IMP Alone Organizes the Active Site of Adenylosuccinate Synthetase from *Escherichia coli*

Zhenglin Hou‡, Wenyang Wang§, Herbert J. Fromm, and Richard B. Honzatko¶

From the Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, Iowa 50011

A complete set of substrate/substrate analogs of adenylosuccinate synthetase from *Escherichia coli* induces dimer formation and a transition from a disordered to an ordered active site. The most striking of the ligand-induced effects is the movement of loop 40–53 by up to 9 Å. Crystal structures of the partially ligated synthetase, which either combine IMP and hadacidin or IMP, hadacidin, and Mg$^{2+}$-pyrophosphate, have ordered active sites, comparable with the fully ligated enzyme. More significantly, a crystal structure of the synthetase with IMP alone exhibits a largely ordered active site, which includes the 9 Å movement of loop 40–53 but does not include conformational adjustments to backbone carbonyl 40 (Mg$^{2+}$ interaction element) and loop 298–304 (L-aspartate binding element). Interactions involving the 5'-phosphoryl group of IMP evidently trigger the formation of salt links some 30 Å away. The above provides a structural basis for ligand binding synergism, effects on $k_{cat}$ due to mutations far from the site of catalysis, and the complete loss of substrate efficacy due to minor alterations of the 5'-phosphoryl group of IMP.

Adenylosuccinate synthetase (IMP-L-aspartate ligase (GDP forming) EC 6.3.4.4) is essential to most organisms (1–3), catalyzing the first committed step in *de novo* biosynthesis of AMP, according to Reaction 1.

\[
\text{IMP + L-aspartate + GTP} \rightleftharpoons \text{adenylosuccinate + GDP + P}_i
\]

**REACTION I**

The kinetic mechanism is random sequential (4) with a preference for the binding of L-aspartate to the preformed enzyme-IMP-GTP complex (5, 6). The enzymic reaction proceeds in two steps (3, 7–9). First, the γ-phosphoryl group of GTP is transferred to the O-6 atom of IMP. His$^{41}$ is presumably a catalytic acid, transferring a proton to the β-phosphoryl group of GDP as it forms in the active site. Asp$^{13}$ abstracts a proton from the N-1 atom of IMP, thereby facilitating the formation of the 6-oxyanion of the nucleotide. In the second step of the reaction, the α-amino group of L-aspartate attacks the C-6 atom of 6-phosphoryl-IMP. His$^{43}$ again probably transfers a proton but this time to inorganic phosphate as it leaves the C-6 atom of the purine base. The β-carboxyl group of L-aspartate may abstract a proton from its own α-amino group (3). Although the first reaction can occur in the absence of L-aspartate (6), no products are released until after the second reaction (4). In crystallographic complexes, the catalytic metal (Mg$^{2+}$) coordinates the α- and β-phosphoryl groups of IMP, the GDP, and the 6-phosphoryl-IMP, and the N-formyl group of hadacidin (3). (Hadacidin (N-formyl N-hydroxyglycine) is a competitive inhibitor with respect to L-aspartate; see Refs. 1–3). Crystallographic complexes with Mn$^{2+}$ and Ca$^{2+}$, the only other metal cations known to support synthetase activity (1), also bind to the assigned Mg$^{2+}$ site.$^1$

According to the “induced fit” concept (10), some fraction of the enzyme-substrate binding energy stabilizes the transition state. Structures of the ligand-free and ligated forms of the synthetase differ in that several disordered loops of the ligand-free enzyme, which contribute residues essential to catalysis, form ordered structures in the presence of substrates and substrate analogs (11–14). The largest conformational change (~9 Å) occurs in loop 40–53, which folds against the guanine nucleotide, putatively stabilizing the transition state by its interactions with Mg$^{2+}$ and the phosphoryl groups of the nucleotide (12).

