Involvement of the C terminus in intramolecular nitrogen channeling in glucosamine 6-phosphate synthase: evidence from a 1.6 Å crystal structure of the isomerase domain

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Background: Glucosamine 6-phosphate synthase (GlmS) catalyses the first step in hexosamine metabolism, converting fructose-6P (6 phosphate) into glucosamine-6P using glutamine as a nitrogen source. GlmS is a bienzyme complex consisting of two domains that catalyse glutamine hydrolysis and sugar-phosphate isomerisation, respectively. Knowledge of the three-dimensional structure of GlmS is essential for understanding the general principles of catalysis by ketol isomerases and the mechanism of nitrogen transfer in glutamine amidotransferases.

Results: The crystal structure of the isomerase domain of the *Escherichia coli* GlmS with the reaction product, glucosamine-6P, has been determined at 1.57 Å resolution. It is comprised of two topologically identical subdomains, each of which is dominated by a nucleotide-binding motif of a flavodoxin type. The catalytic site is assembled by dimerisation of the protein.

Conclusions: The isomerase active site of GlmS seems to be the result of evolution through gene duplication and subsequent dimerisation. Isomerisation of fructose-6P is likely to involve the formation of a Schiff base with Lys603 of the enzyme, the ring-opening step catalysed by His504, and the proton transfer from C1 to C2 of the substrate effected by Glu488. The highly conserved C-terminal fragment of the chain may play a key role in substrate binding, catalysis and communication with the glutaminase domain. The corresponding sequence pattern DXPXXLAK[SC]VT (in single-letter amino-acid code, where X is any amino acid and letters in brackets indicate that either serine or cysteine may take this position) may be considered as a fingerprint of GlmS.

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Introduction

Glutamine-dependent amidotransferases are responsible for utilisation of the amide nitrogen of glutamine in a variety of biosynthetic reactions [1–3]. Given the fundamental role of these enzymes in cellular metabolism, elucidation of their structure and mechanism of action may have significant implications for the discovery of new therapeutic agents. Glucosamine 6-phosphate synthase (GlmS, L-glutamine:D-fructose-6P amidotransferase, EC 2.6.1.16) catalyses the first step in hexosamine metabolism, converting fructose-6P into glucosamine-6P in the presence of glutamine. The end-product of the pathway, N-acetylglucosamine, is an essential building block of bacterial and fungal cell walls. The mammalian enzyme plays a key role in desensitising the insulin-responsive glucose-transport system [4]. Structural differences between prokaryotic and eukaryotic enzymes may be exploited to design specific inhibitors that might serve as prototypes of antifungal and antibacterial drugs.

GlmS from *Escherichia coli* is a bienzyme complex consisting of two structurally and functionally distinct domains [5]. The N-terminal glutaminase domain formed by residues 1–240 catalyses hydrolysis of glutamine to glutamate and ammonia. The C-terminal isomerase domain encompassing residues 241–608 utilises this ammonia for fructose-6P to glucosamine-6P conversion. The reaction might proceed through the formation of fructosimine-6P that is then isomerised to glucosamine-6P. Unlike other amidotransferases, GlmS cannot use exogenous ammonia as a nitrogen donor. The two domains, separated by controlled chymotrypsin proteolysis or expressed separately, retain their ability to bind substrates and to catalyse glutamine hydrolysis and fructose-6P to glucose-6P isomerisation respectively [6,7]. The three-dimensional (3D) structure of the glutaminase domain revealed the catalytic mechanism of the N-terminal nucleophile type and shed light on the fructose-6P induced activation of glutamine hydrolysis [8].

Now we have solved the crystal structure of the isomerase domain that catalyses the conversion of fructose-6P into glucosamine-6P or glucose-6P, depending on the presence or absence of glutamine. The isomerase activity associated with this domain is unique among amidotransferases and assigns GlmS to a family of aldose/ketose isomerases (EC 5.3.1.9 and EC 5.3.1.10). The best-studied members of this enzyme family are triosephosphate isomerase (TIM) and D-xylose isomerase. Although both are characterised by an $(\alpha\beta)_8$-barrel structure [9,10], their catalytic mechanisms are different. D-xylose isomerase exploits the hydride-shift mechanism [11], whereas TIM follows the enolisation mechanism [12]. The structural diversity among isomerases is remarkable: phosphoribosylanthranilate isomerase has a TIM-barrel fold [13]; the catalytic domain of D-mannose-6P isomerase is folded into an 11-stranded antiparallel β sandwich of a jelly-roll type [14]; L-fucose isomerase, also known as D-arabinose isomerase, has a multidomain αβ structure dominated by a six-stranded antiparallel β barrel [15]; glucosamine-6P deaminase [16] and glucose-6P isomerase [17] display different variants of the nucleotide-binding motif. No sequence homology can be detected between these enzymes and GlmS.

