Feedback Inhibition and Product Complexes of Recombinant Mouse Muscle Adenylosuccinate Synthetase*

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govern the committed step of AMP biosynthesis, the generation of 6-phosphoryl-IMP from GTP and IMP followed by the formation of adenylosuccinate from 6-phosphoryl-IMP and L-aspartate. The enzyme is subject to feedback inhibition by AMP and adenylosuccinate, but crystallographic complexes of the mouse muscle synthetase presented here infer mechanisms of inhibition that involve potentially synergistic ligand combinations. AMP alone adopts the productive binding mode of IMP and yet stabilizes the active site in a conformation that favors the binding of Mg$^{2+}$-IMP to the GTP pocket. On the other hand, AMP, in the presence of GDP, orthophosphate, and Mg$^{2+}$, adopts the binding mode of adenylosuccinate. Depending on circumstances then, AMP behaves as an analogue of IMP or as an analogue of adenylosuccinate. The complex of adenylosuccinate-GDP-Mg$^{2+}$-sulfate, the first structure of an adenylosuccinate-bound synthetase, reveals significant geometric distortions and tight non-bonded contacts relevant to the proposed catalytic mechanism. Adenylosuccinate forms from 6-phosphoryl-IMP and L-aspartate by the movement of the purine ring into the α-amino group of L-aspartate.

Adenylosuccinate synthetase (IMP:L-aspartate ligase (GDP-forming), EC 6.3.4.4) catalyzes the committed step in AMP biosynthesis (1–3). In vertebrates, the synthetase is also a component of the purine nucleotide cycle and may play roles in metabolism beyond the maintenance of adenine nucleotide levels (2). In organisms responsible for malaria and related diseases, adenylosuccinate synthetase plays an integral role in a dominant salvage pathway that transforms hypoxanthine into adenine nucleotides. Potent inhibition of adenylosuccinate synthetase generally terminates growth. Hence, the synthetase is a target in the development of herbicides and in the development of drugs for the treatment of cancer and infectious disease (2).

The reaction for synthetases from Escherichia coli and mouse muscle (and presumably all other synthetases as well) occurs in two stages: First, the γ-phosphoryl group of GTP is transferred to the O-6 atom of IMP, forming 6-phosphoryl-IMP (6PIMP).1 Second, the α-amino group of L-aspartate displaces the 6-phosphoryl group of 6PIMP to form adenylosuccinate (SAMP) (1–7). Data from isotope exchange kinetics support the formation of 6PIMP as an enzyme-bound intermediate (4), and indeed, 6PIMP appears at the active site in crystal structures of the E. coli and mouse muscle synthetases (7, 8). The kinetic mechanism is random sequential with a strong bias in favor of the association of L-aspartate after the formation of an enzyme-IMP-GTP complex (9). The synthetase requires Mg$^{2+}$, but Ca$^{2+}$ or Mn$^{2+}$ also support catalysis, albeit at lower rates (1, 2, 10). Synthetases are subject to several possible mechanisms of metabolite regulation in vivo: inhibition of the E. coli synthetase by stringent effectors, such as guanosine 3′-diphosphate 5′-diphosphate (11, 12); substrate inhibition of the mouse muscle enzyme by high concentrations of IMP (13–15); and feedback inhibition by AMP (16).

Although crystal structures of the synthetase with AMP (17), IMP (8, 18), or 6PIMP (7, 8) are in the literature, complexes with adenylosuccinate or L-aspartate are unavailable. In fact, crystallographic analogues of adenylosuccinate and L-aspartate are not available. In fact, only the first structure of adenylosuccinate-bound synthetase (19) is known. In complexes of hadacidin, the N-formyl-N-hydroxylglyoxime (N-formyl-N-hydroxyglyoxime) is a competitive inhibitor with respect to L-aspartate (K$\text{m}$ $\approx$ 10$^{-6}$ M) (21), but does it faithfully represent interactions of the true substrate? The N-hydroxyl and N-formyl groups of hadacidin together are similar in charge and to protonate the α-carboxyl group of L-aspartate (3). Hence, hadacidin is like the mono-anionic form of succinate. In complexes of hadacidin, the N-hydroxyl group forms hydrogen bonds with an aspartyl side chain essential for catalysis (Asp$^{22}$ and Asp$^{23}$ in E. coli and mouse muscle synthetases, respectively). An unprotonated oxygen atom from the α-carboxyl group of L-aspartate, however, cannot hydrogen bond with this aspartyl side chain. Either the active site has different conformations in its hadacidin and L-aspartate complexes or an additional cofactor (metal cation or proton) must stabilize the interaction between the aforementioned carboxyl groups (3).

