Glutamine Binding Opens the Ammonia Channel and Activates Glucosamine-6P Synthase*

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Glucosamine-6P synthase catalyzes the synthesis of glucosamine-6P from fructose-6P and glutamine and uses a channel to transfer ammonia from its glutaminase to its synthase active site. X-ray structures of glucosamine-6P synthase have been determined at 2.05 Å resolution in the presence of fructose-6P and at 2.35 Å resolution in the presence of fructose-6P and 6-diazo-5-oxo-l-norleucine, a glutamine affinity analog that covalently modifies the N-terminal catalytic cysteine, therefore mimicking the γ-glutamylthioester intermediate formed during hydrolysis of glutamine. The fixation of the glutamine analog activates the enzyme through several major structural changes: 1) the closure of a loop to shield the glutaminase site accompanied by significant domain hinging, 2) the activation of catalytic residues involved in glutamine hydrolysis, i.e. the α-amino group of Cys-1 and Asn-98 that is positioned to form the oxyanion hole, and 3) a 75° rotation of the Trp-74 indole group that opens the ammonia channel.

Glucosamine-6P synthase (GlmS) catalyzes the first and rate-limiting step of hexosamine metabolism, the conversion of d-fructose-6P (Fructose6P) into d-glucosamine-6P using l-glutamine as a nitrogen source. Because the end product of this pathway, UDP-N-acetyl glucosamine, is a major building block of the bacterial peptidoglycan and fungal chitin, inhibitors of the microbial enzymes have potential antifungal and antibiostics properties. More recently, the human enzyme, Gfα, has attracted significant attention because of the observation that hexosamine biosynthetic pathway plays a nutrient sensor role and is a mediator of insulin resistance (1). Indeed, transgenic mice overexpressing glucosamine 6-phosphate synthase in skeletal muscle and fat have been shown to develop insulin resistance with manifestations similar to that observed in type-2 diabetes (2). Therefore, inhibitors directed toward the human enzyme are expected to limit the renal and ocular complications associated with type-2 diabetes in diabetes treatment. Understanding at the molecular level the particular mechanisms of the different enzymes is essential to development of specific drugs against the human, fungal, or bacterial enzymes. GlmS belongs to the glutamine-amidotransferase family that uses the nucleophilic attack of the thiol group of a cysteine residue on the δ-carbonyl group of glutamine to form ammonia, which is then transferred to an NH3 acceptor substrate that differs for each amidotransferase (3, 4). Glutamine amidotransferases provide the major route for incorporation of nitrogen into the biosynthetic pathways of amino acids, amino sugars, purine and pyrimidine nucleotides, coenzymes, and antibiotics. Together with glutamine synthase, glutamine phosphoribosylpyrophosphate amidotransferase, and asparagine synthetase B, GlmS belongs to the N-terminal nucleophile (Ntn) subclass of amidotransferases that uses the α-amino group of the terminal nucleophilic cysteine residue to activate its thiol group (5). GlmS from Escherichia coli consists of a 27-kDa glutaminase domain (GSm, residues 1–239) that catalyzes glutamine hydrolysis and a 40-kDa synthase domain (GSyn, residues 249–608) that catalyzes Fructose6P amination and isomerization (Fig. 1) (6, 7). The x-ray structures of the individual domains have been previously determined for the glutaminase domain in the presence of l-glutamate or γ-glutamyl hydroxamate (8) and for the synthase domain in the presence of glucosamine-6P, glucose-6P, Glc6P, or 2-amino-deoxyglucitol-6P (9, 10). The binding site residues were identified, and a mechanism at each active site was proposed (Fig. 1) in agreement with the biochemical data (11). The structures also revealed the homodimeric nature of the enzyme but could not give any information about the communication between the two domains. The next crystal structure at 3.1 Å resolution of the whole enzyme crystallized in the presence of Fructose6P showed the existence of an 18 Å-long channel connecting the glutaminase and isomerase sites (12). The walls of the ammonia channel were constituted by the side-chain of Trp-74 and the main-chain of Arg-26, both from the glutaminase domain, and by residues 601–607 belonging to the C-terminal nonapeptide (C-tail, residues 600–608), as well as a loop of the synthase domain of the neighboring subunit (residues 503–505*) (12). Unexpectedly, the channel was totally blocked by the Trp-74 indole group. This provided a clue to explain why GlmS, together with glutamate synthase, are the only amidotransferases that cannot use exogenous ammonia as a nitrogen donor instead of glutamine (13, 14).