The 3D structure of GlmS is essential for understanding the general principles of catalysis and the evolutionary relationships among ketol isomerases. The structure is also expected to disclose the mechanism of nitrogen transfer in glutamine amidotransferases. In this paper we describe the high-resolution crystal structure of the isomerase domain of GlmS in complex with the reaction product glucosamine-6P.

Results and discussion

Quality of the model

The structure of the isomerase domain of GlmS has been refined to an R factor of 18.5% for all data in the resolution range 10–1.57 Å. The final model contains residues 243–608 of GlmS (residues 241 and 242 are not visible), 416 water molecules, glucosamine-6P, 2-methyl-2,4-pentanediol (MPD), N-morpholino ethanesulphonate (MES), a sodium ion and five sulphates. The refinement statistics are given in Table 1. The overall G factor calculated by the program PROCHECK [18] as a measure of the stereochemical quality of the model is 0.1, which is better than that expected for a structure refined at such a resolution. None of the residues has a forbidden combination of mainchain torsion angles; 93% fall in the most favoured regions, as defined in PROCHECK. The root mean square deviation (rmsd) of peptide units from planarity is 2.9°; the maximum deviation does not exceed 10°.

Contrary to the published nucleotide sequence [19], residues 418 and 419 are modelled as lysine and leucine, respectively, as is clearly suggested by the electron density (Figure 1). This could be due to a natural mutation of the *E. coli* strain, or due to an error in the DNA sequence (the hexamer AAGCTG coding for the dipeptide Lys–Leu could have been read erroneously as AACGTG, which would give Asn–Val).

Table 1

Refinement statistics.

Resolution range (Å)	10–1.57
R_{cryst}* (%)	18.5
Number of atoms	
protein	2818
heterogen	62
solvent	416
Average B factors (Å^2)	
protein	22.2
solvent	38.7
Rmsd	
bond distances (Å)	0.014
bond angles (°)	1.4
peptide angles (°)	2.9
B factor correlation (Å^2)	
mainchain	3.6
sidechain	7.8

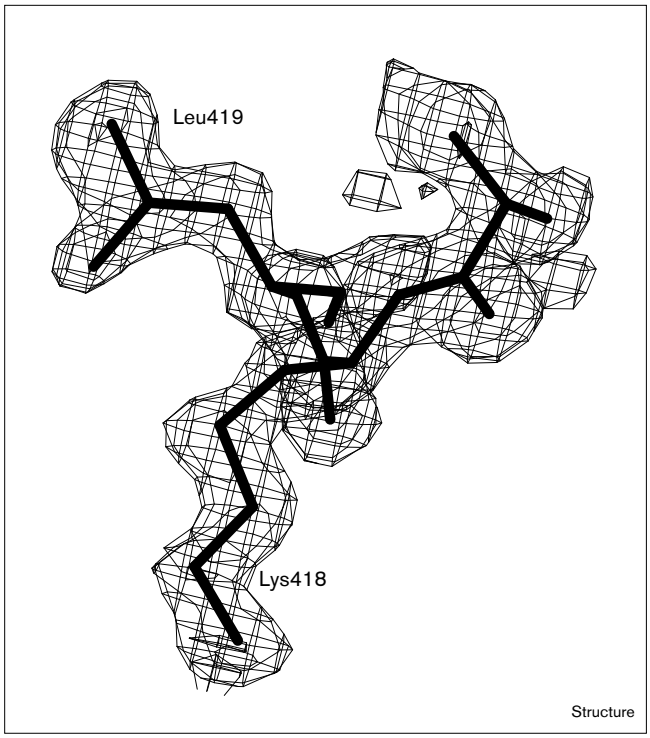
*$R_{cryst} = \Sigma||F_o|-|F_c||/\Sigma|F_o|$.

Overall structure

The isomerase domain of GlmS is bilobal in shape and consists of two topologically identical subdomains of equal size (Figure 2). The N- and C-terminal subdomains comprise residues 241–424 and 425–592, respectively (residues 1–240 of GlmS belong to the glutaminase domain). C-terminal residues 593–608 (C tail) form an irregular loop on the surface of the N-terminal subdomain. In the following discussion and Figure 2, secondary structural elements of the N- and C-terminal subdomains are referred to with the letters N and C, respectively; β strands are numbered consecutively along the chain from 1 to 5 and helices from A to H in each subdomain (Figure 3). Helices CE and CG are 3_{10} helices; the others are all α helices. Loops following β strands are numbered according to these strands, for example N1 for the loop following the first β strand of the N-terminal subdomain.

