Refined Crystal Structures of Guanine Nucleotide Complexes of Adenylosuccinate Synthetase from Escherichia coli*

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Structures of adenylosuccinate synthetase from Escherichia coli complexed with guanosine-5′-[(β,γ-imido)triphosphate (GppNp) and guanosine-5′-[(β,γ-methylene)triphosphate (GppCp) in the presence of Mg2+ have been refined to R-factors below 0.2 against data to a nominal resolution of 2.7 Å. Asp333 of the synthetase hydrogen bonds to the exocyclic 2-amino and endocyclic N1 groups of the guanine nucleotide base, whereas the hydroxyl of Ser426 and the backbone amide of Lys331 hydrogen bond to the 6-oxo position. The side chains of Lys331 and Pro417 pack against opposite faces of the guanine nucleotide base. The synthetase recognizes neither the N7 position of guanine nucleotides nor the ribose base. The synthetaserecognizesneither g

REACTION 1

GTP + IMP + l-aspartate ⇄ GDP + Pi + adenylosuccinate

According to the proposed mechanism of Lieberman (1956), the γ-phosphate of GTP is transferred to the 6-oxoxygen of IMP, after which the amino group of aspartate displaces phosphate from the 6-phosphoryl-IMP intermediate. Studies in kinetics support the Lieberman mechanism (Fröm, 1958; Webb et al., 1984; Bass et al., 1984; Cooper et al., 1986), but alternative reaction mechanisms are in the literature (Markham & Reed, 1977; Miller & Buchanan, 1962). The synthetase from various sources, including Escherichia coli, is subject to feedback inhibition by AMP, adenylosuccinate, GDP, and GMP (Wynagaarden & Greenland, 1963; Rudolph & Fromm, 1969; Van der Weyden & Kelly, 1974; Clark & Rudolph, 1976; Markham & Reed, 1977). Literature regarding the synthetase is reviewed by Stayton et al. (1983).

The synthetase from E. coli is a dimer of identical polypeptide chains of 431 amino acids with Mw = 48,000 (Wolfe & Smith, 1988; Poland et al., 1993). Synthetases from different sources (Wolfe & Smith, 1988; Wiesmüller et al., 1991; Guicherit et al., 1991, 1993; Kusano et al., 1992; Powell et al., 1992; Mäntsälä & Zalkin, 1992; Speiser et al., 1992) show at least 40% overall identity in amino acid sequence. The synthetase family has the consensus sequences commonly associated with GTP binding in the GTPase superfamily (Dever et al., 1987; Guicherit et al., 1991, 1994). Furthermore, adenylosuccinate synthetase adopts a polypeptide fold similar to that of dethiobiotin synthetase from E. coli, an enzyme that couples the hydrolysis of ATP in the conversion of 7,8-diaminopelargonic acid to dethiobiotin (Alexeev et al., 1994; Huang et al., 1994). Adenylosuccinate synthetase from yeast (Zeidler et al., 1993) binds to T-rich, single-stranded DNA. However, the physiological consequences of the observed in vitro binding of the synthetase to DNA remain unclear.

Crystal structures of the unligated synthetase (P21 and P222121 crystal forms) are in the literature (Poland et al., 1993; Silva et al., 1995). The P21 crystal form is disordered by the gradual infusion of Mg2+–GTP and Mg2+–GDP. Most likely guanine nucleotides promote specific conformational changes in residues involved in lattice contacts in the P21 crystal form. In contrast, the P222121 crystal form accepts guanine nucleotides up to concentrations of 0.8 mM (approximately 5-fold in excess of the reported K_m for GTP) without the loss of resolution in the diffraction of x-rays.

The current study presents crystal structures of GTP analogs (GppNp and GppCp)3 complexed with adenylosuccinate synthetase. The structures reveal for the first time key interactions between the base of guanine nucleotides and the enzyme. In spite of the presence of succinate in the buffers of each of the complexes, we find no evidence for the ordered binding of succinate. In comparison with preliminary structures of the synthetase complexed with IMP, GDP, Mg2+, and succinate,2 the base of the guanine nucleotide in structures reported here

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1 The abbreviations used are: GppNp, guanosine-5′-[(β,γ-imido)triphosphate; GppCp, guanosine-5′-[(β,γ-methylene)triphosphate; GppN, guanosine-5′-[(β-amidino)diphosphate.

