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The mechanism of sugar phosphate isomerization by glucosamine 6-phosphate synthase

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Abstract

Glucosamine 6-phosphate synthase converts fructose-6P into glucosamine-6P or glucose-6P depending on the presence or absence of glutamine. The isomerase activity is associated with a 40-kDa C-terminal domain, which has already been characterized crystallographically. Now the three-dimensional structures of the complexes with the reaction product glucose-6P and with the transition state analog 2-amino-2-deoxyglucitol-6P have been determined. Glucose-6P binds in a cyclic form whereas 2-amino-2-deoxyglucitol-6P is in an extended conformation. The information on ligand-protein interactions observed in the crystal structures together with the isotope exchange and site-directed mutagenesis data allow us to propose a mechanism of the isomerase activity of glucosamine-6P synthase. The sugar phosphate isomerization involves a ring opening step catalyzed by His504 and an enolization step with Glu488 catalyzing the hydrogen transfer from C1 to C2 of the substrate. The enediol intermediate is stabilized by a helix dipole and the ϵ-amino group of Lys603. Lys485 may play a role in deprotonating the hydroxyl O1 of the intermediate.

Keywords: aldose-ketose isomerase; catalytic mechanism; crystal structure; glucosamine 6-phosphate synthase

Glucosamine 6-phosphate synthase (GlmS, L-glutamine:D-fructose 6-phosphate amidotransferase, E.C. 2.6.1.16) is a key enzyme of hexosamine metabolism responsible for the utilization of the amide nitrogen of glutamine in the biosynthesis of amino sugars (Zalkin, 1993; Massiere & Badet-Denisot, 1998; Zalkin & Smith, 1998). One of the products of the pathway, N-acetylglucosamine, is an essential building block of bacterial and fungal cell walls. The human enzyme has a regulatory function in desensitizing the insulin-responsive glucose transport system (Traxinger & Marshall, 1991). GlmS converts fructose 6-phosphate (Fru-6P) into glucosamine 6-phosphate (GlcN-6P) or glucose 6-phosphate (Glc-6P) depending on the presence or absence of glutamine (Badet et al., 1987; Leriche et al., 1996). The enzyme consists of two structural domains, an N-terminal glutaminase domain, which hydrolyzes glutamine to glutamate and ammonia, and a C-terminal isomerase domain, which catalyzes the ketose-aldose isomerization and utilizes nitrogen for synthesis of GlcN-6P. The two domains of the *Escherichia coli* GlmS separated by controlled chymotrypsin proteolysis or expressed separately retain their ability to bind substrates and to catalyze glutamine hydrolysis and Fru-6P isomerization, respectively (Denisot et al., 1991; Leriche et al., 1996).

The isomerase activity associated with the 40-kDa C-terminal domain is unique among amidotransferases and assigns GlmS to a class of aldose-ketose isomerases (E.C. 5.3.1.9 and 5.3.1.10). Extensive structure-functional studies on triosephosphate isomerase (TIM) and D-xylose isomerase have revealed that in spite of a common $(\alpha/\beta)_8$-barrel fold they have different catalytic mechanisms. D-Xylose isomerase exploits the hydride shift mechanism (Collyer & Blow, 1990). TIM follows the enolization mechanism (Rose, 1975), which has also been accepted for several other isomerases including both metal-dependent (mannose-6P, L-arabinose, and L-fucose isomerases) and metal-free enzymes (phosphoglucose isomerase (PGI), GlcN-6P deaminase). The isomerases processing phosphosugars are usually metal-independent, whereas sugar isomerases require Mg, Mn, or Co for activity. The only exception is mannose-6P isomerase, which contains Zn. No sequence or structural similarity has been detected between these enzymes and GlmS.

GlmS sequences have been determined for more than 20 organisms including eukarya, bacteria, archea, and viridae. The enzyme from *E. coli* is the best characterized and the only one for which the three-dimensional information is available (Isupov et al., 1996). The X-ray structure of the isomerase domain has previously been solved in complex with GlcN-6P (Teplyakov et al., 1998). Here we

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Abbreviations: Fru-6P, fructose 6-phosphate; Glc-6P, glucose 6-phosphate; GlcN-6P, glucosamine 6-phosphate; GlcNol-6P, 2-amino-2-deoxyglucitol-6-phosphate; GlmS, glucosamine 6-phosphate synthase; PGI, phosphoglucose isomerase; TIM, triosephosphate isomerase.

report two crystal complexes of the isomerase domain, with the reaction product Glc-6P and with the transition state analog 2-amino-2-deoxyglucitol-6P (GlcNol-6P), determined at 1.9 and 2.0 Å resolution, respectively. These data allow us to propose a mechanism of sugar phosphate isomerization catalyzed by GlmS.