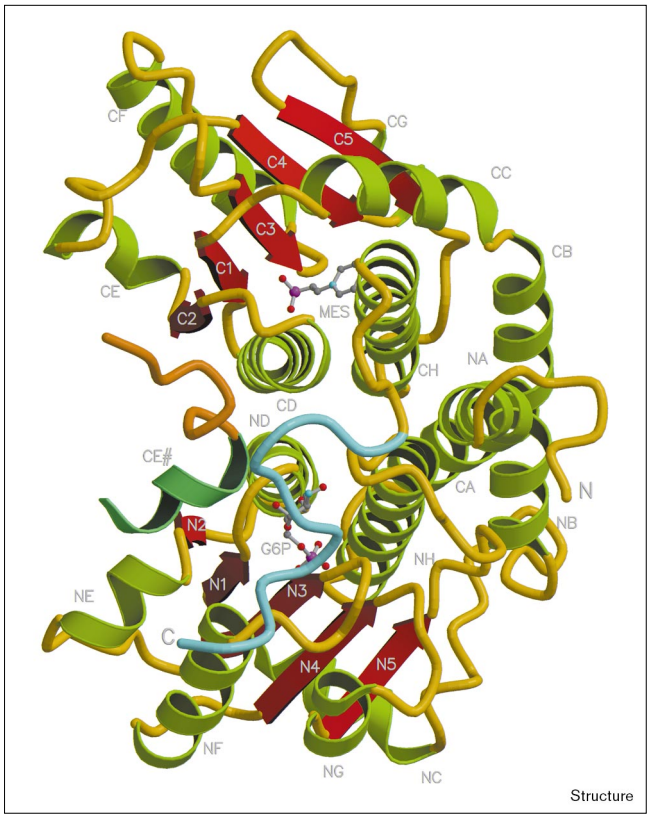
Each subdomain has an αβ structure and is dominated by a five-stranded parallel β sheet flanked on either side by α helices forming a three-layer αβα sandwich. Helices in the loops connecting β strands run approximately antiparallel to the strands. The topology of the β sheet is –1x, 2x,

Figure 1



Residues 418 and 419 are modelled as lysine and leucine, respectively, and not as asparagine and valine, as the DNA sequence suggests. The figure shows the ($3F_o-2F_c$) electron density at residues Lys418 and Leu419 contoured at 1.5σ.

Figure 2



Ribbon representation of the isomerase domain of GlmS. The approximate twofold axis is horizontal. Secondary structural elements (β strands, red; α helices, green) are labelled. The C tail is shown in cyan. Glucosamine-6P and MES are shown as ball-and-stick models. The fragment with helix CE# belongs to the neighbouring monomer.

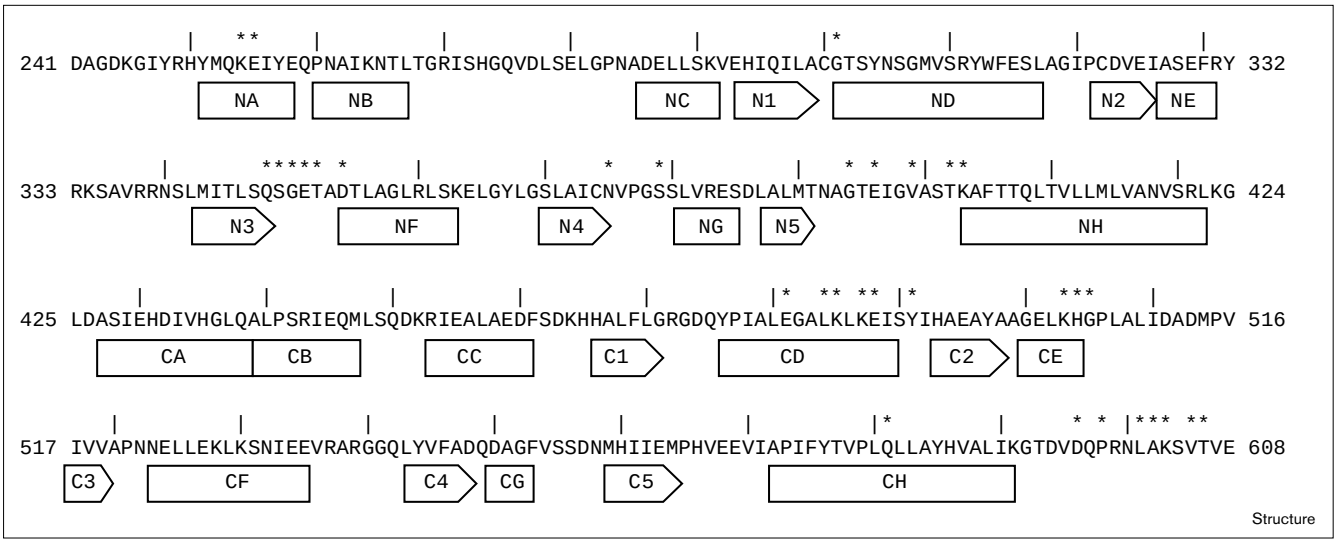
1x, 1x [20]. The fold represents the nucleotide-binding motif of a flavodoxin type [21]. In addition to this motif, there is a 20-residue broken α helix donated by each subdomain to the X-shaped junction at their interface. The two subdomains are related by an approximate twofold axis going through the junction. The two halves of the structure can be superimposed with an rmsd of 3.3 Å for all 164 equivalent α carbons. For the core consisting of the β sheet and inner α helices A, B, D and H (84 Cα atoms), the superposition gives an rmsd of only 1.2 Å. No significant similarity was detected between the corresponding fragments of the sequence on both amino-acid and nucleotide levels, however.