The largest conformational changes and the vast majority of ligand-protein hydrogen bonds are at the guanine nucleotide pocket and, hence, implicate GTP in the structural rearrangements. Data equally compelling, however, suggest an IMP-driven conformational change. IMP is crucial for the dimerization of the synthetase monomers (15). The 5'-phosphoryl group of IMP hydrogen bonds with Arg$^{143}$ from the symmetry-related subunit of the synthetase dimer (12). Mutation of Arg$^{143}$ significantly elevates the $K_m$ for IMP and abolishes ligand-induced dimerization of the synthetase (3). The 5'-phosphoryl group of IMP is essential for catalytic activity; IMP analogs, in which a carbon, nitrogen, or sulfur atom replaces one of the phosphate oxygen atoms, are poor substrates (16). The 5'-phosphoryl group of IMP interacts with the side chain of Asn$^{38}$, and the mutation of Asn$^{38}$ to alanine lowers $k_{cat}$ 200-fold but has no effect on $K_m$ for any of the substrates (17). Evidently, the total binding energy of the Asn$^{38}$-IMP hydrogen bond goes toward the stabilization of the transition state.

Presented here are crystal structures of adenylosuccinate synthetase from *Escherichia coli* that provide direct evidence of

---

$^1$ Z. Hou and R. B. Honzatko, unpublished observations.
a central role for IMP in organizing the active site. One complex (hereafter the pyrophosphate complex) has IMP, hadacidin (an analog of L-aspartate), and Mg\(^{2+}\)-pyrophosphate (in place of Mg\(^{2+}\)-GDP) at an ordered active site, hence demonstrating that the guanine base is not essential for organization of the active site. A second complex has IMP and hadacidin (hereafter the IMP-hadacidin complex) at an ordered active site that does not bind Mg\(^{2+}\), even at concentrations as high as 100 mM. The association of Mg\(^{2+}\) with the active site therefore requires the phosphoryl moiety of the guanine nucleotide. A third complex has IMP alone (hereafter the IMP complex) and, aside from the L-aspartate binding loop, has an ordered active site. Hence, IMP-enzyme interactions alone suffice in driving the major conformational transitions of the synthetase active site. The above structures provide a basis for understanding ligand binding synergism in adenylosuccinate synthetase, substantial effects on catalytic rates due to mutations well removed from the active site, and the requirement for an unmodified 5'-phosphoryl group of IMP.

**Experimental Procedures**

**Purification of Enzyme from *E. coli***—The synthetase was prepared as described previously (18) from a genetically engineered strain of *E. coli*. Enzyme purity exceeded 95% as determined by SDS-polyacrylamide gel electrophoresis (data not shown).

**Crystallization**—Hadacidin was a generous gift of Drs. Fred Rudolph and Bruce Cooper, Department of Biochemistry and Cell Biology, Rice University. All other reagents came from Sigma. Crystals were grown by the method of hanging drops under conditions similar to those reported previously (12). In growing crystals of the pyrophosphate complex, droplets contained equal volumes (6 μl) of an enzyme solution (HEPES, 30 mM; pyrophosphate, 2 mM; IMP, 4 mM; hadacidin, 4 mM; and protein, 18 mg/ml at pH 6.8) and a crystallization buffer (polyethylene glycol 8000, 16% w/v; caesium chloride/sodium chloroplate, P, 6.5, 100 mM and magnesium acetate, 100 mM). Mg\(^{2+}\) of the crystallization buffer precipitated pyrophosphate at 2 mM. The precipitate was removed by filtration (0.2-μm pore) before crystallization. Hence, the actual concentration of pyrophosphate is significantly lower than 2 mM but saturating under the conditions employed. The final pH of the crystallization buffer was 6.5. Wells contained 500 μl of crystallization buffer. Conditions of crystal growth for the IMP-hadacidin complex and the IMP complex differ from those of the pyrophosphate complex only by the omission of pyrophosphate or both pyrophosphate and hadacidin. Crystals of ~0.5 mm in all dimensions and belonging to the space group \(P_2_1_2_1\) grew in about one week under all sets of conditions. The crystals are isomorphous to those of Poland et al. (12).

**Data Collection and Reduction**—Data were collected on a Siemens area detector at 100 K and were reduced by using XENGEN (19). The data were at least 98% complete to their nominal resolution (where >0.1/0(1): > 2; Table I). One crystal from each complex was used for data acquisition.

