} is not the general base for step II, the other ligands of WAT^{Nuc} identified by the model must be considered as potential general bases. Glu^{446} is hydrogen bonded to WAT^{Nuc}, but the pK_a values of glutamine residues make it an unlikely proton acceptor. Alternatively, the phosphate itself could accept the proton, either directly or via WAT1. The pK_a of free phosphocysteine is about 5.0 (41). The use of the phosphate moiety of a thiophosphate ester to catalyze its own hydrolysis has been proposed for the non-enzymatic hydrolysis of S-n-butylphosphorothioate (42).

WAT1—Based on our modeling studies and our crystal structure, it is unlikely that WAT1 is WAT^{Nuc}. Rather, WAT1 plays a structural role by bridging together a phosphate oxygen (O-1), the tyrosine leaving group oxygen (in step I) or WAT^{Nuc} (in step II). The conserved Glu^{446} and an amide on the general acid-containing WpD-loop. WAT1 may be functionally more flexible than a side chain moiety, since it can adapt to multiple hydrogen bonding conformations necessary between steps I and II. Further insight into this water will be gained from a detailed analysis of a high resolution structure of a PTPase-vanadate complex. 4

Applicability to Other PTPases—Sequence alignment of the PTPase domain from proteins of a wide range of organisms has identified 21 invariant and 29 highly conserved residues (43) (of which only 26 are found in the Yersinia sequence; Ref. 44). Nearly all of the 47 invariant or highly conserved residues are clustered around the active site. An ellipsoidal volume (45) defined by these residues has dimensions of 35 × 30 × 24 Å^3 (compared to 58 × 44 × 38 Å^3 for an ellipsoid defined from all the residues) and contains nearly all of the invariant or highly conserved residues while excluding nearly all the remaining residues. Thus, the roles of these conserved residues are probably predominantly structural, supporting the catalytically essential residues and maintaining the proper dielectric environment for the electrostatic interactions involved in catalysis.

Acknowledgments—We thank Jack Dixon, John Denu, and Zhong-Yin Zhang for many useful discussions. Fig. 1 was made using O (46) and the ray-tracing package VORT (Version 2.10, University of Melbourne, 1992). Fig. 2 was made with O and VORT, with a surface generated by GRASP (47). Figs. 3 and 4 were made using MOLSCRIPT (48) and VORT.REFERENCES

1. Fischer, E. H., Charbonneau, H., and Torks, N. K. (1991) Science 253, 401–406
2. Guan, K., and Dixon, J. E. (1990) Science 249, 553–556
3. Bliska, J. B., Guan, K. L., Dixon, J. E., and Falkow, S. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 1187–1191
4. Guan, K. L., and Dixon, J. E. (1991) J. Biol. Chem. 266, 17062–17030
5. Coleman, J. E. (1992) Annu. Rev. Biophys. Biomol. Struct. 21, 441–483
6. Zhang, Z. Y., and Dixon, J. E. (1993) J. Biol. Chem. 268, 9340–9345
7. Zhang, Z. Y., Palfei, B. A., Wu, L., and Zhao, Y. (1995) Biochemistry 34, 16389–16396
8. Zhang, Z. Y., Wang, Y., Wu, L., Fauman, E. B., Stuckey, J. A., and Schubert, H. L. (1994) Biochemistry 33, 12626–12630
9. Zhang, Z. Y., Wang, Y., and Dixon, J. E. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1624–1627
10. Zhang, Z. Y., Madian, D., McNamara, D. J., Sawyer, T. K., and Dixon, J. E. (1994) Biochemistry 33, 2285–2290
11. Stuckey, J. A., Schubert, H. L., Fauman, E. B., Zhang, Z. Y., and Saper, M. A. (1994) Nature 370, 571–575
12. Schubert, H. L., Fauman, E. B., Stuckey, J. A., Dixon, J. E., and Saper, M. A. (1995) Protein Sci. 4, 1904–1913
13. Zhang, Z. Y., Cieplens, J. C., Schubert, H. L., Stuckey, J. A., Fisher, M. W., and Saper, M. A. (1995) Structure of Yersinia PTPase Anion Complexes 18787

