Crystal Structure of a Dual Activity IMPase/FBPase (AF2372) from Archaeoglobus fulgidus

THE STORY OF A MOBILE LOOP*

Kimberly A. Stieglitz‡‡, Kenneth A. Johnson‡, Hongying Yang‡, Mary F. Roberts‡, Barbara A. Seaton‡*, James F. Head‡*, and Boguslaw Stec‡‡

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Several hyperthermophilic organisms contain an unusual phosphatase that has dual activity toward inositol monophosphates and fructose 1,6-bisphosphate. The structure of the second member of this family, an FBPase/IMPase from Archaeoglobus fulgidus (AF2372), has been solved. This enzyme shares many kinetic and structural similarities with that of a previously solved enzyme from Methanococcus jannaschii (MJ0109). It also shows some kinetic differences in divalent metal ion binding as well as structural variations at the dimer interface that correlate with decreased thermal stability. The availability of different crystal forms allowed us to investigate the effect of the presence of ligands on the conformation of a mobile catalytic loop independently of the crystal packing. This conformational variability in AF2372 is compared with that observed in other members of this structural family that are sensitive or insensitive to submillimolar concentrations of Li+. This analysis provides support for the previously proposed mechanism of catalysis involving three metal ions. A direct correlation of the loop conformation with strength of Li+ inhibition provides a useful system of classification for this extended family of enzymes.

In mammalian cells, inositol monophosphatases are abundant cytosolic enzymes necessary to regenerate the synthesis of myo-inositol for the synthesis of phosphatidylinositols. However, in archaeal hyperthermophilic organisms, e.g. Archaeoglobus fulgidus, an IMPase ortholog is thought to be used in the biosynthesis of a unique osmolyte, di-myo-inositol 1,1-phosphate (1). The archaeal enzymes have another twist as well. Besides catalyzing the hydrolysis of Ins-1-P (needed in response to thermal and salt stress) they also very specifically dephosphorylate FBP at C-1 (2). Enzymes with these characteristics exist as two distinct gene products in mammalian systems and several bacteria (3). The dual specificity of the enzyme from primitive hyperthermophiles strongly suggest that IMPase and FBPase enzymes may have evolved from the same gene product (2).

Structural features of the IMPase/FBPase (MJ0109) from Methanococcus jannaschii (2) are similar to those of mammalian IMPase, inositol polyphosphate phosphatase (IPPase), FBPase, and the yeast enzyme Hal2. These enzymes form an extended family for which the general principles of chemical reactivity are the same (3). Two or three metal ions are necessary for catalysis to occur. From the structure of human IMPase with bound Ins-1-P and Gd3+, it was inferred that two metal ions were needed for catalysis (4). However, crystallographic data for pig kidney FBPase (5) and for MJ0109 (6) are consistent with a three-metal ion-assisted catalytic mechanism.

There is strong biochemical evidence that Li+ inhibits IMPase in mammalian cells where a dose response to this ion correlates with a decrease in the intracellular pool of myo-inositol (7). Li+, by inhibiting mammalian IMPases, is thought to cause a depletion of inositol that attenuates the synthesis of phosphatidylinositol necessary to regenerate the phospholipase C dependent phosphatidylinositol/IP3 signaling pathway (8, 9). There are two other enzymes that are very Li+-sensitive with known three-dimensional structures: human IPPase (10) and yeast 3′-phosphoadenosine-5′-phosphate phosphatase, Hal2 (11). In contrast, the dual specificity thermophilic IMPase/FBPase enzymes from M. jannaschii and A. fulgidus and mammalian FBPase are not inhibited by Li+ in the submillimolar range (Table I). Thus, the IMPase superfamily can be functionally subdivided into those that are strongly inhibited by Li+ and those that are less sensitive to Li+. Structural hints on the mechanism of Li+ inhibition of human IMPase are indirect. In pig kidney FBPase (13), which is inhibited by Li+ but activated by low concentrations of K+, monovalent cations are thought to inhibit the enzyme by distorting the active site geometry or preventing turnover or product release. Despite extensive mutagenesis experiments carried out on several enzymes, no structural evidence for a common mechanism for IMPase/FBPase has been observed. In contrast, the existence of a common mechanism in IMPase/FBPase for Li+ inhibition is suggested in the crystallographic data presented here.