**Model Refinement**—Starting phases were calculated from the fully liganded complex (12), omitting all ligands, solvent molecules, and residues 37–52 (the dynamic loop that undergoes the most significant conformational change). Models of ligands and amino acid residues were fit to the resulting electron density map, followed by a cycle of refinement using XPLOR (20). Constants of force and geometry for the protein came from the work of Engh and Huber (21), and those for hadacidin from Poland et al. (12). Constants of force and geometry for pyrophosphate were based on the phosphoryl groups of GTP and ATP. In early rounds of refinement, all the crystal structures were heated to 2000 K and then cooled in steps of 25 K. In later rounds of refinement, the systems were heated to 1000 to 1500 K but cooled in steps of 10 K. After the slow cooling protocol was complete (at 300 K), the models were subjected to 120 steps of conjugate gradient minimization, followed by 20 steps of individual B-parameter refinement. Individual B-paramaters were subjected to the following restraints: nearest neighbor, main-chain atoms, 1.5 Å; next-to-nearest neighbor, main-chain atoms, 2.0 Å; side-chain atoms, 2.0 Å; and next-to-nearest neighbor, side-chain atoms, 2.5 Å.

Water molecules were added if electron density at a level 2.5σ was present in maps based on Fourier coefficients \((F_{<0} - |F_{calc}|/exp(\pi^2 / 2)\) and acceptable hydrogen bonds could be made to an existing atom of the model. Water molecules were omitted if after refinement they were more than 3.5 Å from their nearest neighbor or if their thermal parameters exceeded 80 Å². Harmonic restraints (50 kcal/mol) were placed on the positions of the oxygen atoms of new water molecules to allow them to relax initially by adjustments in orientation. Occupancies of water molecules were not refined because of the high correlation between occupancy and thermal parameters. Therefore, solvent sites with B values above 150 and 80 Å² probably represent water molecules with occupancy parameters below 1.0 and thermal parameters substantially lower than those reported from refinement.

**RESULTS**

**Quality of the Refined Models**—The models reported here have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics (RSCB), with accessions labels 1KXJ, 1KKB, and 1KKF. The asymmetric unit consists of a monomer subunit; the molecular 2-fold axis of the synthetase dimer coincides with a crystallographic 2-fold axis (12). The method of Luzzati (22) indicates an uncertainty in coordinates of 0.3 Å for the structures. The amino acid sequence used in refinement is identical to that reported previously (14). Results of data collection and refinement are in Table I. The program PROCHECK (23) indicates better stereochemistry than is typical for a structure of 2.5 Å resolution. All residues fall within the generously allowed region of the Ramachandran plot except Gln10 as noted in previous studies (11, 14). Superpositions of the present structures onto the nitrate complex (12) give root mean-square deviations in Ca coordinates of ~0.25, 0.35, and 0.35 Å for the pyrophosphate, IMP-hadacidin, and IMP complexes, respectively. Thermal parameters vary from 5–65, 15–75, and 5–69 Å² for the pyrophosphate, IMP-hadacidin, and IMP complexes, respectively. An overview of the IMP complex is presented in Fig. 1, along with electron density from an omit map of the IMP-ligated complex.

**IMP, Hadacidin, Mg\(^{2+}\)-Pyrophosphate Complex (PDB Accession Label 1KKF)**—A difference map of the pyrophosphate complex using calculated phases based on the coordinates of the nitrate complex (Mg\(^{2+}\), GDP, and nitrate molecules omitted) clearly displays three isolated, ball-like densities at the active site. One spheroid of electron density is at the observed Mg\(^{2+}\) site, whereas the other two are at the putative β- and γ-phosphoryl sites of GTP. Thermal parameters for atoms of pyrophosphate (average thermal parameter of 29 Å²) and Mg\(^{2+}\) (thermal parameter of 42 Å²) refined in this density are comparable with each other and with nearby atoms of the protein (residues 40–41, average thermal parameter of 29 Å²). Hence, even though Mg\(^{2+}\) and pyrophosphate are depleted by precipitation (under “Experimental Procedures”), the above suggests ligands bound at or near full occupancy. The distance between centers of the spheroids is 3 Å, consistent with that of pyrophosphate and too close for the simultaneous binding of two molecules of orthophosphate. Furthermore, orthophosphate is a poor inhibitor of the synthetase (\(K_i\) ~ 5 mM), and its concentration here (as (un)should not exceed 50 μM.

