Interactions of Nucleotides with Fully Unadenylylated Glutamine Synthetase from *Salmonella typhimurium*[Δ]

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ABSTRACT: Glutamine synthetase (GS) catalyzes the ATP-dependent biosynthesis of glutamine from glutamate and ammonia in the presence of divalent cations. To gain insight into the structural basis of the feedback inhibition of GS by AMP, we have studied crystal structures of GS complexes with AMP and the related molecules: AMPPNP (a less hydrolyzable ATP analog), ADP, GDP, adenosine, and adenine. AMP is a feedback inhibitor of GS; ATP and ADP are cofactors, and AMPPNP, GDP, adenosine, and adenine are also GS inhibitors. GS used in this study is from *Salmonella typhimurium* and is free of covalent modification by adenylylation. All of the crystals examined contain two bound Mn^{2+} ions per GS subunit. The X-ray structures show that all nucleotides bind at the same site, the cofactor ATP binding site, as do adenosine and adenine. Thus from X-ray structures, AMP, adenosine, adenine, and GDP would be expected to inhibit GS-Mn by competing with the substrate ATP for the active site. This suggestion from the crystal structures that AMP is competitive with respect to ATP is supported by kinetic measurements using the biosynthetic assay.

The highly regulated enzyme glutamine synthetase (GS)[1] catalyzes the entry of nitrogen into metabolism by combining ammonia and glutamate, with the aid of ATP, to yield glutamine, ADP, and inorganic phosphate (the "biosynthetic reaction"). It also catalyzes the "glutamine hydrolysis", the "γ-glutamyl transfer" reaction in the presence of the cofactors ADP and inorganic phosphate (Stadtman & Ginsburg, 1974), and the formation of pyrophosphate and glutamate from phosphate and glutamine in the presence of AMP (Whitley & Ginsburg, 1980).

The amide nitrogen of glutamine serves as a nitrogen source for the biosynthesis of nitrogen-containing metabolites (Ginsburg & Stadtman, 1973). The original kinetic studies by Woolfolk & Stadtman (1967) showed that AMP is one of nine end products of glutamine metabolism that inhibit GS activity. Their results on *Escherichia coli* GS suggested that ADP and GDP are competitive inhibitors with respect to ATP, whereas AMP is a noncompetitive feedback inhibitor with respect to any substrate. A separate site for AMP, distinct from the ATP (ADP) site, was also inferred by equilibrium binding (Ginsburg, 1972), by NMR (Chock et al., 1977), and by calorimetric studies (Shrake & Ginsburg, 1977). That is, these studies were interpreted in terms of both substrate and allosteric sites on GS.

However, other experiments yielded alternative interpretations of the number of nucleotide binding sites of GS. Dahlquist and Purich (1975) examined interactions of *E. coli* GS with feedback inhibitors using magnetic resonance techniques. They concluded that AMP binds at the nucleotide 5′-monophosphate region of the ATP site. Consistent with this conclusion is the finding of Hunt et al. (1975) that AMP substitutes for ADP in the γ-glutamyl transferase activity. Furthermore, GS catalyzes a reversible AMP-dependent formation of pyrophosphate (Whitley & Ginsburg, 1980). In all three of these GS-catalyzed reactions, AMP might be expected to bind to the ATP/ADP substrate binding site. Therefore the number of distinct nucleotide binding sites on the surface of GS remains an open question.

X-ray crystallography is helpful in locating binding sites. By X-ray methods, a 3.5-Å atomic model for the 5616 amino acid residues of unadenylylated GS from *Salmonella typhimurium* was earlier determined (Almassy et al., 1986; Yamashita et al., 1989), and 2.8-Å atomic models for *E. coli* and for *S. typhimurium* GS were refined (Liaw, 1992). The 2.8-Å atomic model of *S. typhimurium* GS is used here with the difference Fourier method to define the interactions of nucleotides with the enzyme.