The structural and topological similarity of the subdomains suggests that GlmS has evolved through a gene-duplication step. Gene duplication within a single polypeptide chain has been proposed as the mechanism of evolution for a number of proteins which have a dimeric structure with an approximate local dyad axis. Dimerisation is often related to the formation of binding sites at the interface. Gene duplication could allow the two halves of the structure to differentiate, possibly improving the binding of substrates or allowing the second binding site to be converted into a regulatory site.

Despite the internal twofold symmetry of the isomerase domain, it contains only one active site, namely that in the N-terminal half with the bound glucosamine-6P (Figure 2). Another putative binding site at the equivalent location in the C-terminal subdomain is occupied by a molecule of MES, which was present in the crystallisation buffer. Thus, although the fine structure of the second site is different, it retains some substrate-recognition features, such as α helices CD and CF, which have their N termini pointing into the site, providing a favourable electrostatic environment for binding an anion — the sulphonate of MES.

Comparison of the GlmS structure with the functionally related enzymes catalysing aldose/ketose isomerisation reveals some general similarity to glucose-6P isomerase [17] and glucosamine-6P deaminase [16]. All three proteins have a nucleotide-binding fold, although the fold in glucosamine-6P deaminase is a dehydrogenase-like six-stranded variant, whereas glucose-6P isomerase and GlmS share a common five-stranded fold of a flavodoxin

Figure 3



Amino-acid sequence and secondary structural elements of the isomerase domain of GlmS. Helices are shown as boxes (CG and CE are 3_{10} helices, others are α helices) and β strands as arrows. Invariant residues are marked by asterisks.

type. The three enzymes act on the same substrate, fructose-6P, and are thought to use the enolisation catalytic mechanism [22,23]. Thus, although there is no common ancestor for the entire family of isomerases, it is possible that some of them, for example, glucose-6P isomerase and GlmS, are evolutionarily related. A preliminary examination of the structural data available for glucose-6P isomerase has not allowed more definite conclusions to be made.

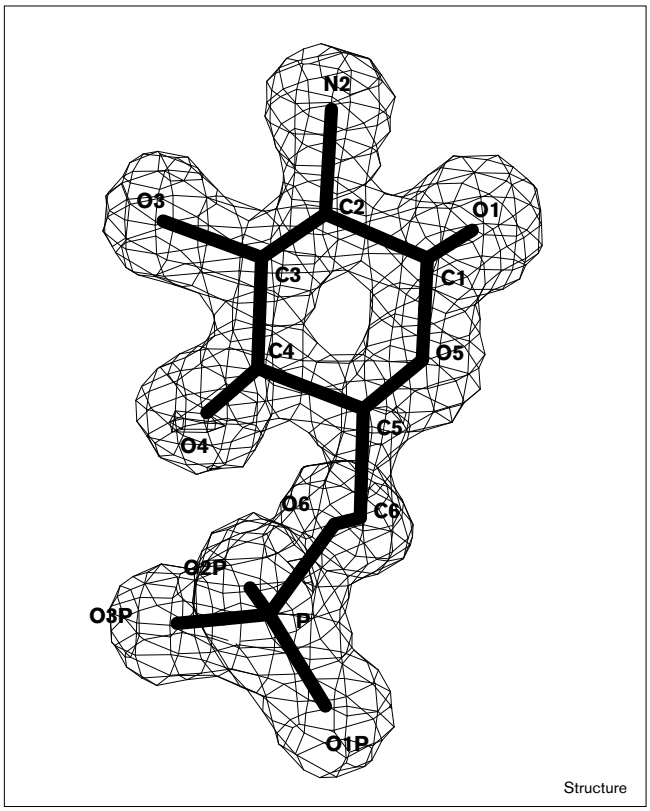
Active site

The isomerase domain was crystallised in the presence of the reaction product glucosamine-6P, which is also a competitive inhibitor of GlmS with K_i = 0.35 mM [24]. Electron density indicates that α-D-glucosamine-6P in the cyclic form is bound at the C edge of the β sheet of the N-terminal subdomain (Figure 4). The binding site is formed by loops N1, N3 and N5, and by the C tail protruding from the other subdomain (Figure 2). Loop N3 (residues 347–352) embraces the phosphate group, whereas the other three polypeptides bind the glucosamine moiety. Phosphate oxygens each form three hydrogen bonds with hydroxyl groups of Ser347, Ser349, Thr352 and Ser303, mainchain amino groups of Ser349 and Gln348, and with water molecules (with low B factors) tightly bound in the pocket (Figure 5). In addition, a dipole of α helix NF directed to the phosphate favours binding of glucosamine-6P. Hydroxyl substituents of glucosamine are bound to the protein mainly through water molecules, except for O4 which forms a hydrogen bond with Thr302. The amino group of glucosamine is hydrogen bonded to carbonyls 399 and 602. Thus, three direct glucosamine–protein interactions involve only mainchain atoms of the three polypeptides of the sugar-binding site, loops N1 and N5, and the C tail.

