Effectors of the Stringent Response Target the Active Site of Escherichia coli Adenylosuccinate Synthetase*

Zhenglin Hou‡, Michael Cashel§, Herbert J. Fromm‡, and Richard B. Honzatko‡‡

From the ‡Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa 50011 and the §Laboratory of Molecular Genetics, NICHHD, National Institutes of Health, Bethesda, Maryland 20892

Guanosine 5'-diphosphate 3'-diphosphate (ppGpp), a pleiotropic effector of the stringent response, potently inhibits adenylosuccinate synthetase from Escherichia coli as an allosteric effector and/or as a competitive inhibitor with respect to GTP. Crystals of the synthetase grown in the presence of IMP, hadacidin, NO₃⁻, and Mg²⁺, then soaked with ppGpp, reveal electron density at the GTP pocket which is consistent with guanosine 5'-diphosphate 2':3':cyclic monophosphate. Unlike ligand complexes of the synthetase involving IMP and GDP, the structure of this complex is occluded with the side chain of Asp¹³ in the inner sphere of the cation. The cyclic phosphoryl group interacts directly with the side chain of Lys⁴⁹ and indirectly through bridging water molecules with the side chains of Asn²⁹⁰ and Arg⁵⁰⁵. The synthetase either directly facilitates the formation of the cyclic nucleotide or scavenges trace amounts of the cyclic nucleotide from solution. Regardless of its mode of generation, the cyclic nucleotide binds far more tightly to the active site than does ppGpp. Conceivably, synthetase activity in vivo during the stringent response may be sensitive to the relative concentrations of several effectors, which together exercise precise control over the de novo biosynthesis of AMP.

Escherichia coli and other bacteria that undergo nutritional stress, for instance amino acid starvation, synthesize guanosine 5'-diphosphate 3'-diphosphate (ppGpp) and guanosine 5'-triphosphate 3'-diphosphate (pppGpp). These nucleotides in turn dramatically influence a broad range of metabolic activities (stringent response), impairing the synthesis of DNA, rRNA, tRNA, proteins, nucleotides, and phospholipids and concomitantly stimulating amino acidbiosynthesis and several other functions (1). Evidently the stringent response stems from a severe shortfall, relative to the demands of ribosomal protein biosynthesis, in one or more of the aminoacylated tRNAs. During the stringent response in E. coli, pools of ATP and GTP decrease, but concentrations of pppGpp and ppGpp rise to millimolar levels. The reduction in the de novo synthesis of purine nucleotides putatively is a consequence of direct inhibition of adenylosuccinate synthetase and IMP dehydrogenase by ppGpp (2–4).

Adenylosuccinate synthetase (IMP:L-aspartate ligase (GDP-forming), EC 6.3.4.4) is an essential enzyme in E. coli (2), catalyzing the first committed step in de novo biosyntheses of AMP as shown in Reaction 1.

\[
\text{IMP + L-aspartate + GTP} \rightarrow \text{adenylosuccinate + GDP + phosphate}
\]

The catalytic mechanism is a two-step process (3–5) as follows: the formation of 6-phosphoryl-IMP by nucleophilic attack of the 6-oxo group of IMP on the γ-phosphoryl group of GTP, followed by a second nucleophilic displacement of the 6-phosphoryl group by aspartate to form adenylosuccinate. ppGpp potently inhibits the synthetase (Kₘ ~ 50 μM; 6–8). The mechanism of inhibition by ppGpp, however, remains unsettled. ppGpp may inhibit the synthetase by binding to an allosteric site, as suggested by its noncompetitive inhibition with respect to GTP (6, 7), or by simply binding to the GTP pocket, as suggested by its competitive inhibition with respect to GTP in a different study (8).