Results

Quality of the models

The structure of the isomerase domain of GlmS co-crystallized with Fru-6P was refined to an *R*-factor of 19.1% for all data in the resolution range 10–1.9 Å. The final model contains all but the two N-terminal amino acid residues. The refinement statistics are given in Table 1. The overall *G*-factor calculated by PROCHECK (Laskowski et al., 1993) as a measure of the stereochemical quality of the model is 0.03, which is better than expected for a structure refined at such a resolution. None of the residues has a forbidden combination of main-chain torsion angles; 95% fall in the most favored regions as defined in PROCHECK. The RMS deviation of peptide units from planarity is 2.7°; the maximum deviation does not exceed 10°.

The structure of the isomerase domain complexed with the inhibitor GlcNol-6P was refined to an *R*-factor of 24.8% for all data in the resolution range 15–2.0 Å. In spite of the high *R*-factor, electron density allowed unambiguous modeling of all but one (N-terminal) residues and revealed the bound inhibitor molecule. The final model also contains one MES molecule, a sodium ion, and three sulfates. Stereochemical parameters correspond to a well-refined structure (Table 1). Given a good quality of the X-ray data, which are characterized by a high redundancy, low *R*-merge, and high completeness (Table 2), the inability to refine the structure to a better *R*-factor may be related to a slight disorder of the crystal lattice as indicated by the thermal factors. An average *B*-factor for the GlcNol-6P complex is 28.2 Å^2 as derived from the Wilson plot, that is higher than for the other complex (25.5 Å^2) (Table 2) although data collection under cryo-conditions usually results in lower *B*-factors. For comparison, the mean *B*-factor for the GlcN-6P complex at 100 K is 18.5 Å^2.

Table 1. *Refinement statistics*

	Glc-6P	GlcNol-6P
Resolution range (Å)	10–1.9	15–2.0
$R_{cryst} = \sum\|\|F_o\| - \|F_c\|\|/\sum\|F_o\|$ (%)	19.1	24.8
R_{free} (5% set) (%)	22.4	28.6
Coordinate error (DPI)[a] (Å)	0.10	0.14
Coordinate error (ML)[a] (Å)	0.06	0.08
Number of atoms		
Protein	2,820	2,824
Heterogen	16	43
Solvent	223	170
Average *B*-factors (Å^2)		
Protein	30.7	35.9
Solvent	37.6	41.1
RMS deviations		
Bond distances (Å)	0.013	0.015
Bond angles (°)	1.4	1.5
Peptide angles (°)	2.7	3.3
B-factor correlation (Å^2)		
Main chain	4.5	6.2
Side chains	8.6	8.6

[a]Coordinate error based on Cruickshank's DPI or on maximum likelihood (ML) (Murshudov et al., 1997).

Table 2. *Data collection statistics*

	Glc-6P	GlcNol-6P
Maximum resolution (Å)	1.9	2.0
Temperature (K)	277	100
Space group	R32	R32
a (Å)	146.1	143.9
c (Å)	173.9	172.8
Unique reflections	55,613	45,848
Redundancy	3.7	9.1
Completeness (%)	99.2	98.7
R_{merge} ($\sum\|I - \langle I\rangle\|/\sum I$) (%)	8.4	5.8
$\langle I\rangle/\langle\sigma\rangle$ at d_{min}	1.8	3.6
B-factor (Wilson plot) (Å^2)	25.5	28.2

Overall structure

The isomerase domain of GlmS belongs to the α/β structural family with a five-stranded parallel β-sheet flanked on either side by α-helices forming a three-layer $\alpha\beta\alpha$-sandwich (Fig. 1). Helices in the loops connecting β-strands run approximately antiparallel to the strands. The fold represents the nucleotide binding motif of a flavodoxin type. The isomerase domain contains two such motifs and displays a nearly exact internal twofold symmetry. The N- and C-terminal subdomains comprise residues 241–424 and 425–592, respectively (residues 1–240 of GlmS belong to the glutaminase domain). Residues 593–608 (the C-tail) protrude from the C-terminal subdomain to the active site located in the N-terminal subdomain at the carboxy-edge of the β-sheet. Two isomerase domains related by a crystallographic twofold axis form a tight dimer, which is a functional unit of the enzyme. The second subunit of the dimer provides an invariant histidine (His504), which is thought to play a catalytic role. No significant differences are observed between the protein structures of the complexes described in this paper and the GlcN-6P complex determined earlier (Teplyakov et al., 1998). Shrinkage of the unit cell by 1.5% along *a* upon freezing of the crystals (Table 2) has no effect on the structure.

Complex with glucose-6P

The isomerase domain was crystallized in the presence of the substrate Fru-6P. However, the electron density clearly indicated a pyranose rather than a furanose form of the sugar bound to the active site (Fig. 2). The ligand molecule was interpreted as Glc-6P. This is consistent with an observation that GlmS as well as the separated isomerase domain can catalyze the conversion of Fru-6P into Glc-6P (Leriche et al., 1996). Moreover, GlmS cannot produce GlcN-6P in the absence of Gln or the glutaminase domain (Badet et al., 1987). Therefore, the observed ligand cannot be GlcN-6P, which is indistinguishable crystallographically from Glc-6P.