The solvent-inaccessible ammonia channel in the amidotransferase family provides a great advantage for catalysis as it prevents the formation of non-reactive ammonium ions and the loss of the reaction intermediates in solution. To achieve this goal, the enzymes must synchronize their catalytic sites through large domain movements triggered by substrate binding (15–17). The different corresponding conformational states of the proteins can be visualized by the crystal structures of the enzymes in the presence of various ligands. GlmS uses an ordered bi bi mechanism with Fructose6P binding prior to glutamine (18). A model for substrate binding and enzyme activation through conformational changes has been proposed based on the crystal structure at 3.1 Å resolution of GlmS in complex with the sugar (12). The rearrangement of the C-tail occurring upon Fructose6P binding would close the synthase site.
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EXPERIMENTAL PROCEDURES

Protein Preparation, Purification, and Crystallization—GlmS was purified from recombinant E. coli HB101 cells at 4 °C. After growth at 37 °C under strong aeration during 17 h in 4 liters of Luria Bertani medium supplemented with 100 μg ml⁻¹ ampicillin, cells were resuspended in 100 ml of buffer A (20 mM Bis Tris Propane, pH 7.2, 1 mM EDTA, 1 mM dithiothreitol) and sonicated. The supernatant was loaded onto an SP-Sepharose fast flow column (5 × 15 cm) of buffer A. Active fractions eluting at 0.35 M NaCl were pooled, concentrated, and loaded onto a Superdex 200 HiLoad gel filtration column previously equilibrated in 20 mM KPO₄, pH 7.2. The active fractions (972 mg) were pooled and concentrated.

The DON-inactivated GlmS was formed by incubating GlmS with 2 mM DON and 10 mM Fru6P for 1 h in 50 mM KPO₄, pH 7.2, loaded onto a Mono Q HR 10/10 column, and eluted with a linear gradient of 0–1 M NaCl in 10 mM Fru6P, 20 mM HEPES, pH 7.2. Crystals were grown at
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18 °C in hanging drops by vapor diffusion for several days. 1 μl of the DON-modified protein (7.5 mg ml⁻¹ in 20 mM HEPES, pH 7.2, 10 mM Fru6P, 0.3 mM NaCl) was mixed with 1 μl of a 1-ml reservoir solution (12% polyethylene glycol 8000, 0.1 mM KCl, 5% glycerol). Although the crystals (0.05 × 0.1 × 0.4 mm) were obtained in the presence of Fru6P, the best fit of the electron density was for glucose-6P in its linear form, which likely resulted from the phosphoglucose isomerase activity of GlmS (7). This complex was called GlmS-Glc6P-DON. Crystals of the GlmS-Fru6P complex (0.1 × 0.1 × 0.8 mm) were obtained by mixing 5 μl of protein solution (30 mg ml⁻¹ in 20 mM HEPES, pH 7.2, 0.3 mM NaCl, 10 mM Fru6P) with 5 μl of a 1-ml reservoir solution (3% polyethylene glycol 400, 0.1 mM HEPES, pH 7.5, 0.8 mM LiCl).

