Crystal Structure and Tartrate Inhibition of *Legionella pneumophila* Histidine Acid Phosphatase

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ABSTRACT

Histidine acid phosphatases (HAPs) utilize a nucleophilic histidine residue to catalyze the transfer of a phosphoryl group from phosphomonoesters to water. HAPs function as protein phosphatases and pain suppressors in mammals, are essential for *Giardia lamblia* excystation, and contribute to virulence of the category A pathogen *Francisella tularensis*. Herein we report the first crystal structure and steady-state kinetics measurements of the HAP from *Legionella pneumophila* (LpHAP), also known as *Legionella* major acid phosphatase. The structure of LpHAP complexed with the inhibitor L(+)-tartrate was determined at 2.0 Å resolution. Kinetics assays show that L(+)-tartrate is a 50-fold more potent inhibitor of LpHAP than of other HAPs. Electrostatic potential calculations provide insight into the basis for the enhanced tartrate potency: the tartrate pocket of LpHAP is more positive than other HAPs because of the absence of an ion pair partner for the second Arg of the conserved RHGXRX P HAP signature sequence. The structure also reveals that LpHAP has an atypically expansive active site entrance and lacks the nucleotide substrate base clamp found in other HAPs. These features imply that nucleoside monophosphates may not be preferred substrates. Kinetics measurements confirm that AMP is a relatively inefficient *in vitro* substrate of LpHAP.

Keywords:

X-ray crystallography

Histidine acid phosphatase

Histidine phosphatase superfamily

Substrate recognition

Abbreviations used: HAP, histidine acid phosphatase; LpHAP, *Legionella pneumophila* histidine acid phosphatase; FtHAP, *Francisella tularensis* histidine acid phosphatase; hPAP, human prostatic acid phosphatase; *p*NPP, *p*-nitrophenyl phosphate.

Introduction

The histidine phosphatase superfamily comprises phosphoryl transfer enzymes that share a common catalytic core, featuring a nucleophilic histidine that is phosphorylated during the catalytic cycle[1].The superfamily has two main branches. Branch 1 includes several functionally diverse enzymes, such as cofactor-dependent phosphoglycerate mutases and a variety of phosphatases. Branch 2 contains mostly phytases and histidine acid phosphatases (HAPs). The latter is the subject of this report.

HAPs catalyze phosphoryl transfer from phosphomonoesters to water optimally at acidic pH. The accepted mechanism begins with attack by the conserved histidine on the substrate P atom forming a phosphohistidine intermediate and liberating the alcohol of the phosphomonoester substrate. A conserved Asp residue facilitates this step of the mechanism by protonating the leaving group. In the second step, hydrolysis of the phosphohistidine produces inorganic phosphate and regenerates the enzyme for another round of catalysis.

Only three HAPs have been characterized biochemically and structurally. Pioneering work by Van Etten and coworkers using mainly human prostatic acid phosphatase (hPAP) identified key active site residues and established the catalytic mechanism[2-6].Crystal structures of rat PAP (84% identical to hPAP)[7-9] and hPAP[10-12] revealed the fold, domain architecture, and active site structure. More recently, we determined several structures of a bacterial HAP from the category A pathogen *Francisella tularensis* (FtHAP), including the structure of a substrate-trapping mutant of FtHAP complexed with 3'-AMP[13].In the substrate-trapping mutant, the conserved Asp that protonates the leaving group has been mutated to Ala (D261A). The structure of D261A complexed with 3'-AMP (PDB 3IT3) revealed a hydrophobic clamp that binds the nucleotide base of the substrate.

The biological roles of HAPs are diverse and continue to emerge. Early work suggested that the cellular form of hPAP functions as a protein tyrosine phosphatase, with potential substrates including c-ErbB-2[14] and the epidermal growth factor receptor[15]. More recently, the transmembrane isoform of hPAP has been shown to suppress pain by dephosphorylating extracellular 5'-AMP to adenosine[16, 17]. The HAP Api m 3 is the major allergen of honeybee venom[18, 19]. In *Giardia lamblia*, dephosphorylation of cyst wall proteins by the lysosomal HAP known as AcPh is required for excystation, the process by which trophozoites emerge from cysts ingested by the host[20]. FtHAP and other acid phosphatases are thought to contribute to the virulence of *F. tularensis*, and a mutant strain of *Francisella* lacking functional genes for FtHAP and three other acid phosphatases showed promising protective capacity as a single-dose live vaccine[21]. Although the *in vivo* substrates of bacterial HAPs are unknown, our studies of FtHAP implicated small molecule phosphomonoesters rather than phosphoproteins as potential substrates[13].