Reported here are crystal structures of adenylosuccinate synthetase from E. coli complexed with IMP, NO₃⁻, Mg²⁺, hadacidin, and guanosine 5'-diphosphate 2':3':cyclic monophosphate (hereafter the ppG2':3':p complex), and with IMP, NO₃⁻, Mg²⁺, hadacidin, and GDP (hereafter the GDP complex). Hadacidin, a fermentation product of Penicillium frequentans (9), is a competitive inhibitor (Kᵢ ~ 10⁻⁶ M) with respect to aspartate (10, 11). Even though ppGpp was used as a ligand in crystal soaking buffers, the electron density in the guanine nucleotide pocket is consistent only with ppG2':3':p. Evidently, ppG2':3':p binds with much greater affinity to the synthetase than does ppGpp. Crystals either absorb a minor impurity of the cyclic nucleotide from solution or the synthetase itself transforms ppGpp to ppG2':3':p. Regardless of its mechanism of formation, the ppG2':3':p complex here represents the first structure of an effector of the stringent response with one of its target enzymes. Results here and from past investigations raise the possibility of several effectors of the stringent response acting in a coordinated way to modulate synthetase activity.
Adenylosuccinate Synthetase

| Table I | Refinement statistics for adenylosuccinate synthetase |
|---------|-----------------------------------------------------|
|         | ppG2'-3'p complex | GDP complex |
| Nominal resolution (Å) | 2.5 | 2.3 |
| Number of measurements | 134,672 | 154,316 |
| Number of unique reflections | 23,427 | 29,374 |
| Completeness of data |            |            |
| Overall (%) | 99 | 99 |
| Last shell (%) | (2.36–2.60 Å) | (2.18–2.36 Å) |
| Rmerge | 9.2 | 8.5 |
| Refinement resolution (Å) | 5–2.5 | 5–2.3 |
| Number of reflections in refinement | 15,550 | 14,929 |
| Number of atoms | 5,220 | 5,386 |
| Number of solvent sites | 154 | 110 |

\[ R_{\text{merge}} \equiv \frac{\sum_i |I_i| - \langle I_i \rangle}{\sum_i |I_i|}, \text{ where } i \text{ runs over multiple observations of the same intensity, and } j \text{ runs over all crystallographically unique intensities.} \]

All data are in the resolution ranges indicated.

† Includes hydrogens linked to polar atoms.

‡ Represents a single crystal.

§ Represents a single crystal.

Models Refinement—Starting phases were calculated from the GDP complex (13), omitting all ligands and solvent. Refinement procedures are as described previously (13). The ligand models were fit to omit electron density maps, followed by a cycle of refinement using XPLOR (16). Constants of force and geometry for the protein came from Eng and Huber (17), and those for hadacatin from Poland et al. (13). Refinement parameters for ppG2'-3'p were based on those of GTP and the crystal structures of 2':3'-monophosphate nucleotides (18–20). In early rounds of refinement, models were heated to 2000 K and then cooled in steps of 25–300 K. In later rounds of the refinement, the systems were heated to 1000 or 1500 K and cooled in steps of 10 K. After the slow-cool 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-parameters were subject to the following restraints: nearest neighbor, main chain atoms, 1.5 Å; next-to-nearest neighbor, side chain atoms, 2.0 Å; nearest neighbor, side chain atoms, 2.0 Å; and next-to-nearest neighbor, side chain atoms, 2.5 Å. Criteria for the addition of water molecules were identical to those of previous studies (5, 13).

RESULTS

Quality of the Refined Models—The method of Luzzati (21) indicates an uncertainty in coordinates of 0.3 Å. The amino acid sequence used in refinement is identical to that reported previously (22, 23). Results of data collection and refinement are in Table I. A overview of the ppG2'-3'p complex is in Fig. 1, and a schematic of ppG2'-3'p is in Fig. 2. Gln10 is the only serious violation of the Ramachandran plot as identified by PROCHECK (24). The conformation of Gln10 is enforced by its hydrogen bonding environment as described in previous work (22, 23). The models have better stereochemistry than is typical for structures based upon data of comparable resolution. Thermal parameters vary in the GDP and ppG2'-3'p complexes from 10 to 64 Å² and 10 to 65 Å², respectively. The variation in thermal parameters as a function of residue is comparable to that of other structures of the ligated synthetase (5, 13). Thermal parameters for individual ligands average to less than 30 Å² in each of the structures. Supperposition of the GDP complex onto the ppG2'-3'p complex results in a root mean square deviation of Cα coordinates of 0.3 Å, comparable to the estimate of coordinate uncertainty.