2 B. W. Poland, H. J. Fromm, and R. B. Honzatko, unpublished results.

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is recognized by the enzyme, but the polyphosphate moiety is not. The complexes do not lead to conformational changes that occur in the enzyme when IMP, Mg\(^{2+}\), and succinate are present along with the guanine nucleotide, thus suggesting that other ligands (minimally Mg\(^{2+}\) and/or IMP) are necessary for complete recognition of the guanine nucleotide by the synthetase.

**MATERIALS AND METHODS**

Preparation of Enzyme—Adenylosuccinate synthetase was prepared from a genetically engineered strain of E. coli according to published isolation procedures (Bass et al., 1987; Poland et al., 1993; Silva et al., 1995). The protein migrates as a single band on SDS-polyacrylamide gel electrophoresis with an apparent relative molecular weight of 48,000 (data not shown).

Growth of Crystals—Conditions for the growth of the P2\(_{1}2\(_1\)2\(_1\)2\(_1\) crystal form were discovered by use of the sparse matrix of Jancarik and Kim (1993) and the method of hanging drops. Droplets contained 2 \(\mu\)l of enzyme solution (20 mg/ml in protein) dialyzed against a solution (pH 7.0) consisting of imidazole (20 \(\mu\)M), succinate (75 \(\mu\)M) and 2-mercaptoethanol (70 \(\mu\)M), and 3 \(\mu\)l of a crystalization buffer, which contained 25% (w/v) polyethylene glycol 3350, 100 \(\mu\)M acetic-acid/sodium-acetate, pH 5.2, and 200 \(\mu\)M ammonium acetate. The final pH of the crystalization buffer was 6.5. Wells contained 500 \(\mu\)l of the crystallization buffer, contained approximately 0.5 \(\mu\)M and imidazole/succinate (pH 7.0) at concentrations equivalent to those belonging to the space group P2\(_{1}2\(_1\)2\(_1\)2\(_1\) (unit cell parameters a = 73.42, b = 93.63, c = 119.47) grew in about one week. The asymmetric unit consists of an entire dimer.

Preparation of Ligand Complexes—Guanine nucleotides GppNp and GppCp came from Sigma. The samples were checked for purity by thin layer chromatography, using polyethyleneimine plates and 1.6M LiCl solutions as eluents. Each of the samples was checked for purity by thin layer chromatography, using polyethyleneimine plates, developed in 1.6M LiCl, and visually inspected for UV-absorbing components.

Crystallographic Refinement—The structure of the P2\(_{1}2\(_1\)2\(_1\)2\(_1\) crystal form was determined by molecular replacement (Silva et al., 1995) using the preliminary structure of the P2\(_2\) crystal form (Poland et al., 1993). The unligated P2\(_{1}2\(_1\)2\(_1\)2\(_1\) crystal form served as the starting model for the interpretation of guanine nucleotide complexes. The refinement of models for ligand complexes of the P2\(_{1}2\(_1\)2\(_1\)2\(_1\) crystal form involved manual fitting of the model to the electron density, using a Silicon Graphics 4D–25 and the program TOPE (Camillibus & Horjus, 1987), followed by a cycle of refinement using XPLOR (Brünger, 1992) on a Silicon Graphics 4D-35. Constants of force and geometry for the protein came from Eng & Huber (1991). In the refinement process, the system was heated to 1000 or 1500 K and then cooled in steps of 10 K. After the slow-cooling protocol was completed (at 300 K), the models were subjected to 200 steps of conjugate gradient minimization, followed by 20 steps of individual B-parameter refinement. Individual B-parameters were subject to the following restraints: nearest neighbor, main chain atoms, 1.5 \(Å^2\); next-to-nearest neighbor, main chain atoms, 2.0 \(Å^2\); nearest neighbor, side chain atoms, 2.0 \(Å^2\); and next-to-nearest neighbor, side chain atoms, 2.5 \(Å^2\).