On the basis of model building, the β-carboxyl group of L-aspartate may have a catalytic function in abstracting a proton from the α-amino group of L-aspartate and in orienting the lone pair orbital of the α-amino group toward the C-6 atom of IMP (3, 5). This putative catalytic role for the β-carboxyl group may account for the absence of measurable synthetase activity with other amino acids such as glycine, serine, and asparagine. On the other hand, hydroxylamine is a substrate of the synthetase (22, 23), and in a buffer system of comparable ionic strength, it supports a maximal catalytic rate comparable with that of L-aspartate.5 Hence, the participation of the β-carboxyl group of L-aspartate as a base catalyst is at best speculative.

1 The abbreviations used are: 6PIMP, 6-phosphoryl-IMP; SAMP, adenylosuccinate; PDB, Protein Data Bank.
2 E. Underbakke and R. B Honzatko, unpublished.
The mechanism of feedback inhibition of the synthetase by AMP is also not fully resolved. Only a single AMP complex is available for the E. coli enzyme (17), and in that complex the enzyme itself is modified near the active site by an adduct of 2-mercaptoethanol. Potent inhibition of the synthetase, such as that observed for AMP, generally involves synergistic combinations of two or more ligands. For instance, nitrate enhances inhibition by GDP (5, 23) and GDP/Pi enhances inhibition by hydantocidin 5'-phosphate (19, 24). A complex of AMP alone therefore may not provide a comprehensive model for feedback inhibition of the synthetase.

Presented here are crystal structures of recombinant mouse muscle adenylosuccinate synthetase in complexes with AMP alone (AMP complex), AMP-GDP-P,Mg2+ (AMP-product complex) and SAMP-GDP-SO42-Mg2+ (SAMP-product complex). The first two complexes reveal distinct binding modes for AMP alone and in the presence of GDP/Pi. The SAMP-product complex offers the first instance of an enzyme active site ligated by SAMP (10 mM in the SAMP-product complex), potassium phosphate (25 mM in the AMP-product complex), SAMP (10 mM in the SAMP-product complex), magnesium acetate (10 mM in both the AMP and AMP-product complexes) and/or magnesium sulfate (15 mM in the SAMP-product complex). The precipitant solution contained 100 mM Heps, pH 7, polyethylene glycol 8000, and 200 mM magnesium acetate (for both the AMP and AMP-product complexes) or 100 mM magnesium sulfate (for the SAMP-product complex). Prisms of equal dimensions appeared in the following concentrations of polyethylene glycol 8000:16% (w/v) for the AMP complex, 19% (w/v) for the AMP-product complex and 14% (w/v) for the SAMP-product complex. Crystals were transferred to cryogenic solutions as described previously (25). The final cryogenic solution contained 21% (v/v) glycerol and equal parts of the above protein solution (less the protein) and the precipitant solution.

**Materials**—Magnesium sulfate and magnesium acetate came from Fisher, and all other reagents were from Sigma.

**Purification and Crystallization of the Recombinant Mouse Muscle Isozyme**—The enzyme was prepared as described previously (25) and was at least 95% pure on the basis of SDS-polyacrylamide gel electrophoresis. Crystals were grown by the method of hanging drops. Wells contained 500 μl of precipitant solution, and droplets had equal volumes (3 μl) of protein and precipitant solutions. The protein solution (10 mg/ml enzyme in 50 mM Heps, pH 7, 50 mM NaCl, 1 mM dithiothreitol, and 0.5 mM EDTA) contained AMP (10 mM and 5 mM in the AMP and AMP-product complexes, respectively), GDP (5 mM in AMP-product and SAMP-product complexes), potassium phosphate (25 mM in the AMP-product complex), SAMP (10 mM in the SAMP-product complex), magnesium acetate (10 mM in both the AMP and AMP-product complexes) and/or magnesium sulfate (15 mM in the SAMP-product complex). The precipitant solution contained 100 mM Heps, pH 7, polyethylene glycol 8000, and 200 mM magnesium acetate (for both the AMP and AMP-product complexes) or 100 mM magnesium sulfate (for the SAMP-product complex). Prisms of equal dimensions appeared in the following concentrations of polyethylene glycol 8000:16% (w/v) for the AMP complex, 19% (w/v) for the AMP-product complex and 14% (w/v) for the SAMP-product complex. Crystals were transferred to cryogenic solutions as described previously (25). The final cryogenic solution contained 21% (v/v) glycerol and equal parts of the above protein solution (less the protein) and the precipitant solution.