The reaction product observed in the present crystal structure is an α-anomer of D-Glc-6P in a cyclic form. The location of the

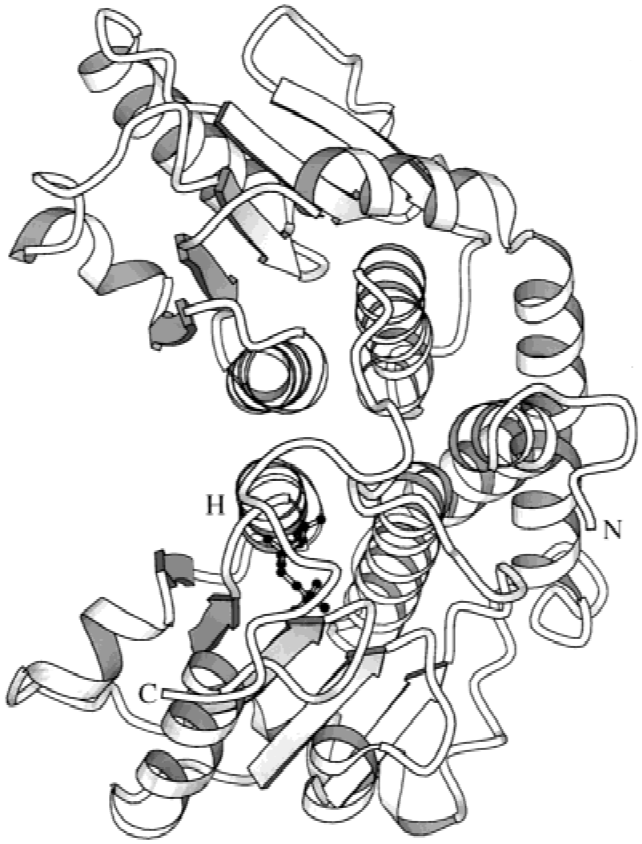

Fig. 1. Ribbon representation of the isomerase domain of GlmS. The reaction product Glc-6P (ball-and-stick model) indicates the active site, which is formed by the three elements of the structure: the parallel β-sheet shown as arrows, the α-helix denoted by letter H, and the C-terminal fragment (C-tail) denoted by letter C.

molecule is exactly the same as was found for GlcN-6P in the corresponding complex. The loop of residues 347–352 embraces the phosphate group and forms numerous hydrogen bonds to the phosphate oxygens. The glucose moiety is bound to the protein through direct H-bonds to the main-chain atoms of Thr302, Val399, and Ala602 and through five bridging water molecules (Fig. 3). The hydroxyl group O1 forms an H-bond to the imidazole Nδ of His504 from the other subunit of the dimer.

Complex with 2-amino-2-deoxyglucitol-6P

The complex was obtained by co-crystallization of the ligand-free enzyme with GlcNol-6P. Electron density reveals an inhibitor molecule bound to the active site in an extended conformation (Fig. 4). The phosphate group is anchored in the loop of residues 347–352 in the same way as was observed for Glc-6P. The sugar part of the inhibitor is located deeper into the binding pocket so that in contrast to the cyclic form it does not interact with the C-tail but instead forms direct H-bonds to the carboxylate of Glu488 and the ϵ-amino group of Lys485 (Fig. 5). The amino group N2 and the hydroxyl group O4 of the sugar bind the carbonyl 399 and the amino group 302, respectively, as do the corresponding groups of Glc-6P. The hydroxyl O5 is H-bonded to the imidazole Nδ of His504. The shift of the O1 group between the cyclic and the open chain form of the ligand is almost 6 Å. The corresponding atomic shifts in the protein at the active site do not exceed 0.7 Å.

Discussion

The isomerase domain is responsible for two activities of GlmS, the conversion of Fru-6P into GlcN-6P in the presence of glutamine (the synthase activity), and the isomerization of Fru-6P into Glc-6P (the PGI-like activity) in the absence of glutamine. A possible reaction mechanism may include the following steps: formation of the enzyme-substrate complex, amination of the sugar, and isomerization. In addition, the enzyme may catalyze the opening of the sugar ring of a substrate and the closure of the ring of a product.