Water molecules were added if (i) electron density at a level of 2.5\(σ\) was present in maps based on Fourier coefficients \(|F_{obs}| - |F_{calc}|\) and \(2|F_{obs}| - |F_{calc}|\) and (ii) acceptable hydrogen bonds could be made to an existing atom of the model. If after refinement a site for a water molecule fell beyond 3.3 \(Å\) from its nearest neighbor, that site was omitted from the model. In addition, water molecules were deleted from the model if their thermal parameters exceeded 80 \(Å^2\). Harmonic restraints (50 kcal/mol) were placed on the positions of oxygen atoms of water molecules in order to allow new water molecules to relax by adjustments in orientation. Occupancies of water molecules were not refined, because of the high correlation between occupancy and thermal parameters for data of a nominal resolution of 2.0–2.5 \(Å\). Thus solvent sites were deleted because of the high correlation between occupancy and thermal parameters substantially lower than those reported from the refinement.

**RESULTS AND DISCUSSION**

Quality of the Refined Models—The models for guanine nucleotide complexes of adenylosuccinate synthetase have been deposited in the Protein Data Bank, Brookhaven National Laboratory. The method of Luzzati (1952) indicates an uncertainty in coordinates of 0.25 \(Å\). Segments 121–130 and 298–304 (37.5 \(µM\)) and are accessible to the enzyme in the active site, while the N-terminal region (1–37) is less well ordered. The overall quality of the models in terms of stereochemistry is better than the “typical” structure as defined by PROCHECK at a resolution of 2.3 \(Å\).

The asymmetric unit of the P2\(_{1}2\(_1\)2\(_1\)2\(_1\) crystal form has a complete dimer. Thus, we anticipate two binding sites for guanine nucleotides in the synthetase.
nucleotides, one for each of two monomers. The binding site associated with chain B, however, is occupied to a lesser extent than the binding site associated with chain A. In fact, the density for nucleotides at chain B is so weak in the GppNp and GppCp complexes that models for nucleotides initially placed in the binding site at chain B were deleted in the final stages of refinement due to high thermal parameters (approximately, 70 Å²). Lattice contacts are largely responsible for the difference in occupancies of the guanine nucleotide binding sites of the dimer. The side chain of Glu155 from a symmetry related dimer encroaches upon the nucleotide binding site of chain B. Furthermore, lattice contacts involving atoms of chain B are greater in number than those involving chain A, an observation consistent with a lower average thermal parameter for chain B relative to chain A (Table I; Silva et al. (1995)). The interactions, reported here, focus on nucleotides at chain A. The interactions involving the nucleotide at chain B in the GppCp complex are subject to high uncertainty, but nonetheless are similar to nucleotide-protein interactions at chain A.

Electron density associated with GppCp at chain A appears in Fig. 1. Although the electron density at a level of 3σ is continuous over the entire ligand, the thermal parameters of the polyphosphate moiety average to 50 Å², reflecting substantial uncertainty in the atomic coordinates. Atoms of the base, however, have thermal parameters comparable with atoms of the surrounding protein. Under the conditions of the soak (GppCp, 0.8 mM), the nucleotide saturates its binding site at chain A. Nonetheless, higher concentrations of GppCp lead to a significant loss of resolution in the X-ray diffraction data, suggesting either a ligand-induced conformational change or a direct influence at a point of contact between protein molecules in the crystal lattice (for instance, binding to chain B). Electron density for the GppNp complex indicates an approximately equal mixture of GppNp and GppN (hydrolyzed GppNp) at chain A, whereas the Mg²⁺-GppNp complex reveals moderate density for only GppN at chain A, consistent with a ligand occupancy of not more than 0.50.

Thin layer chromatography of GppNp in the presence of enzyme, with or without Mg²⁺, revealed a slow chemical transformation of the GppNp. After 24 h in the presence of the enzyme, alone or with Mg²⁺, GppNp appeared as an equal mixture of two substances, one of which corresponded to GppNp, the other to a fast-migrating substance. After 72 h only the fast-migrating component appeared. The fast-migrating component had an R₁ value similar to that of diphosphate nucleosides. Adenylosuccinate synthetase apparently catalyzes the hydrolysis of GppNp. Given our current understanding of the enzyme, we favor an attack by water on the γ-phosphate of GppNp, thus producing GppN. A complete chemical analysis of the product is necessary, however, in order to confirm the mechanism; the X-ray data alone are consistent with the formation of GppN or GDP. No degradation was observed for GppCp under identical conditions.