X-ray Data Collection and Processing—Crystals were soaked for a few minutes in a cryoprotectant solution (25% glycerol, 16% polyethylene glycol 8000, 0.1 mM KCl for the GlmS-Glc6P-DON crystal and 25% polyethylene glycol 400, 0.1 mM HEPES, pH 7.5, 1 mM LiCl for the GlmS-Fru6P crystal) before flash freezing in a cold nitrogen stream at 100 K. The diffraction data sets were collected on beam line ID14-EH2 for the GlmS-Glc6P-DON crystal or ID14-EH1 for the GlmS-Fru6P crystal at the European Synchrotron Radiation Facility in Grenoble and processed with DENZO and SCALEPACK (21) (Table 1).

Structure Determination—The GlmS-Glc6P-DON and GlmS-Fru6P structures were solved by molecular replacement with AMoRe (22) using, respectively, the individual GlmS domains (Protein Data Bank codes 1GDO and 1MOQ) (8, 9) or monomer A of the native protein (Protein Data Bank code 1X1A) (12) as model.

Refinement and Model Building—In the GlmS-Glc6P-DON structure, the two monomers in the asymmetric unit are related by a non-crystallographic 2-fold axis to form a compact dimer. In the GlmS-Fru6P structure, monomers B and C in the asymmetric unit form a dimer, whereas monomer A forms a dimer with its counterpart in the neighboring asymmetric unit. The refinement consisted of alternating rounds of manual fitting of the model to electron density maps using O (23) and isotropic refinement in CNS (24). The relatively high R-factors and large differences in the mean isotropic B-factors for the different domains of the GlmS-Glc6P-DON structure (Table 1) prompted us to use translation libration screw parameterization in REFMAC (25) to take into account translations and librations of pseudo-rigid bodies within the asymmetric unit of the crystal (26). Each synthase and glutaminase domain was treated as different TLS groups. Non-crystallographic symmetry restraints were used during the refinement of the GlmS-Fru6P structure in CNS and were released in REFMAC. Use of TLS parameters is reasonable as the GlmS-Fru6P structure is loosely packed with a solvent content of 60.8% and as the glutamine domain of chain C makes only one crystallographic contact with its symmetric molecule in the neighboring asymmetric unit. The analysis of the structures is based on monomer A, which is best defined in the electron density maps of both structures. 99.7% of the residues in both structures are in the allowed regions of the Ramachandran plot. Residues in the disallowed regions include residues of the linker region between the two domains and Glu-91 (ϕ, 65.4°; ψ, −116°), as previously observed (8, 12), that is located at the edge between two β-sheets. Superposition of the structures have been made with SUPERPCK. 3

RESULTS

The GlmS-Glc6P-DON structure is the first GlmS structure for which both sites contain a ligand, and this ternary complex represents the active conformation of the enzyme before the ammonia transfer step.

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TABLE 1

| Data collection and refinement statistics | GlmS-Glc6P-DON | GlmS-Fru6P |
|------------------------------------------|---------------|------------|
| Space group                              | P2₁2₁2₁       | C2         |
| Crystal dimensions                       | a, b, c (Å)   | 83.3, 91.2, 185.0 | 132.2, 109.7, 176.3 |
| α, β, γ (°)                              | 90, 90, 90    | 90, 91.7, 90 |
| Resolution (Å)                           | 30.00–2.35 (2.39–2.35) | 30.00–2.05 (2.09–2.05) |
| Rwork/Rfree before TLS refinement        | 0.097 (0.446) | 0.083 (0.72) |
| Rwork/Rfree after TLS refinement         | 0.097 (0.446) | 0.083 (0.72) |
| No. atoms                                | 4909          | 14037      |
| Protein                                  | 56911         | 145921     |
| Redundancy                               | 405           | 717        |
| Water                                    | 36.2          | 38.2       |
| R.m.s.d.                                 | 0.0064        | 0.021      |
| Bond lengths (Å)                         | 1.347         | 1.93       |
| Bond angles (°)                          | 48.1 (B)      | 38.7 (°GlmS, A) |
| Ramachandran statistics (%)              | 68.1 (B)      | 30.2 (°GlmS, A) |
| Most favored                             | 36.1 (°GlmS, B) | 53.4 (°GlmS, B) |
| Additionally allowed                     | 19.9 (A)      | 71.9 (°GlmS, C) |
| Generously allowed                       | 45.7 (°GlmS, C) | 53.7 |
| Disallowed                               | 0.2           | 0.6        |
| Solvent content (%)                      | 52.9          | 60.9       |

* A total of 5% of the data was set aside to compute Rfree.
* Upper case letters designate chain.