GDP Complex (Protein Data Bank Accession Code 1icb)—

Conditions of crystallization for the GDP complex reported here differ from those of an earlier study (13) in the elimination of acetate from the crystallization buffers. Acetate and GDP synergistically inhibit the synthetase, as does nitrate and GDP (25). Although the combination of GDP/nitrate exhibits greater synergism than GDP/acetate, the total concentration of acetate in the previous study was some 40-fold higher relative to the nitrate anion, hence the potential exists for substantial compe-
Adenylosuccinate Synthetase

17507

**TABLE II**

Selected contacts involving ligands of the GDP and ppG2\(^{\cdot3\cdot}p\) complexes

| Ligand atom | Contact atom | Distance, ppG2\(^{\cdot3\cdot}p\) complex | Distance, GDP complex |
|-------------|--------------|--------------------------------------|----------------------|
| IMP         | O-6          | Nitrate N3 | 2.95 | 2.81 |
|             | Gln\(^{224}\) N2 | 2.75 | 2.74 |
| N-1         | Asp\(^{13}\) OD1 | 3.07 | 2.63 |
| N-7         | Gln\(^{224}\) N2 | 3.35 | 2.74 |
| O-2         | Val\(^{773}\) O | 2.71 | 2.73 |
| O-1A        | Arg\(^{303}\) NH2 | 2.57 | 2.98 |
| O-2A        | Asn\(^{38}\) ND2 | 3.11 | 3.32 |
| O-3A        | Thr\(^{239}\) OG1 | 2.72 | 2.67 |
| Mg\(^{2+}\) | GDP O1A      | 2.19 | 2.17 |
|             | GDP O2B      | 2.09 | 2.08 |
|             | Hadacidin O  | 2.08 | 2.19 |
|             | Nitrate O2   | 2.80 | 2.48 |
|             | Asp\(^{13}\) OD1 | 2.47 | 2.73 |
|             | Gly\(^{40}\) O | 2.04 | 1.95 |
| Nitrate     | O-1          | Lys\(^{16}\) NZ | 3.11 | 3.27 |
|             | Gln\(^{224}\) N2 | 2.72 | 2.61 |
|             | O-2          | Mg\(^{2+}\) | 2.3 | 2.48 |
|             | Gly\(^{40}\) N | 2.82 | 2.73 |
|             | Asp\(^{13}\) N | 2.94 | 3.10 |
|             | Lys\(^{16}\) NZ | 2.54 | 2.56 |
| N-3         | IMP O-6      | 2.95 | 2.81 |
| ppG2\(^{\cdot3\cdot}p\) | O-1A        | Asp\(^{333}\) OD1 | 3.15 | 2.81 |
|             | O-2          | Asp\(^{333}\) OD2 | 2.80 | 2.78 |
|             | O-6          | Ser\(^{14}\) OG | 2.02 | 2.52 |
|             | O-1B         | Gly\(^{40}\) N | 3.34 | 3.32 |
|             | O-2B         | Wat\(^{605}\) O | 3.50 | 2.53 |
|             | O-4'         | Lys\(^{16}\) NZ | 3.43 | 3.11 |
|             | O-5'         | Thr\(^{239}\) OG1 | 3.40 |  |
|             | Arg\(^{305}\) NH1 | 3.80 |  |
|             | O-1A         | Mg\(^{2+}\) | 2.29 | 2.17 |
|             | Arg\(^{305}\) NH2 | 3.03 | 3.14 |
|             | Thr\(^{42}\) N | 3.26 |  |
|             | Arg\(^{305}\) NH1 | 3.21 |  |
|             | Gly\(^{37}\) N | 2.86 | 2.77 |
|             | His\(^{41}\) ND1 | 2.97 | 2.85 |
|             | Mg\(^{2+}\) | 2.09 | 2.08 |
|             | His\(^{41}\) ND1 | 3.36 | 2.92 |
|             | Gly\(^{34}\) N | 3.52 | 3.47 |
|             | Gly\(^{15}\) N | 3.06 | 3.03 |
|             | Lys\(^{16}\) N | 2.95 | 2.74 |
|             | Lys\(^{49}\) NZ | 2.65 |  |

\(^a\) Residue from symmetry related monomer.