Glucosamine-6P is likely to occupy the catalytic site of the isomerase domain in the crystal. There are three lines of evidence in support of this idea. Firstly, all enzymes with a nucleotide-binding fold have an active site at the C-terminal edge of the parallel β sheet [21]. Secondly, 30 out of 33 invariant residues cluster around the glucosamine-6P-binding site and actually form this site; the only exception is the tripeptide Lys503–His504–Gly505 in loop C2, the function of which is clarified below. Thirdly, Lys603 located in the pocket has been implicated in playing a catalytic role [25]; in addition, the proximity of Cys300 to the bound glucosamine-6P is consistent with the results of inhibition studies using anhydro-1,2-hexitol 6-phosphate [26].

Numerous hydrogen bonds and salt bridges suggest that the framework of the active site is quite rigid. Conformational freedom required for substrate binding and transformation may be provided by water molecules that mediate the enzyme–substrate interactions. Relative flexibility in binding of the sugar moiety is compensated by the tight anchoring of the phosphate group that defines the proper orientation of the substrate with respect to catalytic elements. Such a mode of substrate binding may be typical for all sugar-phosphate isomerases and has implications for the catalytic mechanism.

Figure 4



Glucosamine-6P in the cyclic form is bound at the C-terminal edge of the β sheet of the N-terminal subdomain. The figure shows ($3F_o-2F_c$) electron density at glucosamine-6P contoured at 1.5σ.

Catalytic mechanism

The reaction catalysed by GlmS is thought to proceed via abstraction of the C1 hydrogen of fructose-6P (or fructosimine-6P) to form a *cis*-enediol (respectively, *cis*-enolamine) intermediate which, upon reprotonation of C2, gives rise to the product glucose-6P or glucosamine-6P [23]. It is not known whether GlmS catalyses ring opening or binds selectively the open-chain form of the substrate. Ring opening has been considered to occur for the functionally related enzymes glucose-6P isomerase [27], D-xylose isomerase [28] and L-arabinose isomerase [29]. The pyranose form of glucosamine-6P observed in the present crystal structure indicates that the active site is well suited to binding a cyclic hexose. On the other hand, the active site is postulated to complement the transition state that has the sugar in the open form. If GlmS did not catalyse ring opening, the binding site would perhaps be more selective for the 'active', open-chain form of fructose-6P, which constitutes only 2.2% of the available fructose-6P *in vivo*. These considerations support the idea of the ring-opening step being catalysed by GlmS. The ability of the enzyme to accommodate both cyclic and open-chain forms of a substrate may be provided by the flexible character of sugar binding achieved through bridging water molecules, rather than by conformational changes in the protein.

It is impossible to identify a residue that might play the role of a general base in the ring-opening step, unless the oligomeric structure of the enzyme is taken into consideration. Two isomerase domains related by a crystallographic twofold axis form a compact dimer with an extensive interface (Figure 6). The solvent-accessible area buried between subunits in the dimer is 2600 Å^2, which is 17% of the total surface of one subunit. This value is close to the upper limit of 3200 Å^2 observed for dimers of comparable molecular weight [30]. Most inter-subunit interactions occur between helices NE and NF of one subunit and helices CE and CF of the other. The C-terminal end of helix CE points to the active site of the other subunit, such that His504 takes its place just above the substrate-binding site (Figure 5). In the enzyme–product complex, His504 is hydrogen bonded to the O1 hydroxyl of glucosamine-6P. This structure probably represents the final stage of the reaction, the situation after the O1 group has been reprotonated from His504. The same residue is an obvious candidate for initiating ring opening by abstracting a hydrogen from O2 of the cyclic fructose.