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‡‡ Present address: Dept. of Biochemical Sciences, University of Rome “La Sapienza,” I-00185 Rome, Italy.
‡ To whom correspondence should be addressed. Tel.: 713-348-3346; Fax: 713-348-5154; Email: stec@bio.rix.edu.
* The abbreviations used are: IMPase, inositol monophosphatase; FBP, fructose 1,6-bisphosphate; FBPase, fructose-1,6-bisphosphatase; IPPase, inositol polyphosphate phosphatase; IPn, inositol 1,4,5-trisphosphate; Ins-1-P, inositol 1-phosphate; PEG, polyethylene glycol; r.m.s.d., root mean square deviation(s); Hal2, yeast 3′-phosphoadenosine-5′-phosphate phosphatase Hal2; BPNTase, bisphosphate nucleotidase; F-6-P, fructose 6-phosphate.

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zymes in the family, the mechanism of Li$^+$ inhibition is still not entirely understood.

This work presents the results of structural studies for another dual specificity IMPase/FBPase from the hyperthermophilic sulfate reducer A. fulgidus. Like MJ0109, this archaeal enzyme (AF2372) has sequence features close to human IMPase but key structural similarities more akin to pig kidney FBPase. A comparison of the structures of the two archaeal Li$^+$-insensitive enzymes (AF2372 and MJ0109), a phoshaptase of intermediate sensitivity (pig kidney FBPase), and three very Li$^+$-sensitive members of this superfamily (human IMPase, IPPase and yeast Hal2) provides a strong correlation between the level of Li$^+$ sensitivity and the conformation of a critical catalytic loop. The disposition of this catalytic loop may serve as a predictor of Li$^+$ sensitivity for other IMPase family members. Furthermore, changes in the loop conformation of AF2372 induced by ligand binding shed some light into the possible mechanism of Li$^+$ inhibition in the human IMPase and into how this phenomenon might have developed from the more primitive archael enzymes.

### EXPERIMENTAL PROCEDURES

**Chemicals**—d$_1$-Ins-1-P, EDTA, sodium chloride, magnesium chloride, zinc chloride, ammonium chloride, malachite green oxalate, ammonium molybdate, isopropyl-1-thio-β-D-galactopyranoside-isopropyl-1-thio-β-D-galactopyranoside, and SDS-PAGE molecular weight markers, gel filtration molecular weight markers Trizma base, and PEG 8000 were obtained from Sigma. Glycine, myo-inositol, and ammonium sulfate were obtained from Aldrich. Ampicillin was obtained from Fisher. Tryptone and yeast extract were obtained from Difco. Q-Sepharose fast flow, phenyl-Sepharose, and Sephacryl S-200HR resins were obtained from Pharmacia. l-Ins-1-P was synthesized enzymatically using an l-Ins-1-P synthase cloned from A. fulgidus as described previously (14). The l-Ins-1-P and the other small molecule fractions were separated from the synthase with Centricron concentrators. The filtrate was evaporated under high vacuum; a known amount of glucose 6-phosphate was used to quantify the concentration of purified l-Ins-1-P by $^31$P NMR spectroscopy. Further purification of the sugar required chromatography on an AG5-X8 anion exchange column (elution with a gradient of 0.2–1.0 M formic acid); fractions containing l-Ins-1-P were collected and the pH value was adjusted to 7.5 with NH$_4$OH. The purified l-Ins-1-P was stored at 4 °C and used for enzymatic assays and crystal soaks. All other chemicals were reagent grade.