Although succinate is present in all of the nucleotide complexes, electron density for bound succinate could not be located in difference maps. Given that initial velocity kinetics indicate a random equilibrium mechanism for the synthetase (Rudolph & Fromm, 1969; Cooper et al., 1986), succinate should bind in the absence of IMP. Kang and Fromm (1995) have suggested, however, that aspartate is recognized by the synthetase as the Mg²⁺-aspartate complex, rather than as free aspartate. Presumably succinate, a competitive inhibitor of the synthetase with respect to aspartate, is recognized by the enzyme in the form of Mg²⁺-succinate. The high acetate concentration (300 mM in total acetate) in the P2₁2₁2₁ crystal form will reduce the pool of Mg²⁺ (10 mM) available to the formation of a Mg²⁺-succinate complex. The absence of electron density corresponding to succinate in the present structures, then, is entirely consistent with the hypothesis of Kang and Fromm (1995).

### Table II

| Ligand atom | Protein atom | Distance GppN complex | Distance GppN/GppNp complex | Distance GppCp complex |
|-------------|--------------|-----------------------|-----------------------------|------------------------|
| N1          | D333 O01     | 3.64                  | 2.90/2.89                   | 3.17                   |
| N2          | D333 O02     | 2.76                  | 2.56/2.62                   | 2.62                   |
| O6          | S414 O      | 2.87                  | 2.81/2.83                   | 2.68                   |
| O1A         | K331 N       | 3.13                  | 2.78/2.75                   | 3.00                   |
| O1A         | K16 N        | 3.44                  | 3.10/4.03                   | 4.20                   |
| O3A         | G17 N        | 2.93                  | 3.13/3.41                   | 3.66                   |
| O3A         | G16 N        | 5.86                  | 5.64/3.14                   | 3.72                   |
| O3A         | G17 N        | 4.89                  | 4.66/3.28                   | 3.42                   |
| O1B         | G17 N        | 3.32                  | 3.43/3.58                   | 5.47                   |
| O2B         | E14 N        | 9.91                  | 9.72/3.14                   | 4.08                   |
| O2B         | G15 N        | 8.58                  | 8.25/2.73                   | 3.13                   |
| O2B         | K16 N        | 7.48                  | 7.07/3.46                   | 2.66                   |
| O2B         | T42 N        | 2.59                  | 2.81/8.84                   | 8.40                   |
| N3B (or C3B)| G40 O        | 2.83                  | 2.61/4.51                   | 3.51                   |
| O2G         | K16 N        | /2.53                 | 4.53                         |
| O3G         | K16 N        | /3.69                 | 2.81                         |
The synthetase recognizes the 6-oxo group of the base by forming hydrogen bonds to OG of Ser$^{414}$ and backbone amide 331. Ser$^{414}$ must function as a proton donor in its hydrogen bond to the 6-oxo group. The hydroxyl of serine, however, can serve as a proton donor or acceptor in hydrogen bonds. A hydrogen bond between Lys$^{18}$ and Ser$^{414}$ orients the dipole moment of the hydroxyl group of Ser$^{414}$ toward the 6-oxo group, thus making the hydroxy of Ser$^{414}$ an obligatory proton donor. Two other dipole moments, backbone amides 331 and 416, are oriented toward the 6-oxo group, creating a local environment decidedly in favor of a substituent with partial negative charge at position 6 of the purine ring. Indeed, the synthetase has an overwhelming preference for GTP over ATP as a substrate (Lieberman, 1956). In addition to the 6-oxo group, Asp$^{333}$ of the synthetase hydrogen bonds to the endocyclic N1 and exocyclic 2-amino group of the base (Table II). The interactions of Asp$^{333}$ in the crystal structure are consistent with the properties of the Asn$^{333}$ mutant, which no longer recognizes GTP as a substrate, presumably due to the inability of the NH$_2$ group of the amide side chain of Asn$^{333}$ to serve as a proton acceptor (Kang et al., 1994). The structures reported here are also consistent with the explanation provided by Kang et al. (1994) for the ability of the Asn$^{333}$ mutant to recognize XTP as a substrate. The proper orientation of the amide side chain of the Asn$^{333}$ mutant would result in hydrogen bonds to the exocyclic 2-oxo group and the endocyclic N1 of XTP. Finally, the side chains of Lys$^{333}$ and Pro$^{417}$ pack against opposite faces of the base. The interactions involving Lys$^{333}$, Asp$^{333}$, Ser$^{414}$, and Pro$^{417}$ were predicted by modeling studies of Poland et al. (1993).