MATERIALS AND METHODS

Purification of GS. GS for crystallization was isolated from cells of *S. typhimurium* by ammonium sulfate precipitation and a Cibacron blue affinity column (Liaw, 1992) in the presence of Mn^{2+}, whereas GS for kinetic measurements was purified by ammonium sulfate precipitation in the presence of Mg^{2+} (Woolfolk & Stadtman, 1967). In the former method, ATP is used to elute GS from the affinity column, and ATP cannot be removed completely by extensive dialysis due to its high affinity to GS. Bound ATP on GS could conceivably affect kinetic measurements and was avoided. Different metal ions were used in the two methods of protein purification because GS crystals were grown in the presence of Mn^{2+} and because completely unadenylylated GS from *E. coli* and from *S. typhimurium* has no biosynthetic activity in the presence

[Δ] The atomic model of GS-AMP has been deposited in the Brookhaven Protein Data Bank (filename 1LGR).

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[1] Abbreviations: GS, glutamine synthetase; AMPPNP, 5′-adenylyl imide diphosphate; P_i, inorganic phosphate.

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Table 1: Summary of X-ray Diffraction Data for GS–Effectors Containing Nucleotide Components

crystal	resoln (Å)	unique/ total reflns	R_{sym}[a]	$\langle \Delta F \rangle / \langle F \rangle$[b] (%)
GS–Ado[c]	2.9	95 610/154 441	8.4	15.6
GS–Adn[e]	3.0	67 238/94 346	10.8	16.8
GS–AMP	2.8	99 219/146 242	6.6	12.0
GS–GDP	2.8	85 105/119 716	6.7	11.3
GS–ADP[d]	2.8	86 071/126 045	7.2	16.1
GS–AMPPNP[d]	3.0	74 798/107 472	17.9	20.0

[a] On intensity. A measure of the precision of data collection. [b] Mean fractional isomorphous difference, $\sum||F_{PH}|-|F_P||/\sum|F_P|$. [c] Ado: adenosine. [d] From Liaw and Eisenberg (1994). [e] Adn: adenine.

of Mn^{2+} (Ginsburg, 1972; Liaw et al., 1993a). AMPPNP was purchased from Boehringer Mannheim.

Crystal Soaking and Data Collection. Fully unadenylylated crystals of GS from *S. typhimurium* were grown (Liaw et al., 1993b) by the hanging drop method of vapor diffusion. Each effector was dissolved in synthetic mother liquor (containing 15 mM imidazole hydrochloride (pH 7.0), 3 mM Mn^{2+}, 3 mM spermine tetrahydrochloride, and 10% MPD) and added to crystal-containing drops (Liaw et al., 1993a). Adenine, adenosine, AMP, ADP, GDP, and AMPPNP were added to GS crystals and allowed to diffuse for 12 h. Estimated final concentrations of adenine, adenosine, AMP, ADP, GDP, and AMPPNP were 10, 10, 5, 1.3, 2, and 1.3 mM, respectively. X-ray data from these GS–effector complexes were collected with an RAXIS-II (Rigaku), image plate detector (Table 1). All of these complex crystals were isomorphous with respect to native GS crystals, belonging to space group $C2$ with unit-cell dimensions a = 235.5 Å, b = 134.5 Å, c = 200.1 Å, and β = 102.8°. X-ray data for GS complexed with AMPPNP and ADP were reported earlier (Liaw & Eisenberg, 1994). Data for GS complexed to adenine, adenosine, AMP, and GDP are given in Table 1.

Difference Fourier Maps. Difference Fourier maps, 12-fold averaged, using Fourier coefficients $F_{o(GS-effector)} - F_{o(GS)}$, were calculated by using CCP4 programs (a suite of programs for protein crystallography, SERC, Daresburg Laboratory, Warrington, England) implemented on a DEC VAX 4000 at UCLA. Phases of the 2.8-Å native GS model were used as phases of the complexes, as justified by the crystal isomorphism.

Enzyme Activity Assay. The enzymatic activity of GS can be measured by phosphate formation in the biosynthetic reaction (Boyer et al., 1959). In order to compare with kinetic data of Woolfolk and Stadtman (1967), kinetic measurements were performed under conditions which were virtually identical to their conditions: For the biosynthetic assay, 80 μL of reaction mixture containing 100 mM imidazole (pH 7.0), 100 mM $MgCl_2$, 100 mM NH_4Cl, 200 mM L-Glu, and variable concentrations of ATP is mixed with 80 μL of GS at 37 °C to initiate the reaction. After 9 min, the reaction is terminated by adding the stop mixture of 640 μL of 1% $FeSO_4$ in 0.015 N H_2SO_4; 10 s later, a faint blue color is developed by the addition of 60 μL of 6.6% ammonium molybdate in 7.5 N H_2SO_4. The amount of phosphate produced is estimated from the absorbance at 660 nm on a Beckman DU-7 spectrophotometer, 1 min after molybdate reagent is added.