The discovery that hPAP is a pain suppressor has renewed interest in studying HAPs [16, 17, 22-26]. Studies show that hPAP markedly reduces sensitivity to painful stimuli (antinociception) and is eight times more potent than morphine. The antinociceptive function of hPAP is due to its ability to catalyze the dephosphorylation of 5'-AMP to adenosine, which activates A₁-adenosine receptors in the dorsal spinal cord. These studies have led to the idea of using recombinant HAPs as a treatment for chronic pain, such as injection of enzymes at acupuncture points ("PAPupuncture") [23].

To gain additional molecular information for HAPs, we targeted the HAP from *Legionella pneumophila* (LpHAP) for crystal structure determination. *L. pneumophila* is a Gram-negative, intracellular pathogen of freshwater protozoa and human alveolar macrophages. In the latter

context, *L.pneumophila* is the etiologic agent of Legionnaires' disease[27]. Also known as the major acid phosphatase, LpHAP is a 39 kDa enzyme that is secreted in a *pilD*-dependent process[28]. LpHAP shares 29 % global sequence identity with hPAP and 39% identity with FtHAP. Herein we report the 2.0 Å resolution structure of LpHAP complexed with the inhibitor L(+)-tartrate, along with measurements of L(+)-tartrate inhibition and kinetic parameters for adenosine monophosphate substrates.

Experimental procedures

Cloning, expression, and purification

The gene for LpHAP (NCBI RefSeq WP_027265797.1) was cloned from genomic DNA into pET-20b using NcoI and XhoI restriction sites. The cloning was performed such that the *pelB* leader peptide of pET20b replaced the natural N-terminal export signal peptide. The expressed protein contains an N-terminal hexahistidine tag.

LpHAP was expressed using a modified autoinduction method[29]. Briefly, the cells were grown in BL21(AI) at 37°C for ~3h, then 0.2% arabinose was added and the temperature was reduced to 18°C. The cells were harvested after 28 hrs and frozen at -80 °C until further use.

The protein was purified using immobilized metal (Ni^{2+}) affinity chromatography and anion exchange chromatography as follows. Frozen cells were thawed and ruptured using sonication. The cell debris was removed by centrifuging the lysate at 16,500 rpm for 60 minutes at 4 °C. The clarified supernatant was loaded onto Ni^{2+} -charged HisTrap (GE Healthcare Life Sciences) column equilibrated with 20 mM phosphate and 150 mM NaCl at pH 7.0 (Buffer A). The column was washed with buffer A supplemented with 20 mM imidazole; LpHAP was eluted with buffer A supplemented with 300 mM imidazole. Fractions were pooled and dialyzed against

50 mM Tris, 50 mM NaCl, and 1 mM EDTA at pH 7.0 (Buffer B) and loaded onto a HiTrap Q anion exchange column (GE Healthcare Life Sciences) pre-equilibrated with Buffer B. The protein was eluted with linear 0-1 M NaCl gradient over 25 column volumes. Based on acid phosphatase activity and SDS-PAGE analysis, fractions were pooled and dialyzed against 50 mM Tris, 50 mM NaCl, and 1 mM EDTA at pH 7.5. The dialyzed protein was concentrated to 8 mg/mL using centrifugal devices having a molecular weight cutoff of 10 kDa (Millipore Amicon Ultra). The protein concentration estimated using the bicinchoninic acid assay (Pierce kit). Typically this procedure produced approximately 2 mg of 99% pure protein per liter of culture.