\(^b\) Corresponding atom not present in GDP.

A stereospecificity between nitrate and acetate for the putative γ-phosphoryl pocket of GTP. The GDP complex presented here provides data to higher resolution relative to that of the previous GDP complex (2.3 Å as opposed to 2.8 Å), and more significantly, the active site in the vicinity of the nitrate anion has stronger electron density and lower thermal parameters. The average distance of the six oxygen atoms nearest to the Mg\(^{2+}\) in the present GDP complex has decreased by 0.16 Å relative to that of the previous one.

The conformation of the synthetase in the GDP complex is essentially identical to that of the GDP complex (Figs. 3 and Table II). Interactions of IMP, nitrate, and hadacidin are unchanged from previous structures. Significant differences occur, however, in the ligation of Mg\(^{2+}\) and at the guanine nucleotide pocket. Within the uncertainty of the coordinates, Mg\(^{2+}\) exhibits octahedral coordination, in contrast to the GDP complex, where it exhibits quasi-octahedral coordination. The oxygen atoms that define the equatorial plane of the Mg\(^{2+}\) (one each from 5′-β- and 5′-α-phosphoryl groups of ppG2\(^{\cdot3\cdot}p\), from NO\(_3\)), and from the N-formyl group of hadacidin) average to 2.1 Å, as opposed to 2.3 Å for the corresponding bonds in the GDP complex (Table II). In the GDP complex, OD1 of Asp\(^{13}\) tightly hydrogen bonds (2.6 Å) to N-1 of IMP and is 2.7 Å from the Mg\(^{2+}\). In the ppG2\(^{\cdot3\cdot}p\) complex, however, the distance between OD1 of Asp\(^{13}\) and N-1 of IMP is 3.1 Å,...
whereas the distance separating the Mg$^{2+}$ and OD1 of Asp$^{13}$ is 2.5 Å. Evidently, the synthetase coordinates Mg$^{2+}$ more tightly in the ppG2*:3’p complex than in the GDP complex; the tighter coordination occurring, however, at the expense of a weakened interaction between Asp$^{13}$ and N-1 of IMP. The significance of the above to the mechanism of catalysis and inhibition of the synthetase is discussed below.

The difference electron density in the guanine nucleotide pocket clearly indicates an analog of GDP (Fig. 4). Although electron density similar to that of the GDP complex is present for the base and 5’-pyrophosphoryl moieties, additional electron density extends from both the 2’- and 3’-hydroxyl groups of the stringent effector. The density can accommodate a single phosphoryl group but not the pyrophosphoryl group expected at the 3’ position. A difference map, based on observed data from the effector and GDP complexes, and calculated phase angles from the GDP complex, reveals a strong and well defined peak of electron density in the vicinity of the 2’- and 3’-hydroxyl groups of GDP (Fig. 4). The positive difference density overwhelms any negative difference density due to water molecules bound to the 2’- and 3’-hydroxyls of GDP. Hence the bound nucleotide cannot be GDP. Furthermore, a 3’ (or 2’) phosphoryl group cannot occupy the density without causing severe steric conflict with the remaining 2’ (or 3’)-hydroxyl group of the ribose. The only acceptable fit to the electron density was provided by ppG2*:3’p. Thermal parameters for the refined cyclic nucleotide are comparable to those of the surrounding protein, suggesting full occupancy of the ligand at the guanosine pocket.