RESULTS

Model Building of Effectors. Electron density difference maps were computed for the following effectors bound to GS: GDP, AMP, adenosine, and adenine. The strongest density peak in each difference map at the 1.2σ contour level occupies the same general region in the active site funnel of GS (Figure 1). Atomic models of the effectors were built into these strong peaks starting with adenosine and AMP. These are clear maps, computed with Fourier coefficients $F_{o(GS-adenosine)} - F_{o(GS)}$ and $F_{o(GS-AMP)} - F_{o(GS)}$ at the 1.5σ contour level (Figure 1a,b). The chemistry of nearby residues was also considered during model building, as is necessary at 2.8-Å resolution.

In the AMP model, the adenine ring is nearly parallel with the benzene ring of Phe 225, approximately 3.5 Å away. The N1 atom and the 6-amino group of the adenine ring interact with Ser 273. The hydroxyl group at C2′ of the ribose ring donates a hydrogen bond to the carbonyl group of Val 223, and the hydroxyl group at C3′ also donates a hydrogen bond to the side chain of Glu 207. The hydroxyl group at C5′ of adenosine forms one hydrogen bond with Arg 355 (Figure 1a). The phosphate group of AMP forms a salt bridge to the positively charged guanidino group of Arg 355 (Figure 2a). On the basis of the number of interactions in these models, we would expect that AMP has higher affinity to GS than adenosine and that adenine has a lower affinity than adenosine.

The AMPPNP and ADP models have been very briefly described in a previous paper (Liaw & Eisenberg, 1994). The α-phosphate groups of ADP and AMPPNP interact with His 271 whereas the α-phosphate group of AMP interacts with Arg 355, and their β-phosphate groups interact with Arg 344 and the n_2 metal ion. The γ-phosphate group serves as a ligand for the metal ions at both the n_1 and n_2 sites (Liaw & Eisenberg, 1994). These interactions of the metal ions with ADP and ATP, but not with AMP, are consistent with the equilibrium binding studies (Ginsburg, 1969), and the finding of Hunt et al. (1975) that AMP binds equally to metal-free GS and metallo-GS, yet divalent cations are required for the binding of ADP. Thus chemically reasonable models of GS–AMP, GS–ADP, and GS–AMPPNP can be built into observed electron density maps.

Binding of Guanine Nucleotides to GS. The strongest density peak at the $F_{o(GS-GDP)} - F_{o(GS)}$ difference Fourier map also appears at the ATP site. However, no strong electron density peak for the six-membered ring of the guanine group is observed in the difference map, in which the peaks for the ribose ring and for the diphosphate group are observed clearly. This may suggest that interaction of the guanine ring with GS may not be as strong as that of the adenine ring, perhaps because of the proximity of its 2-amino group to the main chain of the protein in the vicinity of Leu 125. The weak difference density of GMP may be related to the low solubility of GMP in the synthetic mother liquor (less than 10 mM): no GMP peak is observed, even at the saturated GMP concentrations.

Effects of High ADP and ATP on GS. Addition to crystallization drops of more than 2 mM ATP, GDP, or ADP, but not AMP, dissolves GS crystals (Liaw et al., 1993b). This may be caused by the clusters of negative charges around the metal ion sites: three Glu residues and the substrate Glu ligate the n_1 ion, the more tightly bound ion (Ginsburg, 1972), and two Glu residues and ATP or ADP ligate the n_2 ion, the less tightly bound ion (Liaw & Eisenberg, 1994). Earlier we proposed that structural stabilization of GS is provided by the charge neutralization of the metal ions (Liaw et al., 1993c). Therefore, high occupancy of the nucleotide site by any of ADP, GDP, or ATP shifts the charge balance and might be expected to alter the local structure (Liaw et al., 1993b). In fact, crystals grown in high concentrations of ATP require more of the positively charged precipitant spermine. Moreover, they diffract only to lower resolution, and they tend to