**Model Refinement**—Initial phases for all complexes reported here came from molecular replacement using the 6P1M-GDP-hadacidin complex of the mouse muscle synthetase (PDB accession code 1LON), without ligands and water molecules. Models representing the ligands were fit to omit electron maps using XTALVIEW (26). Refinement of the model, following protocols described previously (25), employed CNS (27). Force constants and parameters of stereochemistry came from Engh and Huber (28). Estimates of coordinate error used the method of Luzzati (29). Stereochemistry of the refined models was analyzed by PROCHECK (30). Superpositions of structure were done with software from the CCP4 package (31).

**RESULTS**

Data collection and refinement statistics are in Table I. The AMP, AMP-product, and SAMP-product complexes have nominal resolutions of 2.7, 2.5, and 2.4 Å, respectively. All crystals belong to the space group P43212 and are isomorphous, having but one subunit of the synthetase dimer in the asymmetric unit. In all structures, electron density is present for the whole sequence save the first 26 amino acid residues of the N termi-
nus. Stereochemistry of the refined models is as good as or better than models derived from data of comparable resolution. No residues fall in unallowed regions of the Ramachandran plot, and \(-90\%\) of all residues lie in most favorable regions. Thermal parameters vary from 27 to 100, 19 to 75, and 15 to 88 Å\(^2\) for the AMP, AMP-product, and SAMP-product complexes, respectively. Root-mean-square deviations in corresponding C\(_\alpha\) atoms are less than 0.5 Å for superpositions of each of the complexes onto the 6PIMP-GDP-hadacidin complex of the mouse muscle synthetase (PDB accession code 1LON).

The active site of the synthetase has six dynamic components, which are in ligated or ligand-free conformations or are disordered, depending on the presence of specific ligands and conditions of crystallization (8, 25). The unligated structure of the mouse muscle synthetase (PDB accession code 1J4B) defines the ligand-free conformations of these dynamic components, whereas the 6PIMP-GDP-hadacidin complex (PDB accession code 1LON) defines their ligated conformations. All dynamic components in the complexes reported here lie in strong electron density. Fig. 1 provides an overview of the SAMP-product complex and defines the dynamic elements of the synthetase. The Switch loop (residues 70–83), the IMP loop (residues 152–165), the GTP loop (residues 448–452), and the Val loop (residues 304–310) are in their ligated conformations. The pre-Switch element (residues 65–69) is in its ligated conformation or nearly so. Small but significant variations in conformation of the pre-Switch element are discussed thoroughly below. The Asp loop (residues 330–336) is in its ligand-free conformation in the AMP and AMP-product complexes and in its ligated conformation in the SAMP-product complex.

**AMP Complex (PDB Accession Code 1MF1)**—The IMP pocket and the \(\beta\)-phosphoryl site of the GDP pocket have appreciable electron density in omit maps. The electron density at the IMP pocket is consistent with a molecule of AMP (Fig. 2). The electron density associated with the \(\beta\)-phosphoryl site of GDP is probably an acetate molecule, given the following observations. (i) The thermal parameter of a water molecule refined at the \(\beta\)-phosphoryl site is approximately one-half the value of thermal parameters for nearby atoms of the protein, whereas the thermal parameters for an acetate molecule are comparable with those of nearby atoms. (ii) The mouse muscle IMP complex (PDB accession code 1IWE; resolution of 2.1 Å) has electron density at the \(\beta\)-phosphoryl site of GDP that clearly represents a bound acetate molecule (8).
The 5'-phosphoryl group of AMP interacts with the backbone amide and side chain of Thr$^{163}$ and with the side chains of Asn$^{68}$, Arg$^{177}$, and Thr$^{271}$ (Arg$^{177}$ comes from the symmetry-related subunit of the dimer). The 2'-hydroxyl group, N-7 atom, and 6-amino group of AMP hydrogen bond with backbone carbonyl 305 (Val loop), the side chain of Asn$^{256}$, and backbone carbonyl 69 (part of the pre-Switch loop), respectively. The pre-Switch loop is almost in its ligated conformation (as defined by the 6PIMP-GDP-hadacidin complex), but peptide linkages between residues 68–70 have significantly different orientations (see below). One oxygen atom of Asp$^{43}$ is 3.4 Å from the N-1 atom of AMP. As neither the N-1 atom nor Asp$^{43}$ should be protonated at pH 7, this interaction is probably a nonbonded contact rather than a hydrogen bond.