**Overexpression and Purification of AF2372 from A. fulgidus**—Cloning the AF2372 gene from A. fulgidus and overexpression of the gene product in Escherichia coli were carried out as described elsewhere (15). Key steps of the purification procedure included heating of crude protein at 85 °C for 30 min, followed by chromatography on Q-Sepharose fast flow (elution with a linear gradient of 0 to 0.5 M KCl). Column fractions were monitored for IMPase activity using 15 mM pNPP and 30 mM MgCl$_2$ in 50 mM Tris and 1 mM EDTA, pH 7.5. Purity of the fractions was assessed with SDS-PAGE; fractions that were greater than 90% pure were collected, pooled, concentrated 10-fold, and dialyzed against two changes of 50 mM Tris, pH 7.5. The concentrated solution was then loaded onto a 1.6 x 72-cm Sephacryl S-200HR gel filtration column pre-equilibrated with 50 mM Tris and 1 mM EDTA, pH 7.5. Fractions containing pure l-Ins-1-Pase activity (as measured by SDS-PAGE and silver staining of the gels) were concentrated to 12 mg/ml and stored in 20 mM Tris HCl and 1.0 mM EDTA, pH 8.0, until used for crystallization.

**Activity Assays**—Production of inorganic phosphate from FBP and Ins-1-P was monitored using a colorimetric assay (16). Substrate concentration was typically 2 mM (except for pNPP, which was 0.5 mM) in 50 mM Tris, pH 8.0, with varying ammounts of divalent cations (Mg$^{2+}$ or Mn$^{2+}$) added. The solution with substrate and enzyme was heated to 85 or 80 °C (the temperature is indicated with each assay set) for 1 min, then 900 μl of dye was added, and the OD$_{590}$ was measured. A standard P$_2$ curve was used to convert OD$_{590}$ to μmol of P$_2$ produced. Assays typically used 1.0–2.0 μg of enzyme.

**Crystallization**—AF2372 was crystallized by vapor diffusion using hanging drops of 5 μl. The apoenzyme was crystallized in either 0.2 M ammonium nitrate and 30% PEG 3350 (P$_2$$_2$$_2$ form) or 0.2 M dihydrogen ammonium phosphate and 30% PEG 3350 (P$_2$ form). Enzyme solution (2.5 μl of ~10 mg/ml) was mixed with an equal volume of mother liquor and equilibrated using the vapor diffusion hanging drop method. To monitor the catalytic loop conformation under various conditions, the apoenzyme crystals were soaked in solutions containing ligands and different divalent cations. In addition, the enzyme was co-crystallized with Ca$^{2+}$ (50 mM) using 5–7% v/v solution of PEG 8000 in 50 mM Tris, pH 8.0, with 100 mM NaCl. These crystals were then soaked with either d-Ins-1-P or FBP. The drops were equilibrated against the reservoir with the concentration of PEG 8000 between 5 and 7%. Crystals appeared in few days and grew to full size within a week.

**X-ray Data Collection**—Crystals having approximate dimensions of 0.5 x 0.4 x 0.4 mm were mounted in glass capillaries directly from the crystallization wells, or flash frozen in a stream of nitrogen gas. Diffraction data were collected both at cryo conditions and at room temperature. Data were collected at Boston University School of Medicine at room temperature on a Rigaku RU-300 rotating anode generator with an R-AXIS IIC imaging plate detector. Data were also collected at Rice University using a Rigaku H3R rotating anode generator with an R-AXIS IV imaging plate detector with the exception of the apo form in P$_2$ space group that was collected at the Brookhaven Synchrotron beamline X8C. Data collected in Brookhaven and at Boston University was indexed and reduced using DENZO and Scalepack (see Ref. 17), while data collected at Rice University was indexed and reduced using Crystal Clear software. In addition to the apoenzyme (P$_3$, or P$_2$), whose crystals diffracted to ~2 Å, several data sets were collected on crystals soaked with ligands or co-crystallized complexes. The data were collected to ~2.2 Å resolution for the ligand soaks.