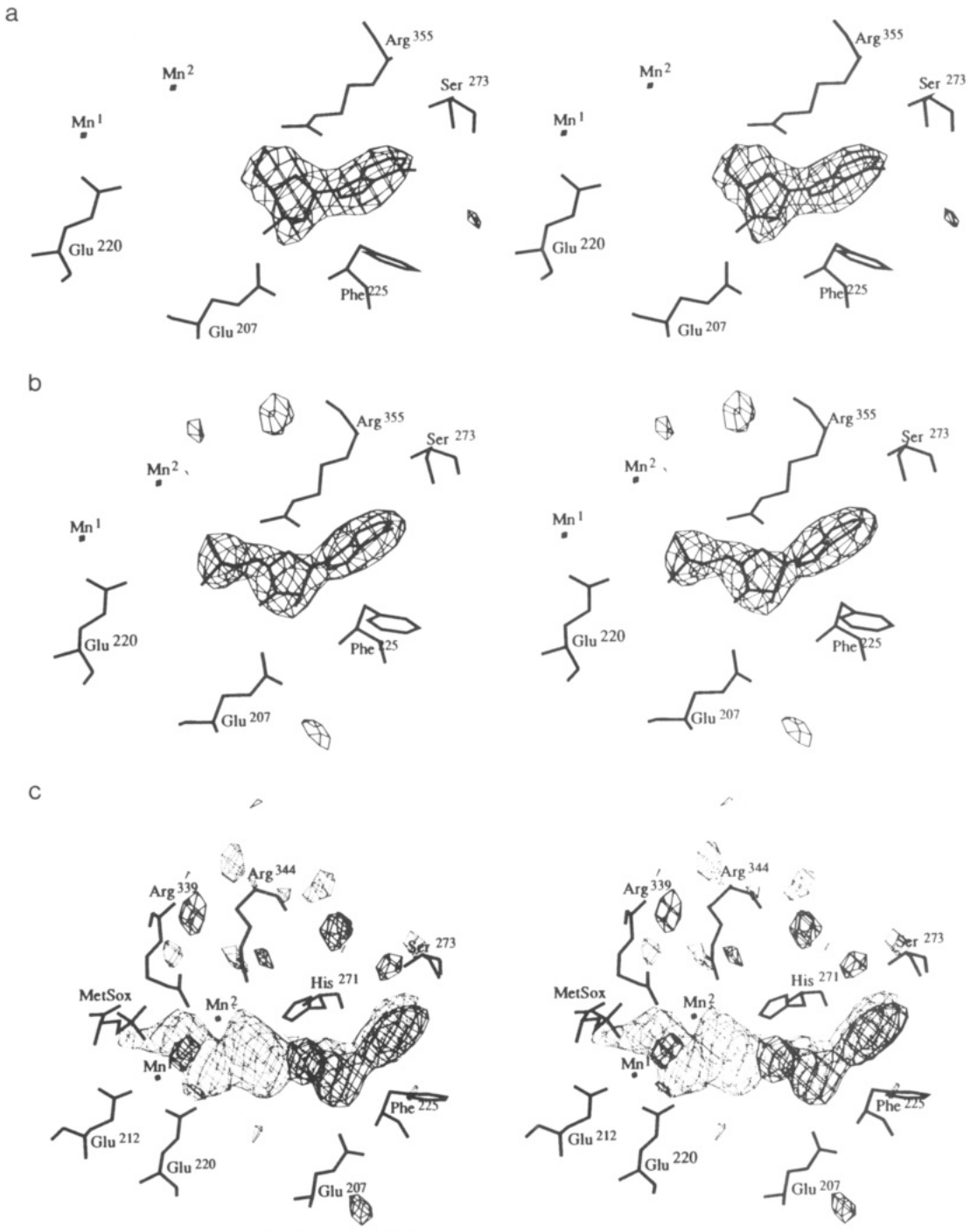

FIGURE 1: The strongest peaks in the 12-fold averaged difference Fourier maps of GS–nucleotide and –nucleoside complexes, using native phases and observed amplitudes in all cases. (a) $F_{o(GS\text{–adenosine})} - F_{o(GS)}$, (b) $F_{o(GS\text{–AMP})} - F_{o(GS)}$, (c) superimposed difference density maps for AMP and ADP: the $F_{o(GS\text{–AMP})} - F_{o(GS)}$ map is shown in solid lines, and the $F_{o[GS\text{–}(ADP+P_i)]} - F_{o(GS)}$ map in dashed lines. The overlapping density peaks suggest that the feedback inhibitor AMP binds at the ADP substrate site.

redissolve. This effect is not seen for AMP, presumably because the greater separation of the α-phosphate group of AMP from the clusters of negative charges at the n_1 and n_2 sites diminishes charge repulsion.