The comparison of this structure to the GlmS-Fru6P structure solved at 2.05 Å resolution, which is the state of the enzyme just before the glutamine binding step, therefore gives information about the conformational changes taking place upon glutamine binding.

Synthase Site—The refinement of the GlmS-Fru6P structure at 2.05 Å resolution using TLS parameters to account for the differences in mean isotropic B-factors between the different molecules in the asymmetric unit resulted in a structure of better quality compared with the previously determined structure at 3.1 Å resolution (12), as judged by the lower isotropic B-factors and the higher number of residues in the allowed region of the Ramachandran plot (Table 1). In addition, it allows visualization of water molecules that might play a significant role in catalysis (Fig. 3A) and assignment of the previously undefined nature of the sugar (Fig. 2A). Indeed, the Fo – Fc omit maps indicate that the bound sugar is in the linear form in both structures. The GlmS phosphoglucose isomerase activity may isomerize Fru6P to Glc6P when GlmS is crystallized in the presence of Fru6P and DON or Fru6P alone (7). The difference between the open-chain forms of Glc6P and Fru6P resides in the hybridization state of C1 and C2, which is difficult to determine at the current resolutions. However, the modeling of the linear forms of Glc6P or Fru6P and the examination of both the positive

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P. Alzari, unpublished data.
and negative contours of the difference density support the majority presence of Fru6P in the absence of DON (GlmS/Fru6P structure) or Glc6P in its presence (GlmS/Glc6P/DON structure).

The overall structure of the synthase domain is very similar in the two structures, although some important differences are noticed at the synthase site (Fig. 2). First, the conformational change of Ser-401 is related to the different positions of the C2 oxygen of the sugar. Second, we observed conformational changes of some residues that constitute the walls of the channel: Lys503*, which participates to the channel of the neighboring subunit and the C-tail, in particular with the flipping of the peptide bond of Lys-603 and the conformational change of Ser-604 that are connected to the movements of Arg-26 and Trp-74 from the glutaminase domain. As discussed below, this opens the ammonia channel and activates the glutaminase function.

Glutaminase Site—In addition to the nucleophilic thiol group of Cys-1, the key catalytic elements for glutamine hydrolysis in GlmS are the α-amino group of Cys-1 and an oxyanion hole formed by Nδ2 of Asn-98 and NH of Gly-99 in order to stabilize the tetrahedral interme-
FIGURE 3. Comparison of the glutaminase sites of the GlmS-Fru6P and GlmS-Glc6P-DON structures. The cavity is lined with residues that are conserved in the Ntn amidotransferases family: Cys-1, Arg-26, Arg-73, Asn-98, Gly-99, and Asp-123 as well as residues Thr-606, Thr-76, and Trp-74. A, GlmS-Fru6P structure. The glutaminase site is empty, and the Q-loop is in an open conformation so that the glutamine pocket is exposed to the solvent. The carbonyl group of Arg-26 and water molecules hydrogen bind to the N-terminal amino group, whereas the carbonyl oxygen atom of Cys-1 hydrogen binds to a water molecule. The water molecules may mimic different positions of the water molecule that is supposed...
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The electron density in the glutaminase binding pocket of the GlmS-Glc6P-DON structure reveals that the thiol group of the nucleophilic cysteine residue is covalently modified by DON (Fig. 3B). In the GlmS-Fru6P structure, the thiol group of the nucleophilic Cys-1 is catalytically competent (Fig. 3A), although it does not point toward the active site in the GlmS-Glu or GlmS-Glu hydroxamate structures (8) (Fig. 3D). Therefore, it is likely that the active orientation of the thiol group of Cys-1 is triggered by acceptor binding. The glutaminase site of Ntn amidotransferase is conserved not only by several conserved residues from the glutaminase site (3, 27) but also by an oxygen atom of a residue of the synthase domain not conserved in this family that makes a direct hydrogen bond with the α-amino group of Cys-1 in the presence of glutamine. By making the corresponding hydrogen bond between GlmS through a conformational change upon DON binding (Fig. 3, B and C), Thr-606 enhances the nucleophilic character of the terminal α-amino group that acts as a general acid/base in glutamine hydrolysis (Fig. 1B) (5). In addition, the active conformation of Cys-1 is ensured by a hydrogen bond between its carbonyl oxygen atom and the guanidinium group of Arg-26 in the GlmS-Glc6P-DON structure (Fig. 3B), an interaction that is conserved in other Ntn amidotransferases where both sites are occupied (19, 20).