**IMP-Hadacidin, Mg\(^{2+}\)**—The interactions of hadacidin and IMP with the synthetase in the pyrophosphate complex are essentially identical to those of hadacidin and IMP in the nitrate complex (12). Hence, only interactions of the pyrophosphate molecule and Mg\(^{2+}\) are presented here. The two phosphoryl groups of pyrophosphate (hereafter called the β- and γ-phosphoryl groups of pyrophosphate) bind to the putative β- and γ-phosphoryl sites of GTP (Fig. 2). The β-phosphoryl group hydrogen bonds with backbone amides 15, 16, and 17, and the γ-phosphoryl group coordinates to Mg\(^{2+}\) (Fig. 2). The γ-phosphoryl group coordinates to Mg\(^{2+}\) and also hydrogen bonds with Lys16, His41, and backbone amides 13, 40, and 224. Mg\(^{2+}\) coordinates with two oxygen atoms of pyrophosphate (one each from the β- and γ-phosphoryl groups), backbone carboxyl 40, and the N-formyl group of hadacidin. The side-chain of Asp13 (2.9 Å away from Mg\(^{2+}\)) is not part of the inner
coordination sphere of the Mg$^{2+}$. Hence, Mg$^{2+}$ in the pyrophosphate complex is four-coordinated. Mg$^{2+}$ in the nitrate complex (12) is five-coordinated; the -phosphoryl group of GDP in the nitrate complex, which has no analogous element in the pyrophosphate complex, provides the additional coordinate bond.

Loop 40–53 of the pyrophosphate complex adopts the closed conformation observed in previous studies (7, 12, 13). Residues 38–41 move about 1 Å toward the -phosphoryl group of IMP, a movement amplified into the 9 Å displacement of the C atom of residue 46. The conformational change of loop 40–53 ruptures the salt link between His41 and Asp21 observed in the ligand-free structure. In the pyrophosphate complex, ND1 of His41 hydrogen bonds with the side-chain of Glu221, whereas NE2 of His41 interacts with the -phosphoryl group of pyrophosphate. The peptide linkage between residues 39 and 40 reorients, allowing the backbone carbonyl of Gly40 to coordinate the Mg$^{2+}$.

The large conformational change in loop 40–53 of the pyrophosphate complex, described above, is accompanied by addi-

---

### Table I

| Complex | Pyrophosphate | IMP-hadacidin | IMP |
|---------|---------------|---------------|-----|
| Resolution (Å) | 2.6 | 2.6 | 2.8 |
| No. of measurements | 85,975 | 121,026 | 99,750 |
| No. of unique reflections | 22,089 | 22,708 | 20,293 |
| Completeness of data set (%) | 99 | 91 | 98 |
| Completeness of last shell (%) | 99 (2.55–2.75 Å) | 90 (2.6–2.9 Å) | 96 (2.6–2.8 Å) |
| R$_{sym}$ | 0.083 | 0.12 | 0.11 |
| No. of reflections in refinement$^a$ | 14,560 | 13,332 | 13,495 |
| No. of atoms$^d$ | 5,153 | 5,021 | 5,039 |
| No. of solvent sites | 344 | 303 | 312 |
| R-factor$^e$ | 0.19 | 0.18 | 0.196 |
| R$_{free}$ | 0.27 | 0.29 | 0.283 |
| Refinement resolution (Å) | 5–2.6 | 5–2.6 | 5–2.8 |
| Mean B (Å$^2$) for protein | 31 | 32 | 30 |

$^a$ See text for the definition of crystalline complexes presented here.

$^b$ R$_{sym}$ = $\sum_i\sum_jI_{ij} - (I_j)\sum_iI_{ij}$, where i runs over multiple observations of the same intensity, and j runs over all crystallographically unique intensities.

$^c$ All data in the resolution ranges indicated.

$^d$ Includes hydrogens linked to polar atoms.