Kinetic Measurements. In order to reconcile our structural studies with previous kinetic measurements (Woolfolk & Stadtman, 1967), the inhibition of the GS activity by AMP was analyzed by using the biosynthetic assay. Kinetic studies were carried out by Woolfolk and Stadtman's (1967) protocol with little modification. The only difference is that we used a smaller volume, 0.16-mL assay mixture and 0.64-mL stop mixture instead of 0.2 and 1.8 mL, respectively, so that the blue color would be less diluted. Our conclusions from the single- and double-reciprocal plots of Figure 3 are the following: (1) The inhibition mode of AMP with respect to ATP is more nearly "competitive" than "noncompetitive" or "uncompetitive" for fully unadenylylated GS from *S. typhimurium*. (2) There seems to be little, if any, cooperativity in AMP binding to GS subunits. This result was also inferred from Ginsburg's data that 12 AMP binding sites are independent and equivalent (Ginsburg, 1969). (3) The K_m value of ATP for fully unadenylylated GS from *S. typhimurium* is calculated to be 0.58 mM, in good agreement with the value of 0.68 mM (Woolfolk et al., 1966) for *E. coli* GS of mixed adenylylation state. (4) The K_i value of AMP for GS from *S. typhimurium* is calculated to be 0.85 mM.

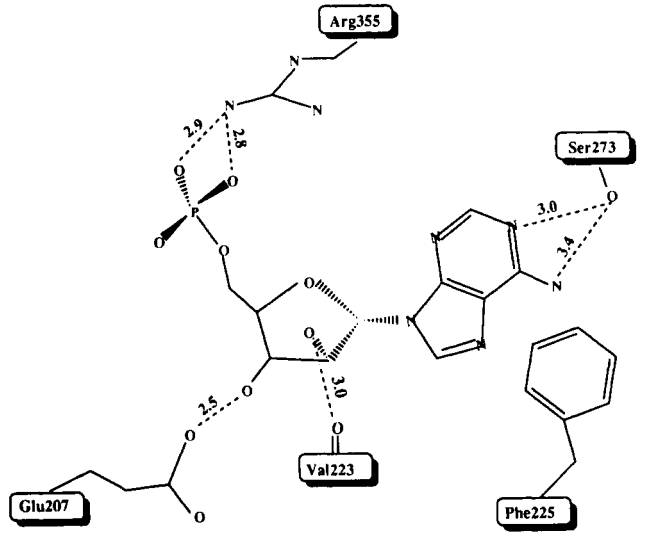

FIGURE 2: An atomic model for interactions of AMP with GS.