Substrate binding

Trapping experiments using a radiolabeled substrate indicated the formation of a Schiff base between C2 of Fru-6P and Nζ of Lys603 (Golinelli-Pimpaneau & Badet, 1991). The crystal structure re-

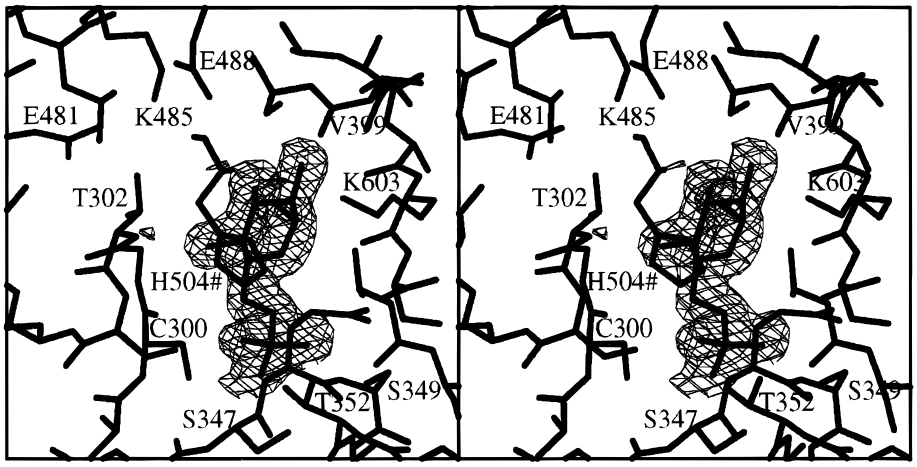

Fig. 2. Stereo pair of the active site with bound Glc-6P. Difference electron density contoured at 1.5σ is shown at Glc-6P, which was excluded from phase calculation.

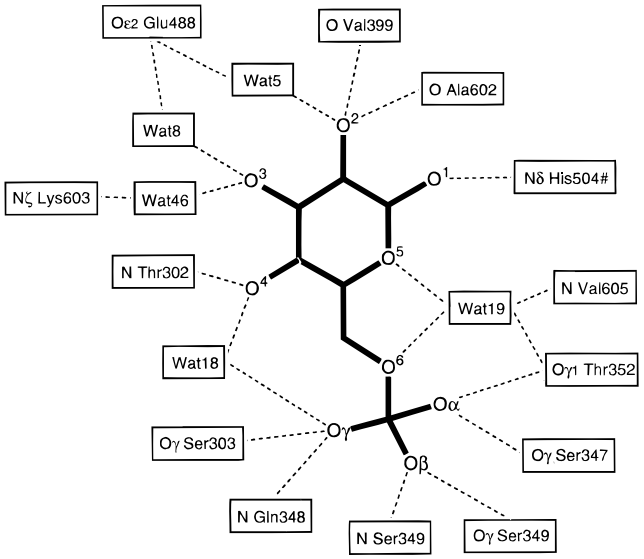

Fig. 3. Schematic representation of Glc-6P interactions at the active site. H-bonds are shown by dashed lines.

veals Lys603 located in the sugar binding site in close proximity to the O2 group of the substrate (Figs. 2, 4). Given the flexibility of the lysine side chain, such a location implies a possibility of direct interaction with the substrate to form a Schiff base. Moreover, Lys603 belongs to the loop of residues 596–603, which is implicated in the formation of an intramolecular channel to transfer ammonia from the glutamine site to the sugar binding site (Teplyakov et al., 1998). The ammonia would enter the sugar site right where the putative Schiff base is expected to be. Thus, the structural arrangement of the active center is consistent with the proposed involvement of Lys603 in substrate binding and catalysis. Participation of this residue in catalysis was also confirmed by site-directed mutagenesis (Badet-Denisot et al., 1993). Replacement of Lys603 by Arg resulted in a 40-fold decrease in the catalytic rate of the synthetic reaction with essentially no effect on K_m. However, the PGI-like activity increased three times. These data suggest that GlmS may follow two reaction pathways corresponding to the "synthetic" and "isomerase" activities in which Lys603 would play different roles. In the presence of glutamine, the reaction would proceed along the "synthetic" pathway through the replacement of Lys603 by ammonia in the Schiff base and subsequent isomerization of fructosimine-6P to GlcN-6P (Fig. 6). In the absence of glutamine, a certain fraction of the enzyme-substrate covalent complex may convert back to Fru-6P, which would be isomerized to Glc-6P. The strong commitment of GlmS for GlcN-6P synthesis is reflected in almost 10^5 times difference in k_{cat}/K_m between the two activities (Leriche et al., 1996), which is probably due to stability of the Schiff base. Lys603 then plays a regulatory role suppressing the glutamine-independent isomerase activity of the enzyme.

Ring opening

There is no evidence whether GlmS catalyzes the opening of a sugar ring as the first step of the reaction or whether it binds the open chain form of Fru-6P. The rate of spontaneous ring opening of Fru-6P is about 20 s^{-1} (Pierce et al., 1985) that is comparable to the catalytic rate of GlmS. The arguments in favor of the occurrence of this step come from the crystal structures with Glc-6P and GlcN-6P, which reveal complementarity of the binding site to the cyclic form of the sugar and indicate His504 as a suitable base to perform the reaction. Ring opening has been considered to occur in other sugar isomerases such as PGI (Malaisse-Lagae et al., 1989), D-xylose isomerase (Lavie et al., 1994), L-arabinose isomerase (Banerjee et al., 1995), and GlcN-6P deaminase (Oliva et al., 1995). A histidine residue was implicated as a ring opening group in GlcN-6P deaminase (His143; Oliva et al., 1995) and D-xylose isomerase (His53; Collyer & Blow, 1990), although a possibility of a different catalyst cannot be ruled out (Lavie et al., 1994).