Crystallization

Crystallization trials were performed using vapor diffusion in 24-well sitting drop trays at 298 K. The protein stock solution contained LpHAP at 8.0 mg/mL in 50 mM Tris, 50 mM NaCl, and 1 mM EDTA at pH 7.5. Drops were formed by mixing 1.5 μ L each of the protein and reservoir solutions. Crystal screening using commercially available kits (Hampton Index, Crystal Screens 1 and 2, and Emerald Wizards 1, 2, and 3) resulted in plate-shaped crystals obtained in 10% (w/v) PEG 8000, 0.1 M imidazole pH 8.0, and 0.2 M calcium acetate. These crystals diffracted weakly to 3.5 Å resolution. The screens were repeated using enzyme that had been incubated with 10 mM of the inhibitor L(+)-tartrate. A 100 mM stock solution of L(+)-tartrate was prepared in the buffer into which the protein had been dialyzed, and 20 μ L of this stock solution was added to 180 μ L of the 8 mg/mL protein solution. These experiments produced crystals shaped like tetragonal bipyramids using a reservoir solution of 20% (w/v) PEG 3350 and 0.2 M sodium acetate at pH 4.5. The crystals were cryoprotected with 25% (w/v) PEG

3350, 0.2 M sodium acetate pH 4.5, and 25% (v/v) PEG 200. The crystals were picked up with Hampton nylon loops and plunged into liquid N₂.

X-ray diffraction, data collection, and refinement

Crystals of LpHAP complexed with L(+)-tartrate were analyzed at Advanced Photon Source beamline 24-ID-C using a Quantum 315 detector, where they diffracted to 2.0 Å resolution. The space group is *C2* with the unit cell dimensions listed in Table. 1. The asymmetric unit contains eight HAP protomers arranged as four dimers. Using the method of Matthews, the solvent content is estimated to be 54 % with V_m of 2.7 Å³/Da[30]. We note that our crystal form is different from the ones reported recently for LpHAP [31]. The data were processed with HKL[32]. Intensities were converted to amplitudes using the French and Wilson[33] method as implicated in Truncate via CCP4i[34]. Data processing statistics are listed in Table. 1

The structure of LpHAP was solved by molecular replacement using MOLREP[35] with a search model derived from the coordinates of FtHAP (PDB entry 3IT1). The initial solution from MOLREP was used to initiate automated model building in Phenix.Autobuild[36]. The structure was completed via several iterative rounds of modeling building in COOT[37, 38] and refinement in PHENIX[39]. The *B*-factor model consisted of an isotropic *B*-factor for each atom and TLS refinement with eight groups (one group per chain). Water molecules were modeled into strong $F_o - F_c$ peaks. The $2F_o - F_c$ density was inspected after refinement, and water molecules with weak or non-spherical $2F_o - F_c$ density were deleted. Non-crystallographic symmetry (NCS) restraints were not used because of the relatively high resolution of the data set (2.0 Å). As a check, an additional round of refinement was performed with 8-fold NCS restraints. The R_{crist}

increased from 0.186 to 0.188, while R_{free} decreased from 0.224 to 0.221. These changes are very small, which suggests that the absence of NCS restraints did not result in overfitting and the addition of NCS restraints does not significantly improve the model. Refinement statistics are listed in Table 1. Atomic coordinates and structure factor amplitudes have been deposited in the Protein Data Bank as entry 5CDH.

Kinetic characterization

The kinetic parameters for 3'-AMP and 5'-AMP were measured using the malachite green assay[40]. Activity was measured at 37 °C in a buffer consisting of 0.2 M sodium acetate at pH 5.5, with 76 pmoles of enzyme present in the assay. The amount of inorganic phosphate generated was measured spectrophotometrically at 625 nm. For each substrate concentration, the reaction was stopped at time points of 15, 75, 135, and 195 seconds using the malachite green-ammonium molybdate reagent. The reaction rate data were fitted to the Michaelis-Menten equation using Origin.

Inhibition by L(+)-tartrate was studied using a discontinuous assay with *p*-nitrophenyl phosphate (*p*NPP) as the substrate [41] at 37°C and pH 5.5 with 6.4 pmoles of enzyme in the assay. The reaction was stopped at time points of 15, 75, 135, and 195 seconds using 0.5 M glycine pH 10. The amount of *p*-nitrophenolate formed was determined spectrophotometrically at 405 nm.