**Structural Solution and Refinement**—The phase problem was solved by using the molecular replacement method with the MJ0109 model with a model of structure of the ortholog (Fig. 1A) as a probe structure. The isotropic and anisotropic searches were performed using the program Amore (18). The searches succeeded with the monomer as a model. The initial solution was refined by rigid body refinement and simulated annealing (crystallography NMR software) with manual rebuilding using O (19). The apoenzyme in the crystal form characterized by the space group P$_3$$_2$$_1$$_2$, initially indexed as P$_2$$_1$$_2$$_1$$_2$, was evaluated for merohedral twinning (20) and found to be nearly perfectly twinned with a twinning ratio of 0.499. The reflections for this trigonal form of the apoenzyme were then sorted and processed in space group P$_3$$_2$$_1$$_2$. Simulated annealing refinement in the program Xplor (21) lowered the R-factor significantly. Several macromolecules including manual rebuilding sessions done on the SGI work station with the program Xtalview (22) led to convergence. The final models were refined by the program Sheld-97 (23). The models were evaluated using Procheck (24). Table II provides a summary of data collection and refinement parameters for both the apoenzyme crystal forms and the ligand-soaked ternary complexes.

## RESULTS

### AF2372 Affinities for Substrate and Metal Ions

At the preparatory stage for crystallographic experiments, the substrate specificity as well as the metal ion affinity of AF2372 were evaluated kinetically (at 85 °C). The substrate concentration, 2 mM, was chosen to be much greater than the $K_m$ for Ins-1-P or FBP (Fig. 2), so that a comparison of activities is likely to reflect differences in $V_{max}$. The $K_m$ for Ins-1-P of AF2372 is similar to that of the M. jannaschii ortholog (Fig. 1A) with Ins-1-P, FBP, β-glycerol phosphate, 2-AMP, and pNPP as substrates. Except for FBP, no other –CH$_2$OPO$_3$$^-$$^2$ $\text{pH}$ of the sugar phosphates examined was removed by the enzyme. The $K_m$ values for l-Ins-1-P and FBP have previously been determined as 0.11 and 0.08 mM, respectively (2). With 2 mM l-Ins-
1-P or FBP (values well above $K_m$), the $K_d$ values for Mg$^{2+}$ and Mn$^{2+}$ with these substrates were found to be 15–30 mM (Table III). There was a 2-fold difference in $K_d$ values for Mg$^{2+}$ using 1-Ins-1-P or FBP as substrate. It is possible that the presence of two phosphate groups in FBP facilitate metal ion interactions within the active site. Similar to the phosphatase from M. jannaschii (25), Ca$^{2+}$ was an inhibitory metal ion for both Ins-1-P and FBP hydrolysis catalyzed by AF2372.

The inhibition of AF2372 phosphatase activity (with 1-Ins-1-P as substrate) by Li$^+$ was very weak with an IC$_{50}$ of 290 mM (Table I). This very weak binding precludes an accurate determination of the mechanism of inhibition as uncompetitive (as many other IMPases) or noncompetitive. Regardless of which mode of inhibition is correct, the $K_i$ would be ~290 mM because the substrate concentration was well above $V_{max}$. For comparison, 300 mM Na$^+$ or K$^+$ had no effect on AF2372 activity indicating that the inhibition by Li$^+$ is specific to that monovalent cation. The AF2372 gene product, like MJ0109 (25) and Thermotoga maritima (26), Ca$^{2+}$ was an inhibitory metal ion for both Ins-1-P and FBP hydrolysis catalyzed by AF2372.

The overall fold of AF2372 is highly conserved in the entire IMPase superfamily from archaea to eukaryotes. IMPases from eukaryotes are organized as homodimers, while FBPases are tetrameric (a dimer of dimers). The AF2372 structure was organized in a similar fashion to that of dimeric MJ0109 with the same domain and global secondary structure organization, although there were some changes at the dimer interface. The dimer interface of AF2372 was large, ~1200 Å$^2$. Hydrophobic contacts constituted 47% of the total interface, polar contacts 14.2%, and charged contacts 33.2% of the interface. There appeared to be 11 hydrogen bonds across the interface. Despite a very extensive nonpolar component coming from a cluster of hydrophobic residues (six phenylalanines), the charged and polar contacts appeared to be key stabilizing interactions for the dimer. Consistent with the structural observations, soaking crystals in high Ca$^{2+}$ (>100 mM) disrupted the dimer interface. More specific interactions in the P$_2$$_1$ apoenzyme included hydrogen bonding at the interface as well as a possible salt bridge between positions Arg-27 and Glu-138.