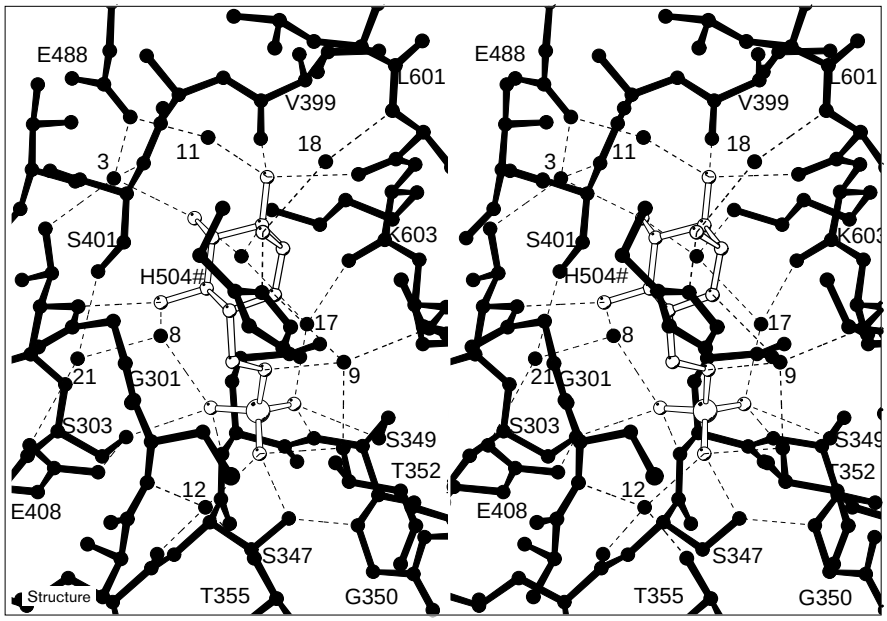
Apart from providing the catalytic base, the dimeric structure explains the conservation of the tripeptide Lys503–His504–Gly505 at the periphery of the isomerase domain. Lys503 and Gly505 flanking the catalytic histidine determine the conformation of the polypeptide by interacting with the C tail (a hydrogen bond Lys503# NZ–O Asn602; where # indicates that the residue belongs to a neighbouring monomer) and by facilitating a sharp turn of the chain at Gly505 (ϕ/ψ = 163/–68°). The dimeric nature of *E. coli* GlmS has been deduced from gel filtration and sedimentation experiments [5]. There is little doubt that the dimer of the isomerase domains observed in the present crystal form corresponds to the functional dimer of the intact protein.

Identification of other residues involved in catalysis is not so straightforward. Lys603 is likely to play a role in the amination reaction. Trapping of a radiolabelled adduct has shown the formation of a Schiff base between Lys603 and a substrate molecule [25]. Replacement of Lys603 by arginine, using site-directed mutagenesis, resulted in a 40-fold decrease in k_{cat} for glucosamine-6P synthesis [31]. The crystal structure shows that Lys603 is indeed located in the active centre close to glucosamine-6P. The possibility of a Schiff base with the substrate cannot be ruled out because the lysine sidechain is flexible enough to reach the substrate.

As there is no other residue in the active site that could function as a general base in the isomerisation step,

Figure 5

Stereoview of the active site of the isomerase domain with bound glucosamine-6P (open lines). Hydrogen bonds are indicated by dashed lines. Residues are labelled using the single-letter amino acid code; water molecules are indicated by numbers only.



Glu488 is likely to be responsible for transferring a proton from C1 to C2 of a substrate. In the crystal structure, the carboxylate group of Glu488 interacts with glucosamine-6P via two bridging water molecules (Figure 5). Mutagenesis studies are underway to determine what role individual residues play in catalysis. It is worth noting that the same three residues (glutamate/aspartate, histidine and lysine) were implicated in catalysis in other sugar isomerases such as TIM, xylose isomerase and glucosamine-6P deaminase [16]. The unique feature of GlmS with respect to other isomerases is that the catalytic residues belong to different polypeptide chains. Although most of the ketol isomerases are oligomers, the catalytic residues are usually located on one of the subunits.