Results

Structure of LpHAP complexed with L(+)-tartrate

The structure of LpHAP complexed with L(+)-tartrate, a known inhibitor of HAPs, was determined at 2.0 Å resolution (Figure 1). LpHAP exhibits the expected HAP fold consisting of two domains (Figure 1A). The core domain consists of a twisted β -sheet flanked by two α -helices on one side and three on the other side. The core domain furnishes the catalytic histidine (His34) and several residues that likely bind the phosphoryl moiety of the substrate. The catalytic histidine is part of the characteristic HAP signature sequence of RHGX_RXP, which is ³³RHGDRT³⁹ in LpHAP. The core domain also contains a long loop that is involved in dimerization. The dimer observed in crystals of PAPs and FtHAP[7-12] is also observed in the LpHAP C2 lattice (Figure 1B), which provides additional support that this assembly represents the solution form of HAPs. The cap domain is smaller and mainly α -helical (Figure 1A). This domain is thought to mediate substrate selectivity in HAPs [13]. In particular, α 3 of the cap domain in FtHAP provides a Tyr residue that is part of the hydrophobic clamp that binds the adenine of the substrate 3'-AMP[13]. As described below, α 3 of LpHAP has an unexpected orientation.

Electron density indicated L(+)-tartrate bound in the active site (Figure 1C). The density representing the entire ligand is very strong in five of the eight chains in the asymmetric unit. In the other chains, the density for the carboxylate distal from His34 (C4) is somewhat weaker. An example of strong electron density for L(+)-tartrate is shown in Figure 1C.

Several residues interact with L(+)-tartrate (Figure 1C). As expected for a highly anionic inhibitor, the interactions are primarily electrostatic. One carboxylate of the inhibitor is anchored in the phosphoryl binding site by Arg33, His34, and Arg37 of the RHGXRRP motif. The hydroxyls interact with Arg101, His280, and Asp281. The carboxylate of L(+)-tartrate distal from the catalytic His ion pairs with Arg101.

Comparison of the tartrate sites of LpHAP and FtHAP

We previously determined the 1.7 Å resolution structure of FtHAP complexed with L(+)-tartrate (PDB entry 3IT1) [13]. Unexpectedly, the tartrate binding sites of LpHAP and FtHAP exhibit notable differences. Although the enzyme-inhibitor interactions described above for LpHAP are also present in FtHAP, the L(+)-tartrate bound to FtHAP makes an additional interaction with Gln132 (Figure 2A). Interestingly, sequence alignments show LpHAP Gln152 aligning with FtHAP Gln132[42], yet Gln152 is 11 Å from the inhibitor because of a conformational difference involving $\alpha 3$ (*vide infra*).

Another difference between the tartrate sites of LpHAP and FtHAP involves the second Arg residue of the RHGXRRP motif. Whereas Arg20 of FtHAP forms an ion pair with Asp185, the equivalent Arg37 of LpHAP is unpaired because Asp185 is replaced by His205 (Figure 2A). (We note that the Arg-Asp ion pair is also present in hPAP.) As a result of this sequence variation, the active site of LpHAP has a cavity at the location corresponding to the carboxylate of FtHAP Asp185 (Figure 2B). The cavity contains a strong electron density feature indicating a solvent molecule; this feature could be modeled satisfactorily as a water molecule in some of the chains

in the asymmetric unit, while the feature may indicate partial occupancy of a larger solvent species in the other chains.