**AMP-product Complex (PDB Accession Code 1MF0)**—Electron density from omit maps is consistent with bound AMP, orthophosphate (labeled $P_i$), Mg$^{2+}$, and GDP from an omit map (coefficients of $2F_{obs} - F_{calc}$, $a_{calc}$ phases) contoured at $3\sigma$ using a cut-off radius of 1 Å (top). Dashed lines represent donor-acceptor interactions and coordinate bonds to the Mg$^{2+}$ (bottom).

**Fig. 3. Stereoview of AMP-product complex.** Electron density covering AMP, orthophosphate (labeled $P_i$), Mg$^{2+}$, and GDP is from an omit map (coefficients of $2F_{obs} - F_{calc}$, $a_{calc}$ phases) contoured at $3\sigma$ using a cut-off radius of 1 Å (top). Dashed lines represent donor-acceptor interactions and coordinate bonds to the Mg$^{2+}$ (bottom).
Asp$_{43}$, P$_i$, the phosphoryl groups of GDP and a water molecule (Fig. 3). The water molecule is 2.8 Å from the Mg$^{2+}$/H$_{11001}$, and also hydrogen bonds with Arg$_{337}$. The oxygen atom from Asp$_{43}$, on the other hand, has a coordination distance to the Mg$^{2+}$/H$_{11001}$ of 2.0 Å. These coordinate and hydrogen bonds are of significance to the proposed mechanism of the synthetase (see "Discussion").

The P$_i$ molecule, in addition to its coordination with Mg$^{2+}$/H$_{11001}$, hydrogen bonds with backbone amides 43, 70, and 256, with the side chains of Lys$_{46}$, His$_{71}$ and Asn$_{256}$, and with the 6-amino group of AMP (Fig. 3).

Although interactions at the 5'-phosphoryl group of AMP are identical in the AMP and AMP-product complexes, the presence of P$_i$, Mg$^{2+}$/H$_{11001}$ and GDP alters the interactions at the base of AMP. By coordinating to Mg$^{2+}$, the side chain of Asp$_{43}$ moves toward the N-1 atom of AMP. Both oxygen atoms of the aspartyl side chain are ~3.0 Å from atom N-1. These contacts, however, for reasons noted above, are probably not hydrogen bonding.

**Fig. 4. Stereoview of SAMP-product complex.** Electron density covering SAMP is from an omit map (coefficients of $2F_{obs} - F_{calc}$, $F_{calc}$ phases) contoured at 3σ using a cut-off radius of 1 Å. Dashed lines represent donor-acceptor interactions and coordinate bonds to the Mg$^{2+}$.

**Fig. 5. Definition of the tilt angle of the base moiety.** The alignment of 6PIMP and SAMP comes from the superposition of C$\alpha$ coordinates of the 6PIMP-GDP-hydridin complex (8) and the SAMP-product complex. The base moiety of 6PIMP defines a tilt angle of 0°.

**Fig. 6. Comparison of SAMP tautomers in solution and SAMP in its ground state enzyme complex.** See "Discussion" for details.

**Fig. 7. Model for the L-aspartate6PIMP-GDP-Mg$^{2+}$ complex.** Interactions involving the two hydrogen atoms of the $\alpha$-amino group of L-aspartate orient the lone pair orbital toward the C-6 atom of 6PIMP.
The coordination of backbone carbonyl 70 to Mg$^{2+}$/H$_{11001}$ evidently triggers a rotation of 180° in the peptide linkage between residues 69 and 70. The pre-Switch loop (residues 65–69) is now in its ligated conformation. Consequently, backbone carbonyl 69 no longer hydrogen bonds with the 6-amino group of AMP (as observed in the AMP complex above), being replaced instead by backbone carbonyl 68. The side chain of Asn68 also shifts by $\pm 1\text{Å}$ but retains its hydrogen bond with the 5-phosphoryl group of AMP. To avoid a close contact with the Pi molecule, the entire base of AMP tilts some 25° relative to that observed in the AMP complex. As a consequence, Asn256 no longer hydrogen bonds with the N-7 atom of AMP. Furthermore, the 2'-hydroxyl group of AMP hydrogen bonds with backbone amide 306, instead of backbone carbonyl 305, as in the AMP complex. In fact, the peptide link between residues 305 and 306 has undergone a rotation of $\pm 180°$.