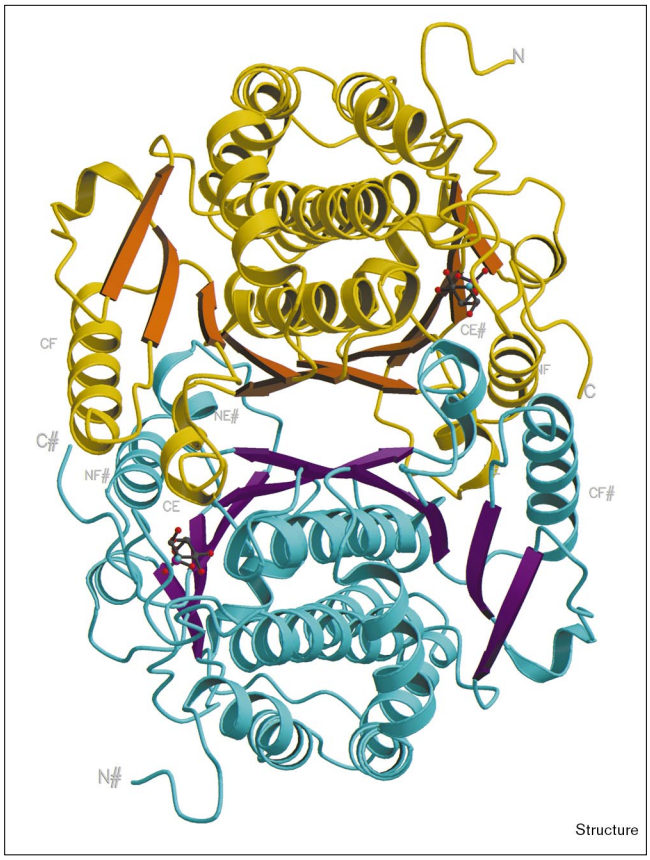
Nitrogen transfer

The ammonia produced by glutamine hydrolysis should remain trapped in the enzyme, in order to avoid protonation and to keep its nucleophilic character. This point of view is consistent with the fact that GlmS cannot use exogenous ammonia for glucosamine-6P synthesis [5]. There are two possible means of transferring NH_3 to the amination site located in the isomerase domain: either the two active sites merge together upon binding of substrates to allow a direct interaction between them, or an intramolecular channel that would isolate the glutamine-derived ammonia from bulk solvent is formed.

Inactivation studies using N-iodoacetyl-glucosamine-6P [32] have shown that either of the substrates, fructose-6P or glutamine, could protect the enzyme from inactivation, suggesting the proximity of the active sites. In a similar experiment, inactivation with a radiolabelled sugar-phosphate analogue 1,2-anhydro-hexitol-6P [26] was shown to affect Cys1, the catalytic residue of the glutamine site, providing another argument in favour of a united active centre. On the other hand, lack of protection by glutamine [26] and the inability of GlmS to bind bisubstrate analogues that consist of glutamine coupled by various spacers to 2-amino-2-deoxy-glucitol-6P [33] corroborate the possibility of separate sites. This is also consistent with the observation that GlmS retains isomerase activity upon inactivation by 6-diazo-5-oxo-L-norleucine (DON), a specific affinity label of Cys1 [7].

The 3D structures of the glutaminase and isomerase domains of GlmS indicate that the merging of the active sites would require substantial conformational changes, supporting the idea of a channel. Such channels connecting the glutaminase site with the synthetic site have been observed in the recently determined crystal structures of carbamoyl phosphate synthetase [34] and phosphoribosyl pyrophosphate amidotransferase [35]. The channels are formed primarily by hydrophobic sidechains and allow diffusion of NH_3 between the two sites. Formation of such a channel in GlmS would involve residues from both the isomerase and glutaminase domains. The 'isomerase part' of the channel is likely to reside in the loop 596–603 (Figure 2). Pro598, Leu601 and Ala602 of this loop together with Val399 from loop N5 form a hydrophobic entrance to the sugar-binding site at the amino group of glucosamine-6P. All of these residues are conserved in the GlmS sequences. The diameter of the entrance is about

Figure 6



Dimer of the isomerase domains as viewed along the crystallographic twofold axis. Helices involved in the intersubunit contacts are labelled. Glucosamine-6P molecules are represented by ball-and-stick models. Different polypeptide chains are coloured blue and yellow, with the β sheets highlighted.

4 Å, as measured between the closest atoms on the opposite sides of the loop. Determination of the structure of the intact GlmS is in progress in order to determine the overall architecture of the NH_3 channel.

The involvement of the C-terminal residues in the interdomain channelling of NH_3 indicates the importance of the C tail in the enzyme function. This role is not restricted to the NH_3 transfer and communication with the glutaminase domain, however. The C tail contributes to the formation of the sugar-binding site, provides the catalytically essential Lys603, and shields the reaction intermediate from the bulk solvent. Not surprisingly, the C-terminal region of the amino-acid sequence is highly conserved among glucosamine-6P synthases (Figure 3); it contains 8 out of 33 strictly conserved residues of the isomerase domain. In addition, five residues are conserved in all but one of the 24 sequences of GlmS reported to date (including three partial sequences). The C-terminal fragment of the sequence can therefore be considered as a fingerprint of the enzyme. A PROSITE [36] search with the consensus sequence DXPXXLAK[SC]VT (single-letter code, where letters in brackets indicate that either serine or cysteine may take this position) detected all GlmS sequences deposited in the Swiss-Prot and EMBL databases, and found no other entries. A shorter pattern PXXLAK[SC]VT also detected all GlmS and no other sequences.

Biological implications

Glucosamine-6P synthase (GlmS) catalyses the first step in hexosamine metabolism, converting fructose-6P into glucosamine-6P or glucose-6P, depending upon the presence or absence of glutamine. The *E. coli* GlmS enzyme consists of a 27 kDa glutaminase domain that catalyses hydrolysis of glutamine to glutamate and ammonia, and a 40 kDa isomerase domain that catalyses fructose-6P amination and isomerisation. Whereas the glutaminase domain shares a common structure and catalytic mechanism with other N-terminal nucleophile hydrolases, the isomerase domain of GlmS is not only unique among amidotransferases, but appears to be dissimilar to the ketol isomerases as well. The structure suggests that the evolution of GlmS involved gene duplication and subsequent dimerisation of an ancestral protein unit characterised by a nucleotide-binding fold. This common structural motif may be considered as evidence of a possible link between amidotransferases and a number of other enzyme families, such as dehydrogenases, flavoenzymes and nucleotide kinases.