The synthetase recognizes the 5’-pyrophosphoryl group and the guanine base of GDP and ppG2*:3’p in the same manner. Atom O-6 of the cyclic nucleotide interacts with OG of Ser$^{414}$ and backbone amide 331. Endocyclic N-1 and exocyclic N-2 of the base hydrogen bond with the side chain of Asp$^{333}$. The 5’-β-phosphoryl group of ppG2*:3’p interacts with backbone amides 15, 16, and 17, the side chain of His$^{41}$, and bound Mg$^{2+}$. The 5’-α-phosphoryl group interacts with the Mg$^{5’}$, backbone amide 42, and Arg$^{305}$. The ribose moiety of ppG2*:3’p, however, differs significantly in location and conformation relative to that of GDP (Fig. 4). The 5’-α-phosphoryl group of ppG2*:3’p rotates ~120° around the torsion angle defined by atoms PB, OA, PA, and O-5’, displacing the entire ribose moiety approximately 1 Å toward loop 42–53. (Loop 42–53 undergoes a 9 Å conformational change upon ligation of the synthetase active site.) As a consequence, the cyclic 2’-3’-phosphoryl group hydrogen bonds with the side chain of Lys$^{49}$ (that side chain is disordered in the GDP complex), and forms water-mediated hydrogen bonds with the side chains of Asn$^{295}$ and Arg$^{305}$. A

Fig. 3. Stereoview of interactions between ppG2*:3’p and the active site. Ligands (only hadacin (Had), nitrate, ppG2*:3’p, and Mg$^{2+}$ are shown) are drawn with bold lines. Donor-acceptor interactions (corresponding distances listed in Table II) are presented with dashed lines.

Fig. 4. Stereoview of electron density associated with ligands in the ppG2*:3’p complex. A, the electron density, contoured at 6σ using a cut-off radius of 0.9 Å, comes from a ligand-excised omit map. ppG2*:3’p and other active site ligands are represented with bold lines. B, difference electron density based on phases from the GDP complex and Fourier coefficients from the ppG2*:3’p and GDP complexes. The contour level is 6σ and reveals only residual electron density in the ppG2*:3’p complex that has no counterpart in the GDP complex. The GDP molecule is represented with bold lines and the ppG2*:3’p molecule with fine lines.
Adenylosuccinate Synthetase

Fig. 5. Stereoview of a model for the putative transition state in the conversion of ppGpp into ppG2'-3'p at the active site of the synthetase. Ligands (hadacidin (Had), Mg2+, and the nucleotide transition state) are drawn with bold lines. Bold, dashed lines represent the geometric relationship of the attacking nucleophile and leaving group with respect to the 3'-α-phosphoryl group. Possible donor-acceptor interactions are represented with dashed lines. A second Mg2+, which could interact with the 3'-pyrophosphoryl group of the nucleotide, is not shown.

Under the conditions of our crystallographic studies ppGpp does not undergo significant hydrolysis. Two possibilities, then, can account for the appearance of ppG2'-3'p in the guanine pocket of the synthetase: either the crystalline synthetase scavenches a minor (and undetectable) impurity of the cyclic nucleotide from our sample of ppGpp or the synthetase itself facilitates the conversion of ppGpp to the cyclic nucleotide. As no build-up of the cyclic nucleotide was observed in the presence of the synthetase, the putative enzyme-mediated process must occur without appreciable turnover. For the latter scenario, ppGpp must bind to the guanine pocket and undergo cyclization, producing an inhibitor that does not readily dissociate from the synthetase.

As a means of better understanding a possible enzyme-mediated mechanism, one can model a transition state complex for the cyclization of ppGpp in the guanine nucleotide pocket (Fig. 5). Mg2+ (total concentration of 100 mM) must be associated with the 3'-pyrophosphoryl group of ppGpp under the conditions of crystal soaking. The water molecule, which bridges backbone carbonyls 42 and 417 and hydrogen bonds with the 2'-hydroxyl of GDP (Table II), may act as a weak catalytic base and abstract a proton from the 2'-OH of ribose (Fig. 5). The activated O-2' atom then attacks the 3'-α-phosphoryl group, displacing the 3'-β-phosphoryl group. The side chain of Lys49, as well as the Mg2+ associated with the 3'-pyrophosphoryl group, would stabilize the development of negative charge on the leaving group and/or the transition state. Lysyl side chains in analogous positions are critical to the catalytic function of adenylylate kinase and GTPases in general.