The common architecture of each monomer consisted of an $\alpha/\beta$ structure organized in two domains of alternating layers of $\alpha$-helices and $\beta$-sheets connected by variable length loops (Fig. 2). The structures of proteins in the IMPase superfamily differ...
mostly by the size of the connecting loops. Both domains in AF2372 were connected by a large hinge-like loop that divided the monomer into two structural fragments. Each domain was organized in an alternating \( \alpha-\beta-\alpha \) motif with one possessing a seven-stranded sheet and the other a five-stranded sheet. The larger sheet was flanked by two helices on both sides, while the smaller one had only a single helix in the top layer (Fig. 2A). The elongated loop that separated the monomer into two domains may provide enough flexibility to cause conformational changes within the monomer that affect ligand binding at the active site. That type of mechanism is used in inhibiting mammalian FBPases (28).

Overall, the shape, volume, and even to the certain degree surface accessible area were conserved for both proteins. However, despite having the same number of amino acids, there were significant conformational differences between AF2372 and MJ0109 (Fig. 2B). The N-terminal helix in the \( A. \text{fulgidus} \) IMPase was slightly elongated relative to that in MJ0109. The second N-terminal helix was also slightly elongated compared with MJ0109 (the MJ0109 helix is from residues 45–54; in AF2372 it comprises residues 44–58). The shorter loop regions in MJ0109 might be one of the major factors that confer greater stability for the \( M. \text{jannaschii} \) homologue (\( T_m = 87 \, ^\circ \text{C} \) for AF2372 versus 95 \( ^\circ \text{C} \) for MJ0109 (15)). The remaining helices of AF2372 were very similar to both MJ0109 and human IMPase in length and positioning (6) with one exception. The first significant difference was at the elongated loop 105–112 in AF2372 that provides most of the crystal contacts in both

![Crystal Structure of AF2372](http://www.jbc.org/)
Crystal Structure of AF2372

Kinetic parameters for L-Ins-1-P and FBP hydrolysis at 80 °C by AF2372

| Substrate \(a\) | \(M^2+\) | \(V_{max}\) | \(K_{cat}^{(app)}\) |
|---|---|---|---|
| L-Ins-1-P | Mg\(^{2+}\) | 3.27 ± 0.67 | 32.9 ± 8.8 |
| L-Ins-1-P | Mn\(^{2+}\) | 2.20 ± 0.39 | 16.0 ± 4.4 |
| FBP | Mg\(^{2+}\) | 1.53 ± 0.30 | 15.1 ± 4.7 |
| FBP | Mn\(^{2+}\) | 1.92 ± 0.27 | 17.3 ± 3.7 |

\(a\) Substrate, 2.0 mm, was dissolved in 50 mm Tris, pH 8.0, at 80 °C. This value is well above the average temperature factors (B) for the entire structure. This ratio drops from 2.8 in the P2\(_1\) crystal form to ~1.2 in P3\(_2\)3.

The third structure was for the enzyme with L-Ins-1-P and two Ca\(^{2+}\) bound at the active site (Fig. 4B). This structure was derived by soaking the P2\(_1\) crystals with 20 mM substrate and 50 mM Ca\(^{2+}\). The occupancy of metal ions as well as the substrate molecule refined around 60%. The precise description of the active site and coordination of the metal ions was hindered by the resolution and the quality of the data. However, general features were easily discernible, and they are described below. There were two Ca\(^{2+}\) metal ions at each active site visible in the structure. Both calcium ions were coordinated by five ligands arranged in a distorted octahedral coordination with one ligand missing. The missing ligand could be easily complemented by a water molecule. The ligands of Ca1 included Asp-82, Asp-85, Asp-200, and two oxygens of the substrate molecule, while Ca2 was coordinated by Asp-82, Glu-67 OE1, and a single oxygen of the phosphate group. The ionotrope moiety was coordinated by the Asp-85, backbone nitrogen of Ala-172, guanidino group of Arg-191, and Tyr-155. The interactions of metal ions and substrates with the enzyme are summarized in Table IV.