In both enzyme-product complexes of GlmS, with Glc-6P and with GlcN-6P, His504 is H-bonded to the hydroxyl group O1 of the pyranose ring, whereas in the complex with the transition state analog GlcNol-6P, the same imidazole Nδ binds the hydroxyl at C5. The switch of an H-bond is due to the change in conformation of the substrate molecule and not of His504. We propose that upon

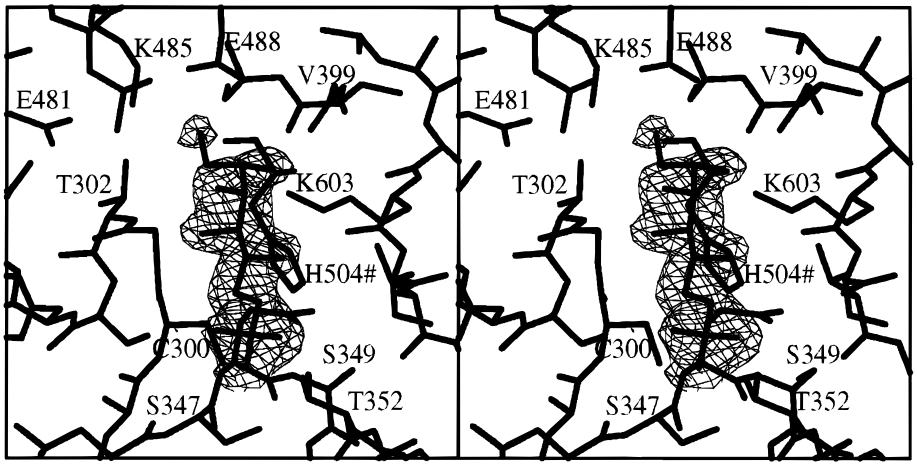

Fig. 4. Stereo pair of the active site with bound GlcNol-6P. Difference electron density contoured at 1.5σ is shown at GlcNol-6P, which was excluded from phase calculation.

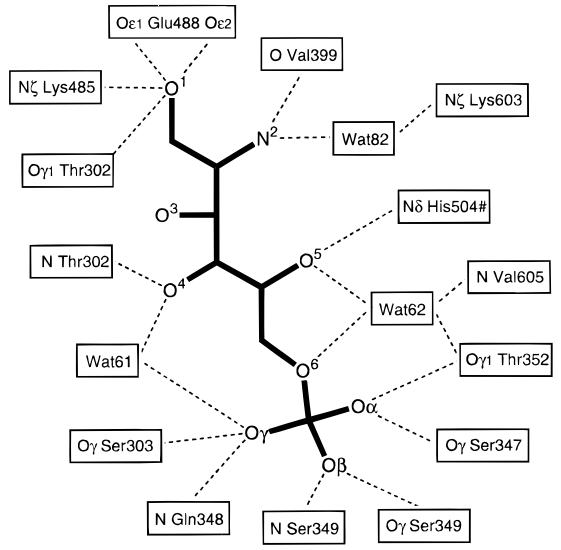

Fig. 5. Schematic representation of GlcNol-6P interactions at the active site. H-bonds are shown by dashed lines.

binding of Fru-6P, the imidazole group of His504 abstracts the O2 hydrogen of the cyclic substrate and then returns it to O5 thus completing the ring opening step (Fig. 7). The substrate molecule in an extended conformation is then ready to be processed further. The structure of the active site and the mode of substrate binding through a number of bridging water molecules would allow the transition of the substrate from the cyclic to the extended form to occur without significant conformational changes in the protein. Ring closure may be catalyzed as the reverse of the ring opening step though it is not really required as the product cyclizes spontaneously under physiological conditions.

Isomerization

Isotope exchange studies (Golinelli-Pimpaneau et al., 1989) provide evidence that GlmS follows the hydrogen transfer mechanism, which involves a *cis*-enolamine (or *cis*-enediol) intermediate (Fig. 6). By use of labeled substrates, GlmS was shown to catalyze the stereospecific abstraction of the *pro*-R hydrogen from C1 of the substrate with the subsequent protonation of C2 at the same *re* face of the intermediate. The crystal structure of the GlcNol-6P complex indicates Glu488 as the catalytic base, which is ideally positioned to perform the hydrogen transfer. The bidentate character of the carboxylate group and its location orthogonal to the enolamine plane allow an efficient hydrogen transfer using a more basic *syn* rather than *anti* orbital. In the crystal complex, the carboxylate group of Glu488 is at a distance of 3.6 Å from C1 and 3.2 Å from C2. The position of a true intermediate with C1 at a hydrogen bond distance from Glu488 would be very close (within fractions of Å) to that observed for GlcNol-6P. Shielding of Glu488 from bulk solvent as observed in the crystal complexes may be important for efficient catalysis as this increases the basicity of the carboxylate group. The level of internal tritium transfer (0.8%) (Golinelli-Pimpaneau et al., 1989) indicates that the proton exchange of the base with the solvent is 125 times faster than the enzyme turnover, which is 930 s^{-1}. This gives an estimation of the pK_a of the base of 7.4 in agreement with the above considerations.