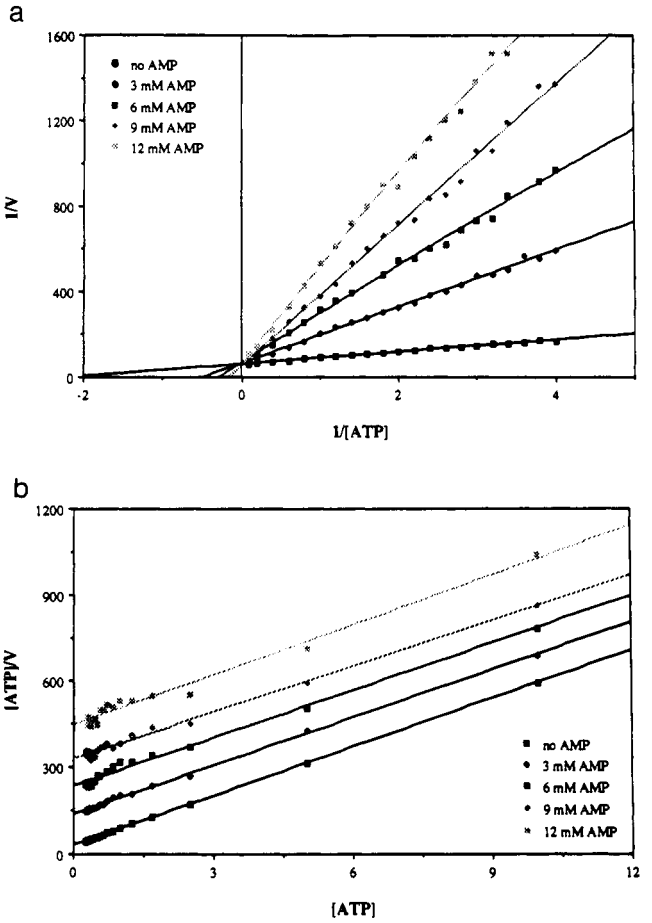

FIGURE 3: Kinetic studies of AMP using the biosynthetic assay. (a) A double-reciprocal plot is shown in which the reciprocals of enzyme reaction velocities are plotted as a function of 1/[ATP]. All lines fit to the data by least squares intersect the y-axis with values $y = 59 \pm 6$. (b) A single-reciprocal plot, $[ATP]/v$ vs [ATP]. Lines fit to the data have slopes with values 56 ± 2. These kinetic data suggest that AMP is a competitive feedback inhibitor with respect to ATP.

DISCUSSION

Structural Basis of Nucleotide Interactions with GS. Studies of kinetic measurements (Woolfolk & Stadtman, 1967) and of equilibrium binding (Ginsburg, 1969) suggested that the adenine ring, especially the 6-amino group, is predominantly responsible for the AMP binding to GS because (1) adenine, adenosine, and AMP have similar inhibition of GS activity; (2) AMP has a higher apparent association constant than GMP, IMP, UMP, and CMP; (3) increased ionic strength does not decrease AMP binding, and inhibition of GS activity by AMP is not changed by pH, suggesting that the phosphate group of AMP does not participate in AMP binding.

However, from the models for GS–AMP, GS–ADP, and GS–AMPPNP (see Results), we would expect the trend of the binding affinity of nucleotides to GS to be ADP > AMP > adenosine > adenine because for these compounds the adenine ring, the ribose ring, and the α- and β-phosphate groups interact with GS. There is a suggestion that the interactions of the phosphate groups with the n_2 metal ion and Arg 355 may be stronger than those of the ribose ring and the adenine ring, based on the height of density peaks in the difference maps. However, the greater number of electrons in phosphorus could account for the stronger density. Also, our soaking studies suggested that adenine has the lowest affinity: only a small peak appears at the position of the six-membered ring in the presence of 10 mM adenine, whereas strong density peaks are observed in the presence of the same or lower concentrations of adenosine, AMP, and ADP. Consistent with this are the measured K_m values by Woolfolk et al. (1966) suggesting that ADP has higher affinity to GS than ATP, and ATP has higher affinity than AMP. The lower affinity of ATP to GS than ADP may be due to its greater number of negative charges repelling the negative-charge clusters at the n_1 and n_2 sites. This may also be the reason for the poorer X-ray data for GS–AMPPNP (a high R merge of 17%). The lower affinity of ATP is also consistent with the absence in our model of positively charged residues interacting with the γ-phosphate group in the GS–AMPPNP complex. On the other hand, Ginsburg et al. (1987) found that AMPPNP binds more tightly to GS than ADP. The absence of direct interactions of metal ions with AMP may result in lower affinity of AMP to the enzyme than of ADP and AMPPNP. Consistent with the lack of direct interactions of metal ions with AMP is Ginsburg's (1969) finding that AMP has the same affinity to GS whether or not it contains metal ions.

Is AMP a Noncompetitive or a Competitive Feedback Inhibitor of GS? The overlapping electron density peaks of AMP, ADP, and AMPPNP in the difference Fourier maps of these nucleotides bound to GS show that AMP binds at the ATP site (Figure 1c). From these maps, AMP would be expected to be essentially a competitive inhibitor with respect to ATP. This conclusion is reinforced by our kinetic data of Figure 3 and by NMR studies of Dahlquist & Purich (1975), and it is consistent with the AMP-supported reactions catalyzed by GS (Levintow & Meister, 1954; Hunt et al., 1975; Whitley & Ginsburg, 1980), which would seem to require AMP to occupy the same enzyme site as ATP. Moreover, the complete inhibition of the ATP $\rightleftharpoons$ P_i exchange but not the Glu $\rightleftharpoons$ Gln exchange by GDP and AMP (Welder & Boyer, 1972) is consistent with our results that GDP and AMP bind to the ATP binding site. However, other data on *E. coli* GS, but not *S. typhimurium* GS, have suggested a separate site for AMP, including the kinetic measurements of Woolfolk and Stadtman (1967), NMR and fluorescence studies (Chock et al., 1977), equilibrium binding (Ginsburg, 1969), and calorimetric measurements (Shrake & Ginsburg, 1977). We consider possible reasons for the different models in the following.