In refined complexes reported here, the nucleotides vary significantly with respect to the covalent structure of their phosphate moieties. We observe no electron density for the $\gamma$-phosphate group of GppNp or for Mg$^{2+}$ in crystals of the synthetase, exposed to Mg$^{2+}$-GppNp. As GppNp is susceptible to hydrolysis under the conditions reported here, electron density for the nucleotide in the Mg$^{2+}$-GppNp soak is represented by GppN. In contrast, the complex of GppNp without Mg$^{2+}$ reveals moderate density close to the position of the $\gamma$-phosphate of GppCp. In addition, electron density for GppNp bifurcates at the $\alpha$-phosphate, suggesting an alternate conformation.
for GppNp or the binding of a second, related guanine nucleotide. The conformation of the α- and β-phosphates of this "alternate conformer" is identical to that of GppN in the Mg²⁺-GppNp complex, where no density for the γ-phosphate is observed. As a consequence, we have interpreted the electron density in the GppNp complex in terms of the mutually exclusive binding of GppN and GppNp.

Under comparable conditions GppCp and Mg²⁺-GppCp give identical results; electron density is present for all three phosphates of the two GppCp complexes. No density is observed, however, for Mg²⁺ in the Mg²⁺-GppCp soak. In fact, we were unable to detect structural differences of any kind in the GppCp complex in the presence and the absence of Mg²⁺. Thus, in Table I we report only the structure for the GppCp complex without Mg²⁺. The presence of 300 mM total acetate in the crystallization buffer may be a significant factor in reducing the pool of Mg²⁺ available to the polyphosphate moieties of nucleotides. The absence of Mg²⁺ in the refined complexes is consistent with studies of Lieberman (1956), which indicate the need for a relatively high concentration of Mg²⁺ (1–2 mM) for maximal activity. In the case of G₃₃¹, Mg²⁺ binds tightly to the GTP-protein complex (Kd of 10⁻¹² M; Gilman, 1987), in stark contrast to the apparent dissociation constant for Mg²⁺ from the synthetase.

The interaction of the phosphate moieties of GppN, GppNp, and GppCp with the synthetase differ significantly from each other (Fig. 3, Table II). For instance, in the GppN complex oxygen O1A of the α-phosphate binds to backbone amide 17 and O2B of the β-phosphate binds to backbone amide 42. In the GppCp complex, oxygen O2B interacts with backbone amides 15 and 16 and oxygen O3G interacts with NZ of Lys₁₆. In the case of the GppNp complex, oxygen O2B binds to backbone amides 14 and 15, whereas oxygen O2G binds to NZ of Lys₁₆. The thermal parameter of atom NZ of Lys₁₆ is high, suggesting that Lys₁₆ may not reside at all times in a single, well defined location, even in the presence of guanine nucleotides.