$^e$ R-factor = $\sum|F_{calc} - |F_{calc}| > 0$.

$^f$ R$_{free}$ based upon 10% of the data randomly culled and not used in the refinement.

---

**FIG. 1. Overview of the IMP-complex.** A single subunit of the IMP-complex, showing the IMP molecule and loop 38–53 in black (left). This illustration was drawn with MOLSCRIPT (35). Stereoview of electron density from a $2F_{calc} - F_{calc}$ omit map covering the IMP molecule and residues 37–53 at a contour level of 1σ (right).
tional conformational changes also observed in the nitrate complex (12). The peptide linkage between residues 223 and 224 reorients, allowing backbone amide 224 to hydrogen bond with the pyrophosphate molecule. The C-terminal end of helix H2 shifts by as much as 3 Å toward loop 40–53. Asp13, Lys31, and Ser114 (residues that interact with the base of guanine nucleotide) and loop 416–421 move as much as 2.5 Å toward loop 40–53. The conformational change in loop 416–421 allows Asp141 to hydrogen bond with backbone amides 46 and 47, backbone carbonyl 419 to hydrogen bond with backbone amide 44, Asp11 to hydrogen bond with Arg119, and backbone carbonyl 417 to hydrogen bond with backbone carbonyl 42 through a bridging water molecule (Fig. 3). A water molecule occupies the same site in fully ligated complexes (7, 12, 13), hydrogen bonding to the 2'-OH of GDP, in addition to the above-named backbone carbonyl groups. Loop 298–304 becomes ordered as Thr300 hydrogen bonds with backbone carbonyls 38 and 39 and to the side chain of His53. Hence, even in the absence of ribosyl and base moieties of the guanine nucleotide, the conformation of the guanine nucleotide pocket in the pyrophosphate complex is identical to that observed in fully ligated complexes.

**IMP-Hadacidin Complex (PDB Accession Label 1KKB)—**IMP and hadacidin interact with the synthetase as observed in the pyrophosphate complex above. No electron density, however, is evident for Mg2+, even though the concentration of Mg2+ in the crystallization solution is approximately 100 mM. Nonetheless, the loss of the coordinate bond between Mg2+ and the N-formyl group of hadacidin does not perturb other interactions between hadacidin and the synthetase.

The conformational differences between the pyrophosphate complex and the IMP-hadacidin complex are in the immediate vicinity of the binding site for pyrophosphate-Mg2+ (Fig. 4). In the IMP-hadacidin complex, His24 interacts only with Glu13. Both side- and main-chain atoms in the vicinity of residues 39–41 possess relatively high thermal parameters in the IMP-hadacidin complex. Backbone carbonyl 40, which coordinates to Mg2+ in the pyrophosphate complex, interacts with OG1 of Thr42 in the IMP-hadacidin complex after a ~90° rotation of the peptide plane between residues 40–41. Lys16, which hydrogen bonds with pyrophosphate, interacts exclusively with the side chain of Glu14 in the IMP-hadacidin complex. The absence of the entire guanine nucleotide then causes only localized conformational change in the vicinity of the Mg2+, β-phosphoryl, and γ-phosphoryl binding sites.

**IMP Complex (PDB Accession Label 1KJX)—**The conformation of the active site of the IMP complex is similar to that of the pyrophosphate and IMP-hadacidin complex in regard to the movement of loop 40–53. In addition, high thermal parameters and hydrogen bonding within the Mg2+ recognition element (residues 39–42), observed in the IMP-hadacidin complex, are retained in the IMP complex. Significant differences in conformation, however, are evident. The base of IMP, stacked against hadacidin in the other complexes, moves 0.8 Å into the hadacidin/L-aspartate pocket. O-6 of IMP hydrogen bonds with NZ of Lys16 and backbone amide 224 by way of a bridging water molecule, which lies in the γ-phosphoryl pocket of GTP (Fig. 5). Asp13 still interacts with IMP, but to maintain the OD1 to N-1 hydrogen bond, its side chain must rotate by ~90° about its Cβ–Cγ bond. In contrast, the base of IMP and the carboxyl group of Asp13 are coplanar in complexes that either have hadacidin or both hadacidin and Mg2+ at the active site (7, 12). The hadacidin/L-aspartate binding element (residues
298–304) is disordered in the IMP complex. Density is present for Arg305 and Arg303, but thermal parameters for these residues are high. The side chain of Arg305, which hydrogen bonds to hadacidin and to the α-phosphoryl group of GDP in fully ligated complexes (7, 12), has a different conformation in the IMP complex, which approximates that of the ligand-free enzyme (11, 14). Arg303, which interacts with the carboxyl group of hadacidin, O$_2^\prime$ of IMP, OG1 of Thr301, and backbone carbonyl 127 in the IMP-hadacidin and pyrophosphate complexes, hydrogen bonds weakly with O$_2^\prime$ of IMP and backbone carbonyl 128.