Analysis of the substrate-binding site of LpHAP

Comparison of the structure of LpHAP to those of FtHAP and hPAP reveals unexpected features in the presumed substrate-binding site. Although the three structures superimpose well (1.6 – 2.2 Å root mean square deviations for C α atoms), significant deviations are evident near α 3 of the cap domain (Figure 3A). In FtHAP, α 3 is tilted toward the tartrate site, which allows the N-terminus of this helix to participate in substrate binding. In particular, the crystal structure of a substrate-trapping mutant of FtHAP showed that Tyr135 of α 3 forms one-half of a hydrophobic clamp that binds the adenine base of the substrate 3'-AMP (Figure 3B)[13]. Also, Gln132 of the loop preceding α 3 hydrogen bonds to the 2'-hydroxyl of 3'-AMP in FtHAP. In contrast, the α 3 helix of LpHAP is tilted away from the tartrate site (Figure 3A), which is similar to hPAP despite the fact that LpHAP is closer in sequence to FtHAP.

LpHAP is atypical in that it appears to lack the nucleotide base clamp. FtHAP exhibits the prototype clamp consisting of Phe23 and Tyr135 (Figure 3B). These residues stack in parallel to the adenine base of 3'-AMP. Docking of 3'-AMP into the active site of hPAP suggests that Ile18 and Phe171 are the clamping residues (Figure 3B). Ile18 is structurally analogous to FtHAP Phe23, while Phe171 is part of the penultimate helix of the cap domain (Figure 1). LpHAP also has an isoleucine residue in one of the clamping positions (Ile40), however; the second clamp residue is missing (Figure 3B). The α 3 helix of LpHAP is too far from the predicted base to provide a clamp residue, while the penultimate helix of the cap domain is

shifted away from the nucleotide site and furthermore does not contain an appropriate clamping residue. For example, Glu198 is the only side chain of the penultimate helix that is in the vicinity of the expected clamp(Figure 3B).

The outward tilt of $\alpha 3$ and incomplete nucleotide base clamp endows LpHAP with an expansive active site entrance compared to FtHAP and hPAP. LpHAP has a crater-shaped active site with diameter ranging from 13 to 19 Å (Figure 4A). The phosphoryl binding site is in the left-hand side of the crater. In contrast, the entrances of FtHAP and hPAP are troughs having lengths of 15 Å and 25 Å, respectively (Figures 4B and 4C). The clamp residues form the middle of the trough in FtHAP and the top of the trough in hPAP.

Kinetic characterization of LpHAP

Inhibition kinetics measurements were performed using L(+)-tartrate as the inhibitor and *p*NPP as the substrate. Three data sets corresponding to different inhibitor concentrations were fit globally to a competitive inhibition model, yielding K_i of $4.3 \pm 0.4 \mu\text{M}$, K_m of $0.55 \pm 0.05 \text{ mM}$, and k_{cat} of $16.1 \pm 0.3 \text{ s}^{-1}$ (Figure 5A).

The unexpectedly wide active site of LpHAP and the lack of a proper nucleotide base clamp suggested that nucleoside monophosphates might not be efficient substrates. We tested this idea by determining the kinetic parameters for 3'-AMP (Figure 5B) and 5'-AMP (Figure 5C). These two substrates have similar kinetic parameters, with k_{cat} in the range of $2 - 3 \text{ s}^{-1}$ and K_m of $3 - 4 \text{ mM}$ (Table 2). The resulting catalytic efficiencies are $0.6 - 0.9 \text{ s}^{-1}\text{mM}^{-1}$. As discussed below, these efficiencies are 200-400 times lower than other HAPs.

Discussion

L(+)-tartrate is a more potent inhibitor of LpHAP than the other two HAPs that have been studied. We found that L(+)-tartrate inhibits LpHAP with a K_i of 4.3 μM . For comparison, the inhibition constants for hPAP and FtHAP are 150 μM and 200 μM , respectively[3, 13].

The crystal structures provide a possible explanation for the higher affinity of tartrate for LpHAP. We noted the Arg-Asp ion pair in FtHAP and hPAP (Figure 2A). The Asp of this ion pair (Asp185) is replaced by His205 in LpHAP, which leaves Arg37 of LpHAP without an anionic partner. We determined the effect of the unpaired Arg on the electrostatic potential field in the tartrate site. LpHAP displays strong positive potential near Arg37 (Figure 2B). In contrast, the tartrate site of FtHAP shows relatively neutral potential on Arg20 and strong negative potential on Asp185 (Figure 2C). Thus, the electrostatic environment around the tartrate anion is substantially more positive in LpHAP than in FtHAP, which may account for the 50-fold higher inhibitor potency.