A charged intermediate would be stabilized by the dipole of helix 301–317, which points with its N-terminus to the active site (helix H in Fig. 1), and by Lys603, which provides a positive electrostatic environment.

TIM, which is known as a nearly perfect enzyme (k_{cat} = 8,700 s^{-1}), uses electrophilic catalysis to speed up the enolization step. His95 is thought to polarize the carbonyl group of the substrate thereby lowering the pK_a of the C1 hydrogen, and may also promote the hydrogen exchange between O1 and O2 (Knowles, 1991). Conversion of His95 to Gln reduces the activity 100 times

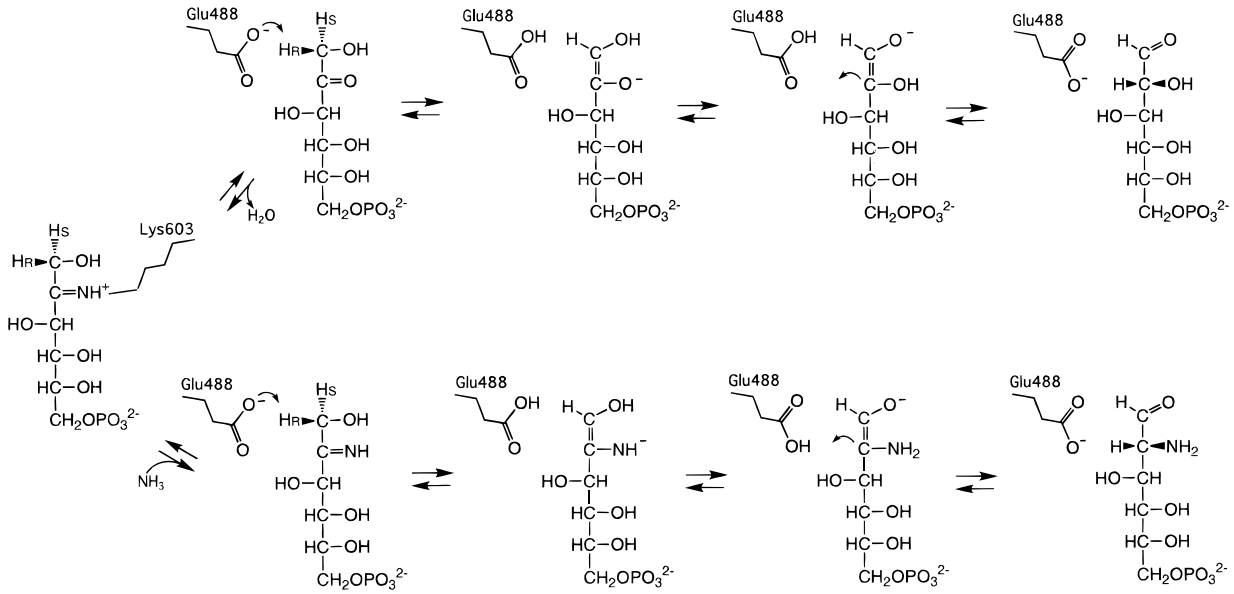

Fig. 6. Mechanism of Fru-6P isomerization catalyzed by GlmS. The upper route is the "isomerase" pathway; the lower route is the "synthetic" pathway.

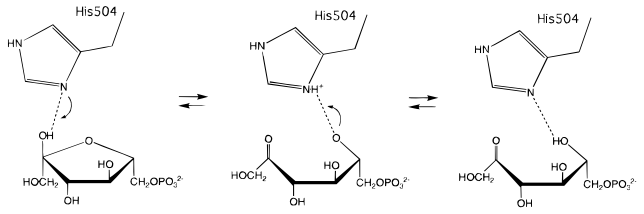

Fig. 7. Mechanism of Fru-6P ring opening catalyzed by GlmS.

(Nickbarg et al., 1988); conversion to Asn slows down the reaction by a factor of 10^4 (Blacklow & Knowles, 1990). In GlmS no residue can play such a role as indicated by the crystal structure. Not surprisingly GlmS is a much less effective enzyme than TIM.

In the absence of an electrophilic residue, Glu488 may not only transfer the hydrogen from C1 to C2 but also promote the exchange between O1 and O2. Such a double role of the catalytic base was ascribed to Glu165 in the H95Q TIM mutant on the basis of isotope exchange data (Nickbarg et al., 1988). GlmS may follow a similar mechanism: Glu488 abstracts a proton from C1 and delivers it to the carbonyl oxygen O2, then deprotonates the hydroxyl group O1 and transfers that proton to C2. This mechanism, however, does not account for the direct hydrogen exchange between C1 and C2, which was detected in the intramolecular tritium transfer experiments (Golinelli-Pimpaneau et al., 1989).