**DISCUSSION**

The structures presented here, along with previously reported complexes of the synthetase, permit a detailed analysis of the conformational changes induced by a particular ligand and the significance of these conformational changes to ligand binding synergism and stabilization of the transition state. The following discussion focuses on conformational transitions, presented schematically in Fig. 6.

IMP alone induces long-range conformational changes in the active site of adenylosuccinate synthetase (Fig. 6, A and B). Loops 40–53, 120–130, and 416–421 attain conformations in the presence of IMP that are similar to those observed in complete ligand complexes of the synthetase. IMP-induced conformational changes in loops 40–53 and 416–421 create the binding pocket for Mg$^{2+}$-GTP and hence provide a structural basis for the binding synergism between ligands at the IMP and GTP sites. Asn38, located at the beginning of loop 40–53, hydrogen bonds with the 5'-phosphoryl group of IMP. The energy of that hydrogen bond must stabilize the transition state because the mutation of Asn38 to alanine reduces $k_{cat}$ up to 200-fold without an effect on the $K_m$ of substrates (17).

Arg419 and Asp21 form a salt-link some 30 Å away from the 5'-phosphoryl group of IMP, which evidently stabilizes the IMP-induced conformation of the synthetase. Mutations at po-

![Fig. 5. Stereoview of the IMP-ligated active site. Dashed lines represent donor-acceptor interactions.](image)

**Fig. 5.** Stereoview of the IMP-ligated active site. Dashed lines represent donor-acceptor interactions.

![Fig. 6. Schematic of conformational changes in the active site of adenylosuccinate synthetase in response to ligand binding. A, ligand-free active site. B, IMP-bound active site. C, IMP/hadacidin-ligated active site. D, fully ligated active site. Crystallographic complexes directly support conformational changes in response to ligand-binding, as described in the text. Labels are defined as follows: Loop–40, residues 38–53; Loop–120, residues 120–130; Loop–300, residues 298–304; Loop–400, residues 416–421; P-loop, residues 8–16; H1, helix 1; Had, hadacidin; P, phosphorus atoms of IMP or GTP; O, oxygen atoms of the phosphoryl groups of IMP or GTP; I, purine base of IMP; and G, purine base of GTP. Dashed lines in panels B, C, and D represent positions of loops in the ligand-free active site. Arg 419* comes from the monomer related by molecular (dimer) symmetry.](image)
sitions 21 and 419 lower catalytic rates by 10- to 20-fold (17). The 5'-phosphoryl group of IMP enhances formation of synthetase dimers from monomers by directly interacting with Arg143 from the subunit related by molecular symmetry (14, 15). Loss of substrate efficacy due to single atom replacements of terminal oxygen atoms of the 5'-phosphoryl group of IMP further emphasizes a strict requirement for an unmodified phosphoryl group (16). The 5'-phosphoryl group of IMP therefore is both a handle by which the synthetase can grasp one of its substrates and a functional group essential to the stability of the transition state.

Mg$^{2+}$-GTP could perhaps elicit conformational movements in loop 40–53 and loop 416–421 comparable with those induced by IMP. Indeed, in crystal structures of adenylosuccinate synthetase from Arabidopsis thaliana and Triticum aestivum, GDP alone is present in ordered active sites (24). Unfortunately, the ligand-free conformations of the plant synthetases are unavailable. Hence, conformational changes induced by GDP in these systems can only be assumed.