The positioning of the side-chain NH2 group of Asn-98 in order to form the oxoanion hole is another consequence of glutamine binding at the glutaminase site (Fig. 3, B and C). In the different structures of GlmS (8) (Fig. 3D) and in the GlmS-Fru6P structure (Fig. 3A), the side-chain of Asn-98 does not adopt such a conformation. The proper structuring of the oxoanion hole requires occupation of both isomerase and glutaminase domains and is driven by a specific hydrogen bond with the guanidinium group of Arg-26, the conformational change of which is linked to the restructuring of loop 24–29 (Fig. 3C). Thus, the conserved Arg-26-Gly-27 dipeptide previously defined as an "anchor" for both the backbone of Cys-1 and the side-chain of the asparagine involved in the oxoanion hole (3) is involved together with Thr-606 in the activation of the glutaminase function through specific hydrogen bonds.

In addition, comparison of the glutaminase sites of the GlmS-Glc6P-DON and GlmS-Fru6P structures shows that DON binding at the glutaminase site leads to a large conformational change of the Q-loop (Fig. 3, C and E). It had been previously anticipated that the glutamine loop would act as a gate keeper in controlling access to the glutaminase site in Ntn amidotransferases (28), but the present work is the first demonstration of the closure of the Q-loop upon DON binding to the acceptor-bound state of an amidotransferase. The most drastic structural change in the GlmS Q-loop concerns Trp-74, which adopts different conformations in the GlmS-Fru6P, GlmS-Glc6P-DON, and GlmS-Glu structures (Fig. 3). These conformations are associated with different hydrogen binding patterns of its indole NH group as well as to a conformational change of Lys-503* resulting from steric hindrance (Fig. 2C). The 75° rotation of the indole ring of Trp-74 is crucial for ammonia transfer because the indole group blocks the channel in the GlmS-Fru6P structure but not in the GlmS-Glc6P-DON structure (see below). Trp-74 is therefore a major actor in coupling glutamine binding to ammonia transfer. In addition, the conformational change of the Q-loop upon DON binding allows a precise positioning of the glutamine analog in the active site. Indeed, it allows an ionic interaction between Arg-73 from the Q-loop and the α-carboxyl group of DON (Fig. 3B). Moreover, Asp-123, which belongs to loop 121–125 that is also involved in the closure of the glutaminase site, provides specific interactions with the α-amino group of the inhibitor (Fig. 3, B and E).

Interdomain Coupling—The C-tail is highly conserved among glucosamine-6P synthases (9). It is a fingerprint of the enzyme and has a fundamental role in the enzymatic function.