**SAMP-Product Complex (PDB Accession Code 1IMEZ)**—Electron density from omit maps is consistent with bound GDP, SO$_4^{2-}$ (at the 6-phosphoryl site of 6PIMP), Mg$^{2+}$, and SAMP at the active site (Fig. 4). All dynamic loops adopt their ligated conformations. Interactions involving GDP, SO$_4^{2-}$, Mg$^{2+}$, and the AMP moiety of SAMP are identical to those above for the AMP-product complex. (The sulfate anion adopts all of the interactions of Pi in the AMP-product complex.) The tilt of the base moiety of SAMP (30°) is approximately the same as that observed for the base of AMP in the AMP-product complex. Interactions involving the succinyl moiety of SAMP maintain the ligated conformation of the Asp loop. One of the oxygen atoms of the “β”-carboxyl group of SAMP hydrogen bonds with the side chains of Arg$^{335}$ and Thr$^{333}$, whereas the other oxygen...
AMP from complexes of the mouse muscle and *E. coli* synthetases superimposes on the productive binding mode of IMP (chain A of PDB accession code 1IWE) (8). Yet the pre-Switch loops of these AMP complexes match the conformation of the pre-Switch loop when it binds IMP nonproductively (chain B of PDB accession code 1IWE). Backbone carboxyl 69 hydrogen bonds with the 6-amino group of AMP, whereas that same carboxyl forces the base moiety of IMP out of its pocket to relieve an unacceptable contact with the O-6 atom (8). In crystallographic complexes, IMP binds to the GTP pocket only when the pre-Switch loop is in its nonproductive conformation. Hence, AMP ligation may favor the binding of IMP to the GTP pocket as well.

GDP, P₈, and Mg²⁺ alter the binding of AMP and the conformation of the pre-Switch loop. The conformation of the protein in the AMP-product complex is identical to that of the 6PIMP-GDP complex (PDB accession code 1LNY) (8), even though recognition of the base moieties of AMP and 6PIMP differ substantially. The hydrogen bond between the 8-amino group of AMP and P₈ (as opposed to the corresponding covalent bond in 6PIMP) forces the base of AMP to tilt some 25° out of the space occupied by the base of 6PIMP. As a consequence, Asn256 cannot hydrogen bond with the N-7 atom of AMP in the AMP-product complex. In fact, AMP in the presence of GDP-P₈-Mg²⁺ binds as an analogue of SAMP, whereas AMP alone binds as an analogue of IMP.

Atoms N-1, C-6, N-6, and C₅ of SAMP in solution probably lie in the same plane, favoring tautomerism of SAMP in which either atom N-6 or atom N-1 bears a proton (Fig. 6). Hence, in solution, a partial double bond covalently links atoms N-6 and C-6. The synthetase, however, stabilizes a conformation of SAMP (C-6 to N-6 torsion angle of ~70°) that precludes the tautomerism of Fig. 6. By reducing the bond order between atoms C-6 and N-6 of SAMP, the synthetase enhances the basicity of the N-6 atom. In fact, the pKₐ of atom N-6 of enzyme-bound SAMP may approximate that of the imidazolium group of histidine. Protonation must occur at some point of the reverse reaction to stabilize the development of negative charge on atom N-6 as l-aspartate forms. Evidently, the synthetase accomplishes proton transfer to atom N-6 in its SAMP ground state complex.

In the forward reaction, therefore, the neutral amino group of l-aspartate probably participates in two hydrogen bonds: It is a proton donor to backbone carboxyl 68 (carboxyl 38 in the *E. coli* synthetase) and a proton donor to a bifurcated hydrogen bond with a pair of oxygen atoms coordinated to the catalytic Mg²⁺ (Fig. 7). This hydrogen bond network orients the lone pair orbital of the neutral α-amino group of l-aspartate toward atom C-6 of 6PIMP. Neither backbone carboxyl 68 nor the oxygen atoms coordinated to Mg²⁺ are effective catalytic bases in the abstraction of a proton from the α-amino group of l-aspartate. Furthermore, the SAMP complex does not support a direct catalytic role for the β-carboxyl group of l-aspartate in proton abstraction (5). Proton abstraction is unnecessary, however, if as suspected the synthetase stabilizes the N-6 protonated form of SAMP.