Efforts to grow crystals of the E. coli synthetase in the presence of Mg$^{2+}$-GDP alone have been unsuccessful. Soaking guanine nucleotides into preformed crystals of the ligand-free enzyme, however, revealed the anticipated interactions between the base of GDP and the active site but with only weak interactions involving the phosphoryl moiety and no evidence for bound Mg$^{2+}$ (25). In soaks of the ligand-free crystals, guanine nucleotides did not move loop 40–53. In contrast, soaks of IMP alone destroyed crystals of the ligand-free synthetase. As lattice contacts in the crystal can selectively restrict conformational responses, the results from the soaking experiments only suggest greater sensitivity toward IMP in triggering a conformational change in loop 40–53.

Fluorescence studies, however, also suggest differences between the GTP-ligated and fully ligated active sites of the E. coli synthetase. Thb$^{3+}$-GTP binds to the synthetase with affinity comparable with that of GTP (26). The addition of IMP to a Thb$^{3+}$-GTP-enzyme complex results in further change in terbium fluorescence emission. As the terbium cation is a potent inhibitor of the synthetase, phosphoryl transfer from GTP to IMP is unlikely. Instead, the IMP-induced change in terbium fluorescence could arise from a conformational change in loop 40–53.

The absence of bound Mg$^{2+}$ in the hadacidin-IMP complex, even though Mg$^{2+}$ is present at a concentration of 100 mM, clearly demonstrates the absolute requirement for the guanine nucleotide in the association of Mg$^{2+}$ with the active site. Yet in the absence of bound cation, hadacidin still interacts with the protein as it does in fully ligated complexes of the synthetase. The binding of hadacidin to the enzyme-IMP complex evidently brings about several small, but important, conformational changes (Fig. 6, B and C). In the presence of hadacidin, IMP now adopts the set of interactions observed in fully ligated complexes. The base of IMP and the carboxyl group of Asp13 are clonar. Hadacidin places Arg305 in a conformation that favors an interaction with the α-phosphoryl group of guanine nucleotides. Loop 298–304 is ordered, with Arg305 participating in the set of interactions of fully ligated complexes. The side chain of Thr306 of that loop, in turn, hydrogen bonds with backbone carbonyls 38 and 53, further stabilizing the conformation of loop 40–53 and the Mg$^{2+}$ binding element. Hence, hadacidin (and presumably l-aspartate) induces conformational change in the active site, which promotes productive binding of IMP and GTP.

The Mg$^{2+}$-polyphosphoryl moiety of the guanine nucleotide evidently finetunes the IMP-induced changes by driving a conformational change in the backbone of residues 40–41 and the side chains of Asp13, Lys16, and His41 (Fig. 6, C and D). (Asp13 and His41 are essential catalytic side chains; see Ref. 27.) In fact, most of the binding energy of the Mg$^{2+}$-polyphosphoryl moiety of the guanine nucleotide probably stabilizes the transition state, rather than promoting substrate affinity. GTP, GDP, and GMP have comparable binding affinities for the synthetase (1). In addition, mutations of Gly12 to valine, Glu14 to alanine, Gly15 to valine, and Gly17 to valine individually reduce $k_{cat}$ but do not affect the $K_m$ of GTP (27, 28). These residues belong to the P-loop (residues 8–16) of the synthetase and, in combination with residues 40–42, define the polyphosphoryl binding site. Asp13 and Lys16 have decidedly different conformations and/or interactions in the IMP complex relative to complexes with more complete ligation of the active site. Although IMP binds and induces significant conformational change in loop 40–53, the ligand itself and much of the catalytic machinery (Asp13, His41, backbone carbonyl 40, and Lys16) are not in their proper places until Mg$^{2+}$-pyrophosphate (or Mg$^{2+}$-GDP) binds to the active site.