An alternative way of the O1 hydroxyl deprotonation may involve the ϵ-amino group of Lys485, which is located at a hydrogen bond distance (3.0 Å) from O1 of GlcNol-6P in the crystal structure (Fig. 4). The ϵ-amino group of Lys485 is H-bonded to the carboxyl of Glu481, hydroxyl of Thr302, and carbonyl 401, all of which act as hydrogen acceptors. Therefore Lys485 can accept a hydrogen from O1 of the enediol or enolamine intermediate. Both Lys485 and Glu481 are strictly conserved in GlmS, which may be due to a particular role of Glu481 in activating Lys485. Following this mechanism, the O2 group of enediol may receive a proton from the solvent. On the other hand, in the "synthetic" route the putative fructosimine-6P, which is the product of transimination of the Lys603:Fru-6P Schiff base, may be in a protonated form (Fig. 6), which would require no hydrogen donor and would thus facilitate the formation of GlcN-6P.

Evolution of isomerases

The isomerization reaction catalyzed by GlmS is thought to proceed via a *cis*-enediol intermediate that is common for most of ketose-aldose isomerases. Comparison to TIM, which is one of the best studied isomerases, also reveals a remarkable similarity in some details of the mechanism. Both GlmS and TIM utilize the carboxylate group of glutamic acid for hydrogen transfer. Its location orthogonal to the enolate plane helps to avoid conformational changes during catalysis and thus speeds up the enolization step. The transition state is stabilized by electrostatic interactions with the helix dipole and a lysine residue (Lys12 in TIM, Lys603 in GlmS). In both enzymes an invariant histidine plays an important role in catalysis. His95 in TIM promotes a hydrogen exchange between O1 and O2. His504 in GlmS is crucial for ring opening. Given the fact that the common catalytic features are based on the totally unrelated three-dimensional structures, the two enzymes provide an example of convergent evolution in the family of ketol isomerases.

Materials and methods

Materials

The isomerase domain of GlmS was overexpressed in *E. coli* and purified to homogeneity as described earlier (Obmolova et al., 1994). The substrate D-Fru-6P was purchased from Fluka (Buchs, Switzerland); GlcNol-6P was synthesized as described (Badet-Denisot et al., 1995).

Crystallization and data collection

For the complex with Fru-6P, the protein was isolated in the presence of Fru-6P. For the GlcNol-6P complex, the protein was isolated in the absence of any ligands. The crystallization solution contained 30% MPD, 0.1 M N-morpholino-ethanesulphonic acid (MES), pH 6.0, 0.5 M $(NH_4)_2SO_4$ and 10 mM of the ligand, either Fru-6P or GlcNol-6P. The crystals belong to the rhombohedral space group R32 and are isomorphous to the crystals of the GlcN-6P complex. There is one 40-kDa protein monomer in the asymmetric unit, which gives 72% solvent content.

X-ray diffraction data were collected at the EMBL synchrotron beamline X11 (DESY, Hamburg, Germany) at a wavelength of 0.91 Å using a MAR Research imaging plate. Two crystals of the isomerase domain co-crystallized with Fru-6P were used to collect the complete data set at 4 °C. The X-ray experiment for the GlcNol-6P complex was carried out at 100 K using one crystal. The crystal was mounted in a cryo-loop after soaking in the mother liquor with 30% glycerol for 1 min and was flash-frozen in the stream of nitrogen. Data were processed with DENZO and SCALEPACK (Otwinowski, 1993). Data statistics are given in Table 2.

Structure determination and refinement

The structures of both complexes were determined by the difference Fourier technique using the atomic model of the GlcN-6P complex (Protein Data Bank entry 1MOQ) as a template. The ligand was not included in the initial model to avoid phase bias. The structures were refined with REFMAC (Murshudov et al., 1997), which utilizes a maximum likelihood approach. Inspection and manual corrections to the models were performed with the program O (Jones et al., 1991). Waters were added using the program WODA (A. Teplyakov, unpubl. results). All other calculations were performed with the CCP4 program suite (CCP4, 1994).

Atomic coordinates and structure factors have been deposited to the Brookhaven Protein Data Bank as entries 1MOR (complex with Fru-6P) and 1MOS (complex with GlcNol-6P).

References

Badet B, Vermoote P, Haumont PY, Lederer F, Le Goffic F. 1987. Glucosamine synthetase from *Escherichia coli*: Purification, properties, and glutamine-utilizing site location. *Biochemistry 26*:1940–1948.

Badet-Denisot MA, Leriche C, Massiere F, Badet B. 1995. Nitrogen transfer in *E. coli* glucosamine-6P synthase. Investigations using substrate and bisubstrate analogs. *Bioorg Med Chem Lett 5*:815–820.