* J. Vijayalakshmi and M. A. Saper, manuscript in preparation.
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Hume, D. M., Saper, M. A., and Dixon, J. E. (1992) J. Biol. Chem. 267, 23759–23766
14. Messerschmidt, A., and Pflugrath, J. W. (1987) J. Appl. Crystallogr. 20, 306–315
15. Kabsch, W. (1988) J. Appl. Crystallogr. 21, 916–924
16. Brünger, A. T. (1987) X-PLOR Version 3.1, Yale University Press, New Haven, CT
17. Bernstein, F. C., Koetzle, T. F., Williams, G. J., Meyer, E. F., Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T., and Tasumi, M. (1977) J. Mol. Biol. 112, 535–542
18. Caughlan, C. N., and Ul-Haque, M. (1967) Inorg. Chem. 6, 1998–2002
19. Glowiak, T., and Szemik, A. W. (1986) J. Crystallogr. Spectrosc. Res. 16, 79–81
20. Glowiak, T., and Wnek, I. (1985) Acta Crystallogr. Sect. C Cryst. Struct. Commun. 41, 324–327
21. Jones, P. G., Sheldrick, G. M., Kirby, A. J., and Abell, K. W. Y. (1984) Acta Crystallogr. Sect. C Cryst. Struct. Commun. 40, 550–552
22. Karle, J. M., and Karle, I. L. (1991) Acta Crystallogr. Sect. C Cryst. Struct. Commun. 47, 1241–1245
23. Kennard, O., Allen, F. H., Bellard, S., Brice, M. D., Doubleday, A., Higgs, H., Hummelink, T., Hummelink-Peters, B. G., Matherwell, W. D. S., Rodgers, J. R., and Watson, D. G. (1979) Acta Crystallogr. Sect. B Struct. Sci. 35, 2331–2339
24. Koster, A. S., Kools, F. X. M. M., and Rieck, G. D. (1969) Acta Crystallogr. Sect. B Struct. Sci. 25, 1704–1708
25. Basch, H., Krauss, M., and Stevens, W. J. (1991) J. Mol. Struct. 235, 277–291
26. Engh, R. A., and Huber, R. (1991) Acta Crystallogr. Sect. A Fundam. Crystallogr. 47, 392–400
27. Zhang, Z.-Y., Malachowski, W. P., Van Etten, R. L., and Dixon, J. E. (1994) J. Biol. Chem. 269, 8140–8145
28. Jia, Z. C., Barford, D., Flint, A. J., and Tonks, N. K. (1995) Science 268, 1754–1758
29. Cleland, W. W. (1990) FASEB J. 4, 2899–2905
30. Benkovic, S. J., and Schray, K. J. (1973) Enzymes 8, 201–238
31. Wwestheimer, F. H. (1987) Science 235, 1173–1178
32. Herschlag, D., and Jencks, W. P. (1989) J. Am. Chem. Soc. 111, 7579–7586
33. Hassett, A., Blättler, W., and Knowles, J. R. (1982) Biochemistry 21, 6335–6340
34. Jones, J. P., Weiss, P. M., and Cleland, W. W. (1991) Biochemistry 30, 3634–3639
35. Weiss, P. M., and Cleland, W. W. (1989) J. Am. Chem. Soc. 111, 1928–1929
36. Herschlag, D., and Jencks, W. P. (1987) J. Am. Chem. Soc. 109, 4665–4674
37. Herschlag, D., and Jencks, W. P. (1990) Biochemistry 29, 5172–5179
38. Kim, E. E., and Wyckoff, H. W. (1991) J. Mol. Biol. 218, 449–464
39. Cho, H., Krishnaraj, R., Kitas, E., Bannwarth, W., Walsh, C. T., and Anderson, K. S. (1992) J. Am. Chem. Soc. 114, 7296–7298
40. Hengge, A. C., Sowa, G. A., Wu, L., and Zhang, Z.-Y. (1995) Biochemistry 34, 13982–13987
41. Åkerfeldt, S. (1960) Acta Chem. Scand. 14, 1980–1984
42. Herr, E. B., J., and Koshland, D. E., Jr. (1957) Biochim. Biophys. Acta 25, 219–220
43. Zhang, Z.-Y., and Dixon, J. E. (1994) Adv. Enzymol. Relat. Areas Mol. Biol. 68, 1–36
44. Michiels, T., and Cornelis, G. (1988) Microb. Pathog. 5, 449–459
45. Bouwer, M. F., Fauman, E. B., and Fletterick, R. J. (1992) Biochemistry 31, 11297–11304
46. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A Fundam. Crystallogr. 47, 110–119
47. Nicholls, A., Sharp, K., and Honig, B. (1991) Proteins Struct. Funct. Genet. 11, 281–296
48. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
49. Ponder, J. W., and Richards, F. M. (1987) J. Mol. Biol. 193, 775–791
50. Pauling, L. (1947) J. Am. Chem. Soc. 69, 542–553