LpHAP exhibits an unexpected orientation of $\alpha 3$. Although closer in global sequence identity to FtHAP (39% identical), the orientation of $\alpha 3$ in LpHAP resembles that of hPAP (29% identical). A local sequence variation common to LpHAP and hPAP possibly contributes to the outward tilt of $\alpha 3$ in these enzymes. Both contain a 3-residue insert in the loop immediately preceding $\alpha 3$ (LpHAP residues 147-149 and hPAP 120-122) (Figure 3A). The $\alpha 3$ helix apparently tilts outward in LpHAP and hPAP in order to accommodate these extra residues. It is possible that $\alpha 3$ of LpHAP and hPAP are flexible enough to adopt a conformation that resembles FtHAP when certain substrates are bound. Additional ligand complex structures would be needed to test this idea.

LpHAP is unique in that it has only one of the two nucleotide base clamping residues seen in FtHAP and hPAP. This feature may explain the relatively low catalytic activity of LpHAP with adenosine monophosphate substrates. We previously measured the parameters of FtHAP with 3'-AMP to be $k_{\text{cat}} = 120 \text{ s}^{-1}$, $K_{\text{m}} = 0.3 \text{ mM}$, and efficiency ($k_{\text{cat}}/K_{\text{m}}$) of $381 \text{ s}^{-1} \text{ mM}^{-1}$ [13] (Table 2). In contrast, we found here that 3'-AMP is a relatively poor substrate for LpHAP. Compared to FtHAP, the k_{cat} of LpHAP is 40 times lower and K_{m} is 10-fold higher. The kinetic parameters of hPAP with 5'-AMP have also been measured (Table 2)[43]; k_{cat} is 15 times lower and K_{m} is 10-fold higher compared to hPAP. Thus, relative to FtHAP and hPAP, LpHAP appears to have very low activity toward adenosine monophosphate substrates. The low apparent catalytic efficiency of LpHAP for adenosine monophosphates is consistent with the lack of a proper nucleotide base clamp. We showed previously that mutation of clamp residue Tyr135 of FtHAP to Ala increases K_{m} for 3'-AMP by a factor of ten to 3.0 mM, which is similar to the K_{m} of LpHAP for 3'-AMP (3.3 mM). This result is consistent with the observation that LpHAP lacks the clamping residue corresponding to Tyr135 of FtHAP.

Taken together, the expansive, crater-shaped active site of LpHAP, the lack of a full clamp, and the apparently low activity with adenosine monophosphate substrates suggest that nucleoside monophosphates may not be the preferred class of *in vivo* substrate for this enzyme. The wide active site perhaps suggests that phosphorylated proteins could be substrates of LpHAP. Finally, the low activity with 5'-AMP suggests that LpHAP is suboptimal for use in pain suppression applications.

Acknowledgements

We thank Dr. Jonathan Schuermann of Advanced Photon Source NECAT beamlines for help with X-ray data collection and processing. This work is based upon research conducted at the Northeastern Collaborative Access Team beamlines, which are funded by the National Institute of General Medical Sciences from the National Institutes of Health (P41 GM103403). This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357.

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Table 1

X-ray diffraction data collection and refinement statistics^a

Space group	<i>C2</i>
Unit cell parameters (Å, °)	<i>a</i> = 217.9, <i>b</i> =134.3, <i>c</i> = 135.8 <i>β</i> = 127.5
Wavelength	0.97903
Resolution (Å)	50.00 – 2.00 (2.07 – 2.00)
Observations	745974
Unique reflections	207120
<i>R</i> _{merge} (<i>I</i>)	0.079 (0.451)
<i>R</i> _{meas} (<i>I</i>)	0.093 (0.680)
<i>R</i> _{pim} (<i>I</i>)	0.048 (0.351)
Mean <i>CC</i> _{1/2}	0.997 (0.755)
Mean <i>I</i> /σ	20.3 (2.7)
Completeness (%)	99.6 (98.6)
Multiplicity	3.6 (3.3)
<i>R</i> _{cryst}	0.186 (0.230)
<i>R</i> _{free} ^b	0.224 (0.280)
No. of protein residues	2609
No. of protein atoms	20192
No. of water molecules	660
Average B-factor (Å ²)	
Protein	31.9
Water	27.9
Tartrate	34.8
rmsd bond lengths (Å)	0.007
rmsd bond angles (°)	0.971
Ramachandran plot ^c	
Favored (%)	98.92
Allowed (%)	1.08
Outliers (%)	0.00
All-atom clashscore ^c	1.9
Coordinate error (Å) ^d	0.22
PDB code	5CDH