Regardless of its mode of formation, ppG2'-3'p must bind at least 200-fold more tightly than does ppGpp to the active site of the synthetase, assuming the cyclic nucleotide is 4% of the concentration of ppGpp in soaking solutions and that the Kf for ppGpp is 50 μM. The estimated Kf for ppG2'-3'p (10⁻⁷ M) compares favorably to the tightest known inhibitors of the synthetase, alanosyal-AICOR (10⁻⁷ M (27)) and hydantocidin 5'-phosphate (10⁻⁸ M (28)). The enhanced binding of ppG2'-3'p may arise from the following three sources. (i) Cyclization of the 2'– and 3'-hydroxyls of the ribose reduces conformational freedom of the nucleotide and hence reduces the entropic penalty associated with ligand binding. (ii) The 2'-3'-phosphoryl group provides additional opportunities for strong hydrogen bonds with the synthetase. (iii) The active site Mg2+ has octahedral coordination, whereas in GDP complexes its coordination is square pyramidal (13) or quasi-octahedral (see above). In fact, an octahedrally coordinated metal cation appears as well in the complex of the tight binding inhibitor hydantocidin 5'-phosphate (29). Asp13 interacts directly with IMP in GDP complexes (see above) and evidently in the absence of an IMP-Asp13 interaction (as in the hydantocidin 5'-phosphate complex (29)) or a weakened IMP-Asp13 interaction (as here in the ppG2'-3'p complex), Asp13 favors direct coordination to the Mg2+. Octahedral coordination of the active site Mg2+, then, may be a hallmark of tight binding inhibitors of the synthetase. Such inhibitors are of potential significance in chemotherapy and the development of herbicides (27, 28).

A cyclic nucleotide at the guanine nucleotide pocket does not explain competitive inhibition between ppGpp and IMP and noncompetitive inhibition between ppGpp and GTP, as observed in two investigations (6, 7). Such phenomena could arise from impure preparations of the synthetase (8). Alternatively, ppGpp could bind to a site distinct from the guanine nucleotide pocket. Synthetase dimers, for instance, putatively bind IMP with an affinity 100-fold greater than that of synthetase monomers. Hence, ppGpp will inhibit competitively with respect to IMP and non-competitively with respect to GTP, by binding to a site that blocks dimerization of the synthetase. The existence of such a site is inferred by covalent modification of the synthetase by pyridoxal phosphate (30) and guanosine-5'-O-[S-(4-bromo-2,3-dioxobuty)thiophosphate] (31) at a cluster of basic residues, exposed in synthetase monomers, but buried between monomers in the synthetase dimer. The experimental protocol developed here reveals ppGpp interactions at the guanine nucleotide pocket (consistent with all kinetics investigations (6–8)) but does not probe for a ppGpp-binding site at the buried interface between subunits of the synthetase dimer.

Potent inhibition of adenylosuccinate synthetase in vivo typically impedes growth of the organism. The pur A- E. coli cell line grows poorly, even in rich medium. Hydantocidin (which is transformed in plants to the tight binding synthetase inhibitor, hydantocidin 5'-phosphate) is a potent herbicide (27). Alanosyal-AICOR and hadacidin are antibiotics (9, 28), the latter having only the synthetase as a known target. Hence, if the stringent response is ultimately a coordinated attempt by E. coli to limit its own growth under unfavorable conditions, then the synthetase is an appropriate target.
Even though ppGpp is an effective inhibitor of the synthetase at its physiological concentration during the stringent response, other effectors (pppGpp and ppG2′:3′p) may also influence synthetase activity. The transformation of ppGpp into ppG2′:3′p, perhaps at the active site of the synthetase, suggests a parallel and more potent mechanism of inhibition, which may be temporally distinct from the inhibition by ppGpp. ppGpp, putatively the first nucleotide generated during the stringent response, could be an inhibitor of the synthetase, its 5′-γ-phosphoryl group being transferred to IMP while the cyclization reaction occurs at the guanine pocket. The result would be an active site complex of 6-phosphoryl-IMP and ppG2pppGpp for the active site.