The crystal structure of the isomerase domain in complex with the reaction product, glucosamine-6P, suggests a catalytic mechanism that combines features of different ketol isomerases. Isomerisation of fructose-6P is likely to involve the formation of a Schiff base with Lys603, a ring-opening step catalysed by His504, and the proton transfer from C1 to C2 of the substrate effected by Glu488. The unique feature of GlmS with respect to other isomerases is that the catalytic residues belong to different polypeptide chains; although most ketol isomerases are oligomers, the catalytic residues are usually located on only one of the subunits.

The present structure supports the idea of an intramolecular channel as a means of transferring the ammonia produced by glutamine hydrolysis to the isomerase site. The probable location of such a channel is a loop of residues 596–603 aligned with the hydrophobic sidechains of Pro598, Leu601 and Ala602 that are accompanied by Val399 from another loop. These residues form an entrance to the sugar-binding site at the amino group of glucosamine-6P, that is, where NH_3 is expected to replace the hydroxyl group of the substrate. All residues involved are strictly conserved in the sequences of GlmS. The highly conserved C-terminal fragment of the chain plays a key role in substrate

binding, catalysis and communication with the glutaminase domain. The corresponding sequence pattern DXPXXLAK[SC]VT may be considered to be a fingerprint of GlmS.

Materials and methods

Crystallisation and data collection

The isomerase domain of GlmS has been overexpressed in *E. coli*, purified to homogeneity and crystallised in the presence of the reaction product, glucosamine-6P [37]. The crystal form described previously and characterised by a large unit cell (334 Å) and strong pseudosymmetry (point group 622 in the space group $P6_1$) was not suitable for X-ray analysis. Each crystal was found to be different in terms of the orientation of non-crystallographic axes, as indicated by a self-rotation function. The structure was solved using another crystal form that was obtained from the 30% MPD solution in 0.1 M N-morpholino-ethanesulphonic acid (MES), pH 6.0, with 0.5 M $(NH_4)_2SO_4$ and 10 mM glucosamine-6P. Both the presence of salt and an additional purification step using hydrophobic chromatography proved to be of crucial importance for successful crystallisation. The crystals belong to the rhombohedral space group R32 with a = b = 146.1, c = 173.9 Å.

X-ray diffraction data were collected at 100K at EMBL synchrotron beamline X11 (DESY, Hamburg) using a MAR Research imaging plate and were processed with the programs DENZO and SCALEPACK [38]. Data statistics are given in Table 2.

Structure determination and refinement

The structure of the isomerase domain was determined by isomorphous replacement using anomalous diffraction from two heavy-atom derivatives, EtHgCl and Pt tetpyridinechloride dihydrate (Table 2). Heavy atoms were located with the program TRAHALO [39]. All other calculations were performed with the CCP4 program suite [40]. Electron density improved dramatically after solvent flattening, due to the unusually high solvent content of 72% (Vm = 4.44 $Å^3$/Da). There appeared to be only one protein molecule in the asymmetric unit. The atomic model was built using the program O [41]. In the first stage, 285 residues, i.e. about 80% of the structure, were modelled into electron density. After a few cycles of restrained least-squares refinement with the program PROLSQ [42], the phases calculated from the current model were combined with the isomorphous phases to produce the electron-density map that showed the rest of the structure. The complete model has been refined with PROLSQ until convergence. The rms coordinate shifts in the last cycles of refinement did not exceed 0.003 Å. No significant residual electron density was detected in the final $(F_o–F_c)$ map.

Table 2

Data collection and phasing statistics*.

	Native	Hg	Pt
Maximum resolution (Å)	1.57	2.5	2.5
Wavelength (Å)	0.91	0.89	0.90
Unique reflections	95 544	24 510	24 393
Redundancy	6.5	5.1	2.7
Completeness (%)	99.5	98.8	98.3
Rmerge† (%)	4.0	6.7	6.1
At dmin‡	5.7	2.4	2.1
Contrast§ (%)	0.0	12.5	16.9
Number of binding sites	–	2	3
Phasing power¶	–	1.4	1.1

*Hg (ethyl mercury chloride) and Pt (platinum(II) tetpyridine chloride dihydrate) derivatives. †Rmerge = $(\Sigma|I–\langle I\rangle|/\Sigma I)$. ‡$\langle I\rangle/\langle\sigma\rangle$. §Contrast = $(\Sigma|F_P–F_{PH}|/\Sigma F_P)$. ¶Phasing power = $(\langle F_H\rangle/E)$.

Accession numbers

Atomic coordinates and structure factors have been deposited with the Brookhaven Protein Data Bank as entry 1MOQ.

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