Role of the C-tail in Closing the Synthase Site—First, the C-tail makes contact between the glutaminase and the synthase domains and contributes to the sugar binding site through residues 603–605 (Fig. 4, A–C). The contact between the sugar and the C-tail is direct in the structures of GlmS in complex with cyclic sugar (9), whereas it is mediated by numerous water molecules in the structures of native GlmS in complex with the open ring sugars (Fig. 4, A and B). Second, the C-tail shields the sugar binding site from bulk solvent when Fru6P is bound (Fig. 4C). Actually, in all the GlmS structures determined so far (9, 10, 12), the isomerase active site is occupied and in a closed conformation. The closure of the isomerase site is supposed to be triggered by Fru6P binding to the enzyme through a 20° rotation of the C-tail around the Ca–C bond of the conserved Pro-598 (Fig. 4C) (12).

Activation of the Glutaminase Function—The C-tail interacts with the glutaminase domain by providing binding pockets for Trp-74 and for the Tyr-28 hydroxyl group through hydrogen bonds with the backbone carbonyl of Pro-598 (Fig. 4C). In addition, the activation of the glutaminase function upon DON binding is ensured by residues of the C-tail: Thr-606, which provides direct hydrogen bonds to the α-amino group of DON makes an ionic interaction with the guanidinium group of Arg-73 and hydrogen bonds to NH and OH of Thr-76, NH of His-77, and Nε2 of His-86 (omitted for clarity). The α-amino group of DON is hydrogen bonded to OH of Thr-76, the carbonyl group of Arg-123, and the carbonyl group of Gly-99. The carbonyl oxygen of DON hydrogen binds to Nε2 of Asn-98 (in r.m.s.d., 2.03 Å for all side-chain atoms). Conformational changes of loop 24–29 (r.m.s.d., 0.95 Å) and the Q-loop (r.m.s.d., 2.77 Å) point toward the solvent and do not interact with the protein in the GlmS-Fru6P and GlmS-Glc6P-DON structures. The superposition of the Cα of each glutaminase domain enlightens the movement of loop 121–125 (r.m.s.d., 1.49 Å for 5 Cαs) and the Q-loop (r.m.s.d., 2.92 Å for 9 Cαs), which closes the glutaminase site upon ligand binding. Surface area calculations with a probe radius of 1.4 Å indicate that the closure of the active site by the Q-loop buries a total area of 279 Å2.
FIGURE 4. Role of the C-tail. A and B, the C-tail acts as an interdomain signal in the activation of the glutaminase function. A, GlmS/Fru6P structure. B, GlmS-Glc6P-DON structure. The communication between the two active sites is mediated by Arg-26 and the C-terminal residues. DON binding triggers a conformational change of Arg-26, the guanidinium group of which hydrogen binds to the carbonyl group of Lys-603 and to OH of Thr-606 in the GlmS/Fru6P structure or to Oδ1 of Asn-98 and the carbonyl group of Cys-1 in the GlmS-Glc6P-DON structure. These specific hydrogen bonds to Arg-26 and Thr-606 activate the glutaminase function by enhancing the nucleophilic character of the α-amino group of Cys-1 and positioning Asn-98 to form the oxyanion hole. C, the C-tail closes the synthase site in the GlmS/Fru6P structure. The hydrogen bond between the carbonyl oxygen of Pro-598 and OH of Tyr-28 is conserved in the GlmS/Fru6P and GlmS-Glc6P-DON structures and may be the hinge point for the opening of the C-tail (in pink) in the absence of Fru6P (12). D, the C-tail forms part of the ammonia channel. Binding of DON at the glutaminase site triggers the opening of the ammonia channel. The channel is formed mainly by main-chain atoms of the C-tail, indicated as a coil, and by the side-chains of residues Leu-601, Ala-602, Val-605, Thr-606, Val-607, and Trp-74, indicated as sticks. The accessible surface of the channel in the GlmS-DON-Glc6P structure calculated with PYMOL and a probe radius of 1.4 Å is represented as a mesh surface and is colored as a function of the atoms forming it (oxygen in red, carbon in orange, and nitrogen in blue). In this structure, the indole group of Trp-74 (in orange) lines the surface of the channel, which is open. The position of the indole group of Trp-74 that blocks the channel in the GlmS/Fru6P structure has been superimposed in blue. The 75° rotation of the indole of Trp-74 is measured by the differences in the C-Cα-Cβ-Cγ dihedral angle. Water molecules in the channel form a hydrogen bond network with the backbone atoms of residues lining the channel as well as OH of Thr-606. These water molecules, which may reflect the position of ammonia in the channel, are likely absent during the ammonia transfer step.
group of Cys-1 and to Asn-98, and Lys-603 and Ser-604, which undergo conformational changes coupled to that of Arg-26, which is involved in the positioning of these two catalytic residues (Fig. 4B).