Adenylosuccinate synthetase is just one of a growing list of enzymes that employ flexible loops in biochemical function (29–33). The precise functional role of flexible loops can vary significantly. AMP, as an allosteric inhibitor, putatively modulates the conformational state of a dynamic loop in fructose-1,6-bisphosphatase (29). A dynamic loop in S-adenosylmethionine synthetase may act as a gate, blocking access to the active site and modulating substrate affinity (30). The role of a dynamic loop in lactate dehydrogenase (31), however, is analogous to that of adenylosuccinate synthetase. Pyruvate induces a conformational change in a loop of lactate dehydrogenase, allowing the side chain of Lys109 to interact with the carbonyl oxygen of the substrate. Directed mutation of Lys109 reduces activity 1500-fold with little effect on substrate affinity (34). In the case of adenylosuccinate synthetase, the large conformational change in loop 40–53 is an amplification of a small conformational change involving the interaction of Aan35 with the 5’-phosphoryl group of IMP. Aan35, then, is a sensor. When IMP binds, the enzyme probably dimerizes, and the active site goes into a state of readiness. Mg$^{2+}$-GTP and l-aspartate, acting alone, may not have the same effect on the global conformation of the synthetase or on its state of oligomerization. Because conformational transitions in the synthetase span a distance of 30 Å, the signal transmission from the sensor (Asn26) is on a scale comparable with that of allosteric enzymes. In a sense, the 5’-phosphoryl pocket is a regulatory/activation site embedded within the active site of the synthetase.
Adenylosuccinate Synthetase

J. Biol. Chem. 273, 16000–16004
18. Wang, W., Hou, Z., Honzatko, R. B., and Fromm, H. J. (1997) J. Biol. Chem. 272, 16911–16916
19. Howard, A. J., Nielsen, C., and Xuong, N. H. (1985) Methods Enzymol. 114, 452–472
20. Brunger, A. T. (1992) XPLOR, version 3, Yale University Press, New Haven, CT
21. Engh, R. A., and Huber, R. (1991) Acta Crystallogr. Sect. A 47, 392–400
22. Luzzati, V. (1956) Acta Crystallogr. 9, 802–810
23. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) J. Appl. Crystallogr. 26, 283–291
24. Prade, L., Cowan-Jacob, S. W., Chemla, P., Potter, S., Ward, E., and Fon涅-Pfister, R. (2000) J. Mol. Biol. 296, 569–577
25. Poland, B. W., Hou, Z., Bruns, C., Fromm, H. J., and Honzatko, R. B. (1996) J. Biol. Chem. 271, 15407–15413
26. Seans, C., and Fromm, H. J. (1991) Arch. Biochem. Biophys. 310, 475–480
27. Kang, C., Sun, N., Poland, B. W., Gorrell, A., Honzatko, R. B., and Fromm, H. J. (1997) J. Biol. Chem. 272, 11881–11885
28. Liu, F., Dong, Q., and Fromm, H. J. (1992) J. Biol. Chem. 267, 2388–2392
29. Choe, J.-Y., Poland, B. W., Fromm, H. J., and Honzatko, R. B. (1998) Biochemistry 37, 11441–11450
30. Fu, Z., Hu, Y., Markham, G. D., and Takusagawa, F. (1996) J. Biol. Chem. 271, 727–733
31. Abad-Zapastero, C., Griffith, J. P., Sussman, J. L., and Rossmann, M. G. (1987) J. Mol. Biol. 198, 445–467
32. Hyde, C. C., Ahmed, S. A., Padlan, E. A., Miles, E. W., and Davies, D. R. (1988) J. Biol. Chem. 263, 17857–17871
33. Lolis, E., and Petsko, G. A. (1996) Biochemistry 29, 6619–6625
34. Clarke, A. R., Atkinson, T., and Holbrook, J. J. (1989) Trends Biochem. Sci. 14, 101–105
35. Kraulis, J. (1991) J. Appl. Crystallogr. 24, 946–950
IMP Alone Organizes the Active Site of Adenylosuccinate Synthetase from *Escherichia coli*
Zhenglin Hou, Wenyan Wang, Herbert J. Fromm and Richard B. Honzatko

*J. Biol. Chem.* 2002, 277:5970-5976.  
doi: 10.1074/jbc.M109561200 originally published online December 12, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M109561200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 12 of which can be accessed free at http://www.jbc.org/content/277/8/5970.full.html#ref-list-1