Comparison of Nucleotide Interactions in the Synthetase with Those of Other Guanine Nucleotide Binding Proteins—In p21ras (Pai et al., 1990), Gα₁ (Coleman et al., 1994), EF-Tu (Kjeldgaard et al., 1993), and the synthetase an aspartate (position 333 of the synthetase) makes hydrogen bonds to N1 and N2 of the guanine base (Table III). In recognition of the O6 position of guanine nucleotides, the synthetase employs a serine hydroxyl (Ser⁴¹⁴) and a backbone amide (of Lys³³¹), whereas the Gα₁ and p21ras employ primarily a backbone amide and EF-Tu primarily a serine hydroxyl. Gα₁ and p21ras, however, have Cys³²⁵ and Ser¹⁴⁵, respectively, each of which is close to the 6-oxo group of the guanine nucleotide. Furthermore, in the complexes of p21ras, Gα₁, and EF-Tu, a backbone amide interacts with Ser¹⁴⁵, Ser¹⁷⁴, and Cys³²⁵, respectively, to orient the dipole of the side chain toward the O6 position. These backbone amides apparently play a role that is analogous to Lys¹⁸ of the synthetase. Opposite faces of the purine base pack against protein side chains of Lys³³¹ and Pro¹⁴⁷ of the synthetase, corresponding to similar packing interactions involving Lys¹⁷ and Phe²⁰ of p21ras, Lys²⁷⁰ and Thr³²⁷ of Gα₁, and Lys¹³⁷ and Leu¹⁷⁶ of EF-Tu. The most significant difference in the interaction of guanine nucleotides with the synthetase in relation to all other known structures of GTP-binding proteins is the lack of recognition of N7 of the base. In p21ras, Gα₁, and EF-Tu the side chain of an asparagine hydrogen bonds to position N7, whereas in the synthetase the closest functional group is the hydroxyl of Ser⁴¹⁴. The lack of recognition of N7 in the case of the synthetase may explain, in part, the large difference in the apparent dissociation constant of GTP from the synthetase (Kd of 10⁻⁵ M; Rudolph and Fromm, 1969), as opposed to other GTP-binding proteins (Kd of 10⁻⁷–10⁻¹¹ M; Bourne et al., 1991). A difference in the mode of recognition of Mg²⁺, suggested by Silva et al. (1995), however, may be a more significant factor in the large difference in affinity of guanine nucleotides to the synthetase relative to G-proteins. Although we suggest below that the nucleotide complexes reported here represent a state of incomplete recognition of the nucleotide by the synthetase, interactions of the guanine base are identical to those of the synthetase in its complex with IMP, Mg²⁺, GDP, and succinate.²

Atoms of the ribose of guanine nucleotides do not interact directly with the synthetase. The weak interaction between the synthetase and the ribose moiety follows the trend of p21ras, Gα₁, and EF-Tu.

In comparison with p21ras, Gα₁, and EF-Tu, each of which recognizes the polyphosphate group of guanine nucleotides by way of a significant network of hydrogen bonds (Table III), the synthetase in complexes reported here interacts weakly with the phosphate moieties of guanine nucleotides. As discussed below, the absence of Mg²⁺ and/or IMP in these structures is

![Superposition of GppN, GppNp, and GppCp in the conformations observed for these nucleotides in their ligand complexes with the synthetase.](image-url)
probably the underlying cause of the poor recognition of the triphosphate group. In the complex of GDP, IMP, succinate, and Mg$^{2+}$, the polyphosphate moiety is involved in an extensive network of hydrogen bonds. Unlike G-proteins, where the observed interactions of the polyphosphate group with Mg$^{2+}$ and the protein contribute significantly to the nanomolar dissociation constant, the polyphosphate moiety of GTP contributes little to the net affinity of the guanine nucleotide to the synthetase. Interactions involving the base of GTP are of primary importance in determining its affinity to the synthetase, as mutations in the region of the phosphate-binding loop have little effect on the $K_m$ of GTP (Liu et al., 1992). Presumably the binding energy of phosphate-protein interactions is used to drive large conformational changes in the synthetase.

Guanine nucleotides adopt a conformation in their complexes with adenylosuccinate synthetase that differs from that of guanine nucleotides in complexes of p21$^{ras}$, Gi$_a$, and EF-Tu (Table IV). The most significant difference involves the torsion angle ($\gamma$) between C4' and C5', which is $\gamma$-syndinal in the synthetase complex but $\gamma'$-synclinal in the G-protein complexes. Perhaps related to this difference in the angle $\gamma$ is a difference in puckering of the ribose ring. The ribose in the synthetase complex differs from that of other G-proteins (Table IV). The most significant difference involves the torsion angle ($\psi$) between C4$^9$ and C5$^9$, which is $2^\circ$ synclinal in the synthetase complex but $1^\circ$ synclinal in the G-protein complexes. Perhaps related to this difference is a difference in puckering of the ribose ring. The ribose in the synthetase complex adopts a C3$^9$-exo conformation as opposed to the C2$^9$-endo conformation of other G-proteins (Table IV). At a resolution of 2.7 Å the puckering of the ribose, however, is only inferred by the x-ray data. The synthetase complex of GDP, IMP, Mg$^{2+}$, and succinate reveals GDP in a conformation that is nearly identical to the one reported here.2 The observed differences in nucleotide conformation in complexes of G-proteins and the synthetase may stem from differences in the mode of recognition of Mg$^{2+}$ by the synthetase relative to G-proteins (Silva et al., 1995).