REFERENCES

1. Cashel, M., Gentry, D. R., Hernandez, V. J., and Vinella, D. (1996) in Escherichia coli and Salmonella: Cellular and Molecular Biology (Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., eds) 2nd ed., pp. 1458–1495, American Society for Microbiology, Washington, D. C.
2. Stayton, M. M., Rudolph, F. B., and Fromm, H. J. (1983) Curr. Top. Cell. Regul. 22, 103–141
3. Lieberman, I. (1956) J. Biol. Chem. 223, 327–339
4. Fromm, H. J. (1958) Biochim. Biophys. Acta 29, 255–262
5. Poland, B. W., Bruns, C., Fromm, H. J., and Honzatko, R. B. (1997) J. Biol. Chem. 272, 15200–15205
6. Gallant, J., Irr, J., and Cashel, M. (1971) J. Biol. Chem. 246, 5812–5816
7. Pao, C. C., and Dyess, B. T. (1981) Biochim. Biophys. Acta 677, 358–362
8. Stayton, M. M., and Fromm, H. J. (1979) J. Biol. Chem. 254, 2579–2581
9. Kaczkas, A. E., Gitterman, C. O., Dulaney, E. L., and Follers, K. (1962) Biochemistry 1, 340–343
10. Clark, S. W., and Rudolph, F. B. (1976) Biochim. Biophys. Acta 437, 87–93
11. Markham, D. G., and Reed, G. H. (1977) Arch. Biochem. Biophys. 184, 24–35
12. Wang, W., Gorrell, A., Hou, Z., Honzatko, R. B., and Fromm, H. J. (1998) J. Biol. Chem. 273, 16000–16004
13. Poland, B. W., Fromm, H. J., and Honzatko, R. B. (1996) J. Mol. Biol. 264, 1013–1027
14. Cashel, M., and Kalbacher, B. (1969) J. Biol. Chem. 245, 2039–2038
15. Engh, A. J., Nielsen, C., and Xuong, N. H. (1985) Methods Enzymol. 114, 452–472
16. Brünger, A. T. (1992) XPLOR: A System for X-ray Crystallography and NMR, Version 3.1, Yale University Press, New Haven
17. Engh, R. A., and Huber, R. (1991) Acta Crystallogr. Sec. A 47, 392–400
18. Særøe, W. (1984) in Principles of Nucleic Acid Structure (Cantor, C. R., ed) pp. 167–173, Springer-Verlag Inc., New York
19. Lapper, R. D., and Smith, I. C. P. (1973) J. Am. Chem. Soc. 95, 2880–2884
20. Reddy, B. S., and Særøe, W. (1978) Acta Crystallogr. Sec. B 34, 1520–1524
21. Luzzati, V. (1956) Acta Crystallogr. 9, 802–810
22. Poland, B. W., Silva, M. M., Serra, M. A., Cho, Y., Kim, K. H., Harris, E. M. S., and Honzatko, R. B. (1993) J. Biol. Chem. 268, 25334–25342
23. Silva, M. M., Poland, B. W., Hoffman, C. R., Fromm, H. J., and Honzatko, R. B. (1995) J. Mol. Biol. 254, 431–446
24. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) J. Appl. Crystallogr. 26, 283–291
25. Markham, D. G., and Reed, G. H. (1978) J. Biol. Chem. 253, 6184–6189
26. Lippman, F. and Sy, J. (1976) Proc. Natl. Acad. Sci. USA 73, 1–14
27. Siehl, D. L., Subramanian, M. V., Walters, E. W., Lee, S.-F., Anderson, R. J., and Toschi, A. G. (1996) Plant Physiol. 110, 753–758
28. Tyagi, A., and Conney, D. (1980) Cancer Res. 40, 4390–4397
29. Poland, B. W., Lee, S.-F., Subramanian, M. V., Siehl, D. L., Anderson, R. J., Fromm, H. J., and Honzatko, R. B. (1996) Biochemistry 35, 15753–15759
30. Dong, Q., and Fromm, H. J. (1990) J. Biol. Chem. 265, 6235–6240
31. Moe, O. A., Baker-Malcolm, J. P., Wang, W., Kang, C., Fromm, H. J., and Colman R. F. (1996) Biochemistry 35, 9024–9033
32. Choe, J.-Y., Poland, B. W., Fromm, H. J., and Honzatko, R. B. (1999) Biochemistry, in press
33. Kraulis, J. (1991) J. Appl. Crystallogr. 24, 946–950