^aValues for the outer resolution shell of data are given in parenthesis.

^b5% test set.

^cGenerated with MolProbity via PHENIX refine.

^dMaximum likelihood-based coordinate error estimate from PHENIX refine.

Table 2
Steady-State Kinetic Parameters

Enzyme	Substrate	k_{cat} (s^{-1})	K_{m} (mM)	$k_{\text{cat}}/K_{\text{m}}$ ($\text{s}^{-1} \text{mM}^{-1}$)
LpHAP	3'-AMP	3.0 ± 0.3	3.3 ± 0.7	0.9 ± 0.2
FtHAP ^a	3'-AMP	120	0.3	381
LpHAP	5'-AMP	2.4 ± 0.1	3.9 ± 0.6	0.6 ± 0.1
hPAP ^b	5'-AMP	37	0.37	100

^aSingh *et al.* [13]

^bLam *et al.*[43]

Figure Legends

Figure 1. Structure of LpHAP. (A) Cartoon representation of the protomer emphasizing domain architecture. The core and cap domains are colored blue and pink, respectively. L(+)-tartrate and catalytic His34 are shown in sticks. (B) Surface representation of the dimer. (C) Electron density and interactions for L(+)-tartrate. The mesh represents a simulated annealing $F_o - F_c$ omit map contoured at 3σ .

Figure 2. Comparison of the L(+)-tartrate pockets of LpHAP and FtHAP. (A) Superposition of LpHAP (cyan) and FtHAP (gold, PDB 3IT1). The residue numbers of conserved amino acids are listed as LpHAP/FtHAP. Black dashes denote unique interactions in FtHAP. Cyan dashes denote a unique hydrogen bond with water in LpHAP. (B) Electrostatic potential surface of the L(+)-tartrate binding site of LpHAP calculated with CCP4mg[44]. L(+)-tartrate is shown in yellow. The orientation is approximately the same as in panel A. (C) Electrostatic potential surface of the L(+)-tartrate binding site of FtHAP calculated with CCP4mg. L(+)-tartrate is shown in yellow. The orientation is approximately the same as in panel A.

Figure 3. Comparison of LpHAP, FtHAP, and hPAP. (A) Superposition LpHAP, FtHAP, and hPAP. The $\alpha 3$ regions are colored cyan (LpHAP), gold (FtHAP), and magenta (hPAP). A bound tartrate ion is shown for reference. (B) Zoomed-in view of the active sites of a substrate-trapping mutant of FtHAP complexed with 3'-AMP (gold with 3'-AMP in green, PDB entry 3IT3), the LpHAP-tartrate complex (cyan), and hPAP (magenta, PDB entry 1ND6).

Figure 4. The active site entrances of (A) LpHAP-tartrate, (B) FtHAP-3'-AMP, and (C) hPAP-phosphate. 3'-AMP from FtHAP (green sticks) has been docked into the structures of LpHAP and hPAP to indicate the predicted nucleotide base clamping residues. The bar indicates the location of $\alpha 3$.

Figure 5. Steady-state kinetics. (A) Inhibition of LpHAP by L(+)-tartrate. The curves are from the global fit of the data to the competitive inhibition model: $V = V_{\max}[S]/\{ K_m(1 + [I]/K_i) + [S] \}$. (B) Reaction rate as a function 3'-AMP concentration. The curve is the fit to the Michaelis-Menten equation. (C) Reaction rate as a function 5'-AMP concentration. The curve is the fit to the Michaelis-Menten equation.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.