**Table IV**

Comparison of guanine nucleotide interactions in adenylosuccinate synthetase, p21$^{ras}$, Gi$_a$, and EF-Tu.

| Ligand atom | GppCp synthetase | Mg$^{2+}$-GppNp p21$^{ras}$ | Mg$^{2+}$-GppSp Gi$_a$ | Mg$^{2+}$-GppNp EF-Tu |
|---|---|---|---|---|
| N2 | D333 OD2 | D119 OD2 | D272 OD2 | D139 OD2 |
| N1 | D333 OD1 | D119 OD1 | D272 OD1 | D139 OD1 |
| O6 | K331 N | A146 N | A326 N | A396 N |
| N7 | N116 ND2 | N269 ND2 | N136 ND2 | N136 ND2 |
| O1A | A18 N | T48 OG1 | T48 N | S47 N |
| O2A | Wat 654 | Wat 172 | Wat 403 | Y47 OH1 |
| O1B | G15 N | S44 N | G23 N | G23 N |
| O2B | G15 N | S17 N | T25 N | T25 N |
| O1G | K16 NZ | K46 N | K24 N | K24 N |
| O2G | K16 NZ | T17 OG | T240 | T240 |
| O3G | K16 NZ | G60 N | G240 | G94 N |

Parameters are defined according to IUPAC convention (Saenger, 1984).
of Pro\(^{17}\)) and in the disordered loops, involving residues 299–304 and 121–130. The loops are disordered in the unligated structure (Silva et al., 1995) and remain poorly ordered in the guanine nucleotide complexes. Differences in these loops are probably due to uncertainties in the interpretation of weak electron density.

In preliminary complexes of the synthetase with GDP, IMP, Mg\(^{2+}\), and succinate,\(^2\) the enzyme undergoes large conformational changes (displacement of some \(\omega\)-carbons in excess of 10 Å) in the region of binding of the guanine nucleotide. The conformational changes involve the loop, spanning residues 38–53. The absence of conformational change in the P2\(_1\)2\(_1\)2\(_1\) crystal form may arise from (i) the inability of the synthetase to relax due to lattice contacts or (ii) a requirement for IMP and/or Mg\(^{2+}\) in combination with the guanine nucleotide to evoke a conformational response. Lattice contacts clearly impact on the guanine nucleotide site of chain B in the P2\(_1\)2\(_1\)2\(_1\) crystal form. As discussed above, lattice contacts are probably responsible for the low occupancy of nucleotide here. The addition of guanine nucleotides to concentrations above 0.8 mM may disrupt lattice contacts in the vicinity of the nucleotide binding site of chain A, even though lattice contacts are remote from the binding site of guanine nucleotides. The addition of IMP (2 mM) and Mg\(^{2+}\) (10 mM) to the P2\(_1\)2\(_1\)2\(_1\) crystal form in the presence of 0.8 mM guanine nucleotide leads to a significant loss of resolution in x-ray diffraction. IMP is apparently necessary in conjunction with guanine nucleotides to evoke a conformational change. Kinetic studies indicate that IMP, GTP, and Mg\(^{2+}\) in combination promote the exchange of the \(\gamma\)-phosphate between bound GDP and bound IMP (Bass et al., 1984). Thus, the conformational changes observed in preliminary structures of the synthetase in its complex with IMP and guanine nucleotides may occur only when the active site contains IMP, Mg\(^{2+}\), and a guanine nucleotide. The complexes reported here, then, represent an incomplete recognition of the nucleotide by the enzyme, the complete recognition requiring the presence of Mg\(^{2+}\) and/or IMP.

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