The fourth structure was derived from the P3\(_2\) crystal soaked with FBP and Ca\(^{2+}\) at concentrations of 20 mM and 50 mM, respectively. The refined occupancy for the metals as well as the substrate was 100%. Three Ca\(^{2+}\) ions were present in this structure (Fig. 4C). The coordination of two calcium ions was analogous to that observed in the L-Ins-1-P–enzyme complex. The third Ca3 metal ion was located more remotely from the active site than the third metal ion in MJ0109. In subunit A it was bound at a classical hepta-coordinated site formed by five water molecules and two protein ligands originating from the mobile loop (Thr-40 Oy1 and carbonyl oxygen of Pro-41). At subunit B two of the water molecules were missing. Ca3 was quite removed from the vicinity of other metal ions with distances ~4.5 Å from Glu-67 and ~4.5 Å from Asp-38. Both of these tentative ligands have a bridging water molecule coordinating Ca3. This implies that if it participates in catalysis, additional motion of the catalytic loop would be required to bring it to the immediate proximity of the tentative nucleophile. Additionally, the structure of MJ0109 suggested that activating and not inhibitory metal ions were necessary to observe a catalytically competent third metal ion binding site.

Therefore, we have obtained a crystal structure of the product complex with activating metal ions (3 Mn\(^{2+}\), fructose-6-phosphate (F-6-P), and inorganic phosphate) in P3\(_2\) crystal form. The occupancy of the metal ions and the phosphate moiety refined to 100%, while the product F-6-P refined at ~60%. As expected, the loop moved toward the active site, and the direct coordination of the third metal ion changed (Figs. 4D and 5D). The Mn3 has an octahedral coordination with three protein ligands (side chains of Asp-38, Thr-40, and Glu-67) as well as three water molecules directly coordinating the metal ion. This is in marked difference with the FBP-calcium complex in which the third metal ion was lacking a direct protein bridge (Glu-67) to the second metal ion. Additionally, one of the inorganic phosphate oxygens was directly coordinated to Thr-84, a feature that clearly suggested the collapsed product complex. This oxygen position was previously suggested to be a site for the nucleophilic water molecule in a precatalytic complex. The detailed description of the ternary complexes will be published separately.

Structural Differences among Two Apo Forms and Complexes with Substrates and Metal ions, a Conformation of the Catalytic Loop—The first loop that directly follows the N-terminal helix (residues 32–43) is a critical catalytic loop. This mobile loop was relatively disordered in the apoenzyme but became more...
ordered upon ligand binding. Thermal factors of the refined apoenzyme structures consistently showed elevated values in this region; this is an indicator of mobility and/or disorder. The structures of AF2372 in complexes with Ins-1-P/Ca\(^{2+}\) or FBP indicated that, in analogy to the behavior of FBPase and MJ0109, the catalytic loop is stabilized by the presence of metal ions and ligands.

The refined conformation of the mobile loop (residues 32–43) was slightly different in the apo form and when a ligand was bound (Fig. 5, A, B, and C). Note the difference in the quality of the electron density comparing the apoenzyme to the liganded structures. The changes in B factors for this region when crystals were soaked in ligand solutions did fully reflect the increased “ordering” of the loop. There was a systematic drop in the B factor for the protein plus ligand structures. The ratio of the average B factor for residues 32–43 to the rest of the molecule approached 1 for both of the substrate/product complexes with three metal ions (Ca\(^{2+}\) or Mn\(^{2+}\)).