Is the AMP Inhibition Mode on E. coli GS Different from That on S. typhimurium GS? The pioneering studies of feedback inhibition of GS by Woolfolk and Stadtman (1967) were on GS from *E. coli*, whereas our studies are on GS from *S. typhimurium*. The amino acid sequences of GS from *E. coli* and *S. typhimurium* differ in 13 positions among the 468 amino acid residues in one subunit (Colombo & Villafranca, 1986; Janson et al., 1986). These 13 replacements represent small structural differences and are apparently unimportant in function. Only two of these differences are of more than two heavy (i.e., non-hydrogen) atoms: Ala 420 in *S. typhimurium* corresponds to Glu in *E. coli* (four heavy atoms difference), and Pro 391 in *S. typhimurium* corresponds to Ala in *E. coli* (two heavy atoms difference). From structural and functional studies to date, none of these 13 residues is known to play an important role in reaction function or in protein folding (Liaw et al., 1993a; Liaw & Eisenberg, 1994). Moreover, GS molecules from both bacteria have nearly identical dodecameric structures, as judged by comparison of their isomorphous crystal structures (Liaw et al., 1993c). Therefore, we do not expect the AMP inhibition mode on *E. coli* to be substantially different from that on *S. typhimurium* GS.

Can the Adenylylation State Affect the Mode of AMP Inhibition? The response of GS to inhibitors depends on the state of GS, particularly its purity, and its adenylylation state (the number of covalently bound AMP moieties/dodecamer). The early kinetic data of Woolfolk and Stadtman (1967) were measured from partially pure GS (15–25% pure). Partially pure GS could conceivably contain other cofactors or proteins affecting kinetic measurements. In fact, Woolfolk and Stadtman (1967) reported that there are differences in the kinetic data of pure GS and partially pure GS. It seems likely that their partially pure GS was 50% adenylylated (Stadtman & Ginsburg, 1974), whereas our GS is completely unadenylylated. Stadtman and co-workers (Kingdon & Stadtman, 1967a; Kingdon et al., 1967) found that kinetic properties of *E. coli* GS were markedly influenced by conditions of cell growth, especially nitrogen sources and harvest time, and later they discovered that the kinetic properties of GS are strongly affected by the adenylylation state of GS, which in turn is affected by growth conditions (Kingdon & Stadtman, 1967b). The different preparations of GS yielded different kinetic measurements because adenylylation of GS affected its response to feedback inhibitors (Stadtman et al., 1968). Adenylylation increases the sensitivity of GS activity to several feedback inhibitors (Kingdon & Stadtman, 1967a; Shapiro et al., 1967); for example, it enhances susceptibility of the biosynthetic activity to inhibition by AMP (Ginsburg, 1969). Also, adenylylation was found to complicate crystallization of GS (Janson et al., 1984). It is thus our suggestion that earlier inferences about the presence of an AMP binding site separate from the ATP/ADP cofactor site were based on complications of GS properties introduced by covalent modification. A full exploration of the effects of GS adenylylation must await the crystal structures of adenylylated GS complexed with nucleotides.

Conclusion. Difference Fourier maps of GS–adenine, GS–adenosine, GS–AMP, GS–ADP, GS–GDP, and GS–AMP-PNP complexes suggest that these nucleotides all bind to the same general site, the ATP substrate binding site, with a maximum of one nucleotide per subunit. Furthermore, our kinetic measurements confirm that AMP is competitive with respect to the cofactor ATP in fully unadenylylated GS from *S. typhimurium*.