The development of negative charge on the N-1 atom of the purine system occurs with the formation of the tetrahedral transition state (Fig. 8). Protonation would stabilize charge development at the N-1 atom, and indeed the nonbonded contact (2.5 Å) between Asp43 and the N-1 atom, observed in the SAMP crystal structure, probably reflects a hydrogen bond that facilitates the placement of a proton on the N-1 atom during the transition state. The transfer of a proton from Asp43 to the N-1 atom of the purine could initiate the reaction through the
formation of a C-6 cation, or the proton transfer could occur later after substantial charge has developed on atom N-1 (Fig. 8). The close contact distance (2.8 Å) between an oxygen atom of the sulfate anion and atom C-6 of SAMP, however, suggests an electrostatic interaction between a negatively charged oxygen atom and an electron-deficient carbon atom. Ultimately the tetrahedral transition state must collapse to the SAMP ground state complex. His71 (His41 in the E. coli synthetase) probably transfers its proton to the leaving group (orthophosphate) as shown in Fig. 8.

The bending distortion imposed by the synthetase on the purine ring increases the energy of SAMP in its ground state complex. The distortion of the purine ring probably disappears in the intermediate tilt position, as observed in the AMP complex, but may be introduced again in the 6PIMP complex. The energy penalties associated with bending distortions in the ground state complexes of SAMP and 6PIMP probably do not carry over to the transition state. The bending distortions in the ground state complexes evidently cannot relax by rigid body motions of their 5'-phosphoribosyl moieties because of tight donor-acceptor contacts involving their 2'-hydroxyl groups and backbone carbonyl 305.

The geometric relationships in the ground state complex of SAMP are fully consistent with the generation of a tetrahedral transition state. An oxygen atom of SO$_4^{2-}$ (as an analogue of orthophosphate) is 2.8 Å from the C-6 atom of SAMP. Furthermore, the vector from the C-6 atom to that oxygen atom is perpendicular to the plane of the purine ring (Fig. 9). The latter represents ideal geometry for an approaching nucleophile. The reaction coordinate is the change in tilt angle of the base moiety and transfers a proton to the leaving group (orthophosphate) as an analogue of the forward reaction, the catalytic aspartate may be more or less tightly coordinated to the Mg$^{2+}$ hydrogen bond with the N-1 position of the purine base, being instead highly coordinated to the Mg$^{2+}$ (7, 8). The N-hydroxyl group of hadacidin, which hydrogen bonds with the oxygen atom of Asp$^{43}$, not coordinated to Mg$^{2+}$, may draw the side-chain of the catalytic aspartate from the N-1 atom of 6PIMP.

Indeed, Asp$^{43}$ is closer to the N-1 atom of 6PIMP in the absence of hadacidin than in its presence, although not so close as to provide unambiguous evidence for the formation of a hydrogen bond (7, 8). Short of such evidence, we can only suspect that the ligation of 1-aspartate to the active site stabilizes a hydrogen bond between Asp$^{43}$ and the N-1 atom of 6PIMP.

The structural data imply a precisely tuned set of interactions that allows Mg$^{2+}$ to modulate the acid/base properties of Asp$^{43}$ (Asp$^{33}$ in the E. coli synthetase) in the sequence of reactions catalyzed by the synthetase. During the first step of the forward reaction, the catalytic aspartate may be more than 3 Å from the Mg$^{2+}$ and hydrogen-bonded with the N-1 atom of IMP (5). By abstracting the proton from the N-1 atom, the catalytic aspartate would generate the 6-oxoanion of IMP, which then displaces GDP from the γ-phosphoryl group of GTP (5). After formation of 6PIMP, the catalytic aspartate moves close to (~2.5 Å away from) the Mg$^{2+}$ and transfers its proton back to the N-1 atom as negative charge develops there during the second transition state. The tetrahedral transition state collapses to the product complex as His71 protonates the orthophosphate leaving group.

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