Soaking in metal ions alone did not improve loop stability significantly. In fact, crystals soaked or co-crystallized with metal ions alone diffracted poorly (>3 Å). Under conditions that gave the P3\(_2\) form of the enzyme, the combination of both substrate and metal ions was needed to stabilize the loop in these Tris/PEG 3350 conditions. When the AF2372 apoenzyme was soaked in D-Ins-1-P, as well as other substrate/metal complexes, a conformational change occurred that shifted the loop on average 2.5 Å closer to the metal ions following the behavior observed in MJ0109 structures that contained ligands at the active.

**Architectural Differences with Other Members of the Family, a Catalytic Loop Variability**—The length of the amino acid peptide chain representing the members of the extended IMPase family differs substantially and extends from 252 residues for MJ109 and AF2372 to 400 for IPPase. Structural
FIG. 4. The active site of AF2372 covered with $2F_o - F_c$ electron densities contoured at 1.5σ level with Asp-82, Asp-85, and Asp200 as the reference points. A, an apoenzyme empty active site in P3$_2$ crystal form. B, P2$_1$ crystal form with d-Ins-1-P and two Ca$^{2+}$ ions; C, P3$_2$ crystal form with FBP and three Ca$^{2+}$ ions. D, P3$_2$ crystal form with F-6-P, inorganic phosphate, and three Mn$^{2+}$ ions.
alignments carried out with SEQUOIA identified the common catalytic core despite relatively low levels of primary sequence homology. However, as inferred from the size difference, extensive external loops and even small domains were formed as additions to the core domain. The superposition in SEQUOIA indicated that AF2372 and MJ109 were the closest structural homologs and could be superimposed with 209 positions to an r.m.s.d. of 1.5 Å (Table V). There were fewer structurally conserved residues and higher r.m.s.d. when AF2372 was superimposed onto the other IMPase or FBPase structures (Table V). However, two clusters of similarities emerged. The structures in the individual clusters had closer similarities to each other than to proteins in the other cluster. AF2372, MJ0109, and FBPase formed one cluster, while Hal2 and human IMPase and IPPase formed the second cluster. The extent of the r.m.s.d. was up to 2.5 Å as measured by superposition of AF2372 with human IPPase.

Despite significant structural additions, the structures superimposed well around the catalytic loop. The two N-terminal helices provided a good anchor for judging the conformational variability of this loop. The five structures of AF2372 determined in different crystal lattices and with different numbers of metal ions bound provided an additional measure of structural divergence. The two apo forms had a more open conformation of the loop, while the metal and substrate bound forms had the loop in a closed conformation. The overall extent of this

| Interaction | Distance (Å) L-Ins-1-P/Ca²⁺ (A/B) | Distance (Å) FBP/Ca²⁺ (A/B) | Distance (Å) F6P/Mn²⁺ (A/B) |
|-------------|---------------------------------|-----------------------------|-----------------------------|
| Metal Ions  |                                 |                             |                             |
| Met₁...O₇P | 2.19/2.79                       | 2.80/2.79                   | 2.91/1.90                   |
| Met₁...O₁P | 2.32/2.92                       | 2.35/2.80                   | 1.99/2.63                   |
| Met₁...O₃P | 2.00/2.78                       | 2.78/2.79                   | 2.29/1.92                   |
| Met₁...O₅P | 1.96/2.78                       | 2.80/2.80                   | 1.98/2.75                   |

Phosphate moieties (6', 1')

* The distance is given in both A and B subunits of AF2372.
motion was similar to that observed for MJ0109 and amounts to a displacement of the tip of the loop by \(2.5 \text{ Å}\) (Fig. 6A).

Separate from changes in conformation of the catalytic loop in the presence or absence of ligand, there were significant differences in the loop conformation in the IMPase superfamily. Superimposing all six known members of the family eluci-
TABLE V