Opening of the Ammonia Channel—The x-ray structures of GlmS reveal that the glutaminase and synthase sites are separated by 18 Å. Residues 601–607 of the C-tail form part of an internal ammonia channel that connects the two active sites (Fig. 4D). In the GlmS-Fru6P structure, the channel is totally closed by the indole ring of Trp-74 (12), and we have shown that DON binding induces the closure of the Q-loop together with a 75° rotation of the Trp-74 indole side-chain (Fig. 3). The consequence of these conformational changes upon glutamine analog binding is the opening of the ammonia channel, allowing the connection between the two active sites (Fig. 4D).

Hinged Movement of the Glutaminase Domain Relative to the Synthase Domain—Another important change between the GlmS-Fru6P and GlmS-Glc6P-DON structures is a hinged movement of 21° between the glutaminase domains when the synthase domains are superimposed (Fig. 5A). The pivot point is near residue Asp-29 and the extremity of the Q-loop (Fig. 5B). Ala-75 and Thr-76 from the Q-loop participate in the dimer interface by interacting with the 524*-539* α-helix and the ionic interaction between Asp-29 and Arg-539* that is crucial for dimerization are conserved in the two structures.

DISCUSSION

In GlmS, the coupling between the two active sites is especially efficient because ammonia cannot be used as a nitrogen donor. Hydrolysis of glutamine occurs significantly only when Fru6P is bound to the synthase site. Thus, the enzyme is supposed to adopt at least three different states (12): (i) The two active sites are empty, the C-tail and the Q-loop are in open conformations, the glutaminase function is inactive, and the ammonia channel is not formed. (ii) Upon glutamine binding, the glutaminase site is fully activated and the ammonia channel is open. In this work, we have visualized the conformational changes that take place during this step by comparing the GlmS-Fru6P and the GlmS-Glc6P-DON crystal structures. These x-ray experiments provide the first structural evidence of glutaminase activation and channel opening upon glutamine binding in amidotransferases. Indeed, the changes observed in GlmS upon glutamine analog binding to the acceptor-bound state of the enzyme were not seen in ferredoxin-dependent glutamate synthase, which is the only other amidotransferase that has been crystallized in the presence of the acceptor alone and in the presence of both acceptor and glutamine analog, because of crystal packing (20). DON binding to GlmS induces the closure of the glutaminase site by a conformational change of the glutaminase domains at the interface.
Q-loop accompanied by a 21° rotation of the glutaminase domain relative to the synthase domain, in order to maintain the dimer interface. These changes restrict the access of solvent to the glutaminase site, protect ammonia from protonation, and prevent it from escaping into the surrounding medium. The Q-loop is therefore a critical element in signaling between the two active sites. We have shown that glutamine analog binding to GlnS activates the glutaminase function by positioning Asn-98 to form the oxyanion hole and enhancing the nucleophilic character of the α-amino group of Cys-1. The communication between the two active sites is mediated by residues forming part of the channel, Arg-26 and the C-terminal residues including Thr-606, the only residue from the synthase domain participating to the glutamine binding site.

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