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REFERENCES

Abell, L. M., Keck, P. J., & Villafranca, J. J. (1989) *Biochemistry 28*, 1940.
Almassy, R. J., Janson, C. A., Hamlin, R., Xuong, N.-H., & Eisenberg, D. (1986) *Nature 323*, 304–309.
Boyer, P. D., Mills, R. C., & Fromm, H. J. (1959) *Arch. Biochem. Biophys. 81*, 249.
Chock, P. B., Rhee, S. G., & Villafranca, J. J. (1977) *Fed. Proc. 36*, 856.
Colombo, G., & Villafranca, J. J. (1986) *J. Biol. Chem. 261*, 10587–10591.
Dahlquist, F. W., & Purich, D. L. (1975) *Biochemistry 14*, 1980–1989.
Ginsburg, A. (1969) *Biochemistry 8*, 1726–1740.
Ginsburg, A. (1972) *Adv. Protein Chem. 26*, 1–79.
Ginsburg, A., & Stadtman, E. R. (1973) in *The Enzymes of Glutamine Metabolism* (Prusiner, S., & Stadtman, E. R., Eds.) pp 9–43, Academic, New York.
Ginsburg, A., Gorman, E. G., Neece, S. H., & Blackburn, M. B. (1987) *Biochemistry 26*, 5989–5996.
Hunt, J. B., Smyrniotis, P. Z., Ginsburg, A., & Stadtman, E. R. (1975) *Arch. Biochem. Biophys. 166*, 102–124.
Janson, C. A., Almassy, R. J., Westbrook, E. M., & Eisenberg, D. (1984) *Arch. Biochem. Biophys. 228*, 512–518.
Janson, C. A., Kayne, P. S., Almassy, R. J., Grunstein, M., & Eisenberg, D. (1986) *Gene 46*, 297–300.
Kingdon, H. S., & Stadtman, E. R. (1967a) *Biochem. Biophys. Res. Commun. 27*, 470–473.
Kingdon, H. S., & Stadtman, E. R. (1967b) *J. Bacteriol. 94*, 949–957.
Kingdon, H. S., Shapiro, B. M., & Stadtman, E. R. (1967) *Proc. Natl. Acad. Sci. U.S.A. 58*, 1703–1708.
Levintow, L., & Meister, A. (1954) *J. Biol. Chem. 209*, 265–280.
Liaw, S.-H. (1992) Ph.D. Thesis, University of California, Los Angeles.
Liaw, S.-H., & Eisenberg, D. (1994) *Biochemistry 33*, 675–681.
Liaw, S.-H., Pan, C., & Eisenberg, D. (1993a) *Proc. Natl. Acad. Sci. U.S.A. 90*, 4996–5000.
Liaw, S.-H., Jun, G., & Eisenberg, D. (1993b) *Protein Sci. 2*, 470–471.
Liaw, S.-H., Villafranca, J. J., & Eisenberg, D. (1993c) *Biochemistry 32*, 7999–8003.
Meister, A. (1980) in *Glutamine: Metabolism, Enzymology, and Regulation of Glutamine Metabolism* (Palacios, R., & Mora, J., Eds.) pp 1–40, Academic Press, New York.
Shapiro, B. M., Kingdon, H. S., & Stadtman, E. R. (1967) *Proc. Natl. Acad. Sci. U.S.A. 58*, 642–646.
Shrake, A., & Ginsburg, A. (1977) *Fed. Proc. 36*, 857.
Stadtman, E. R., & Ginsburg, A. (1974) in *The Enzymes* (Boyer, P., Ed.) Vol. 10, pp 755–807, Academic, New York.
Stadtman, E. R., Shapiro, B. M., Kingdon, H. S., Woolfolk, C. A., Hubbard, J. S. (1968) *Adv. Enzyme Regul. 6*, 257–289.
Welder, F. C., & Boyer, P. D. (1972) *J. Biol. Chem. 247*, 993–1000.
Whitley, E. J., Jr., & Ginsburg, A. (1980) *J. Biol. Chem. 255*, 10663–10670.
Woolfolk, C. A., & Stadtman, E. R. (1967) *Arch. Biochem. Biophys. 118*, 736–755.
Woolfolk, C. A., Shapiro, B. M., & Stadtman, E. R. (1966) *Arch. Biochem. Biophys. 116*, 177–192.
Yamashita, M. M., Almassy, R. J., Janson, C. A., Cascio, D., & Eisenberg, D. (1989) *J. Biol. Chem. 264*, 17681–17690.