| AF2372 | AF2372 | IGOI | ICNO | IAWB | IPAP |
|--------|--------|------|------|------|------|
| HGO    | 1.59 [20] |      |      |      |      |
| (M749) | 2.10 [241] |      |      |      |      |
| ICNO   | 1.94 [173] | 1.58 [153] |      |      |      |
| (FPPase) | 3.15 [260] | 3.25 [243] |      |      |      |
| IAWB   | 1.59 [205] | 1.57 [187] | 2.05 [246] |      |      |
| (Hom Impase) | 3.31 [236] | 3.19 [243] | 3.98 [247] |      |      |
| IPAP   | 1.79 [171] | 1.75 [174] | 1.83 [156] | 1.60 [214] |      |
| (Hal2) | 3.70 [245] | 3.49 [248] | 4.69 [270] | 2.88 [219] |      |
| IPP    | 2.56 [136] | 2.03 [147] | 1.88 [107] | 1.88 [153] | 1.52 [161] |
| (Hom Impase) | 4.97 [245] | 6.17 [248] | 6.86 [272] | 5.97 [256] | 7.13 [304] |

* The upper row indicates the r.m.s.d. and number of residues in final superposition (in brackets) with 3 Å cutoff and 30° continuation. The lower row indicates the r.m.s.d. and number of residues in total superposition with 8 Å cutoff and 80° continuation.

** Successful after weakening the gap penalty to 6.

** The use of mobile loops as sensors of monovalent ions is quite common in proteins. There are many examples of ubiquitous sodium/potassium transporters and ion channels that utilize mobile loops and differential cation sensitivity to operate. Na+ and Cl−-dependent transporters GLYT1 and GLYT2 remove glycine from the synaptic cleft. Extracellular loop 1 in the GLYT2 transporter was identified as the source of structural heterogeneity that is involved in the specific effect of lithium on serotonin transport (30). Similarly, the glutamate transporter GLT-1 has a region identified as loop-like that is coupled to drive glutamate transport. When Ser-440 in this loop was mutated to glycine, the GLT-1 mutant could use Li+ to drive glutamate transport (31). The Na+/Ca2+ exchangers NCX1 and NCX3 reveal structural domains (i.e. loops) important for differential sensitivity to exter-
nal Ni\(^{2+}\) or Li\(^+\) (32). Although the last of these is a totally separate biological system, this transporter has ion sensitivities that parallel those of Li\(^+\)-sensitive IMPases.

In the extended IMPase family the mobile loop appears to have a double role. Its mobility is associated with and controlled by the metal ion/substrate binding. The structures of

**Fig. 6. Analysis of mobile loop conformation in the IMPase superfamily.** A, superposition of all five structures of AF2372 with P\(_2\)_\(_1\) apoenzyme (in blue), P\(_3\)_\(_2\) apoenzyme (in yellow), IMP + 2 Ca\(^{2+}\) (in purple), FBP + 3 Ca\(^{2+}\) (in green), and F-6-P + Pi+3 Mn\(^{2+}\) (in red). B, superposition of Li\(^+\)-insensitive enzymes AF2372 (in red) with MJ0109 (in green) and pig kidney FBPase (in orange). C, superposition of Li\(^+\)-sensitive enzymes human IMPase (in purple), human IPPase (in light blue) with Hal2 (in dark blue). The mobile catalytic loop is indicated by the black arrow.
archaeal dual function IMPase/FBPases suggest that this loop mobility is required for the efficient release of product (i.e. phosphate). The second role of the mobile loop is to differentiate the influence exerted by monovalent metal ions. In Li+-sensitive enzymes, the loop appears to be longer and in a more open (farther away from the active site) conformation, while in Li+-insensitive enzymes, the mobile loop is shorter and in a more closed conformation. This structural differentiation appears to be useful in classifying IMPase-like proteins. It remains to be seen what is the precise role of the loop in Li+ inhibition and how universal is this structural classification. The predictive power of this hypothesis needs to be tested on more representatives of this family of proteins.

Recently, a new human IMPase (35, 36) as well as new human BPNTase (37) were cloned. The new inositol monophosphatase termed A2 appears to have significant sequence homology to BPNTase termed A2. The IMPase termed A2 appears to have significant sequence homology to already characterized Li+-sensitive IMPase. The rat 3'-phosphoadenosine-5'-phosphate termed Hal2 (37). Additionally, during the submission process of this paper another enzyme from this extended protein family was characterized by x-ray crystallography (38).

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