Badet-Denisot MA, Rene L, Badet B. 1993. Mechanistic investigations on glucosamine-6-phosphate synthase. *Bull Soc Chim Fr 130*:249–255.

Banerjee S, Anderson F, Farber GK. 1995. The evolution of sugar isomerases. *Protein Eng 8*:1189–1195.

Blacklow SC, Knowles JR. 1990. How can a catalytic lesion be offset—The energetics of two pseudorevertant triosephosphate isomerases. *Biochemistry 29*:4099–4108.

Collaborative Computational Project No 4. 1994. The CCP4 suite: Programs for protein crystallography. *Acta Crystallogr D50*:760–763.

Collyer CA, Blow DM. 1990. Observation of reaction intermediates and the mechanism of aldose-ketose interconversion by D-xylose isomerase. *Proc Natl Acad Sci USA 87*:1362–1366.

Denisot MA, Le Goffic F, Badet B. 1991. Glucosamine 6-phosphate synthase from *Escherichia coli* yields two proteins upon limited proteolysis: Identification of the glutamine amidohydrolase and 2R ketose/aldose isomerase-bearing domains based on their biochemical properties. *Arch Biochem Biophys 288*:225–230.

Golinelli-Pimpaneau B, Badet B. 1991. Possible involvement of Lys603 from *Escherichia coli* glucosamine-6-phosphate synthase in the binding of its substrate fructose 6-phosphate. *Eur J Biochem 201*:175–182.

Golinelli-Pimpaneau B, Le Goffic F, Badet B. 1989. Glucosamine-6-phosphate synthase from *Escherichia coli*: Mechanism of the reaction at the fructose 6-phosphate binding site. *J Am Chem Soc 111*:3029–3034.

Isupov MN, Obmolova G, Butterworth S, Badet-Denisot MA, Badet B, Polikarpov I, Littlechild JA, Teplyakov A. 1996. Substrate binding is required for assembly of the active conformation of the catalytic site in Ntn amidotransferases: Evidence from the 1.8 Å crystal structure of the glutaminase domain of glucosamine 6-phosphate synthase. *Structure 4*:801–810.

Jones TA, Zou JY, Cowan SW, Kjeldgaard M. 1991. Improved methods for building models in electron density maps and the location of errors in these models. *Acta Crystallogr A47*:110–119.

Knowles JR. 1991. Enzyme catalysis—Not different, just better. *Nature 350*:121–124.

Laskowski RA, MacArthur MW, Moss DS, Thornton JM. 1993. PROCHECK: A program to check the stereochemical quality of protein structures. *J Appl Crystallogr 26*:283–291.

Lavie A, Allen KN, Petsko GA, Ringe D. 1994. X-ray crystallographic structures of D-xylose isomerase-substrate complexes position the substrate and provide evidence for metal movement during catalysis. *Biochemistry 33*:5469–5480.

Leriche C, Badet-Denisot MA, Badet B. 1996. Characterization of a phosphoglucose isomerase-like activity associated with the carboxy-terminal domain of *Escherichia coli* glucosamine-6-phosphate synthase. *J Am Chem Soc 118*:1797–1798.

Malaisse-Lagae F, Liemans V, Yayali B Sener A, Malaisse WJ. 1989. Phosphoglucose isomerase-catalysed inter conversion of hexose phosphates; comparison with phosphomannose isomerase. *Biochim Biophys Acta 998*:118–125.

Massiere F, Badet-Denisot MA. 1998. The mechanism of glutamine-dependent amidotransferases. *Cell Mol Life Sci 54*:205–222.

Murshudov GN, Vagin AA, Dodson EJ. 1997. Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr D53*:240–255.

Nickbarg EB, Davenport RC, Petsko GA, Knowles JR. 1988. Triosephosphate isomerase—Removal of a putatively electrophilic histidine residue results in a subtle change in catalytic mechanism. *Biochemistry 27*:5948–5960.

Obmolova G, Badet-Denisot MA, Badet B, Teplyakov A. 1994. Crystallization and preliminary X-ray analysis of the two domains of glucosamine 6-phosphate synthase from *Escherichia coli*. *J Mol Biol 242*:703–705.

Oliva G, Fontes MRM, Garratt RC, Altamirano MM, Calgagno ML, Horjales E. 1995. Structure and mechanism of glucosamine 6-phosphate deaminase from *Escherichia coli* at 2.1 Å resolution. *Structure 3*:1323–1332.

Otwinowski Z. 1993. Oscillation data reduction program. In: Sawyer L, Isaacs N, Bailey S, eds. *Proceedings of the CCP4 study weekend.* Warrington, UK: Daresbury Laboratory. pp 56–62.

Pierce J, Serianni AS, Barker R. 1985. Anomerization of furanose sugars and sugar phosphates. *J Am Chem Soc 107*:2448–2456.

Rose IA. 1975. Mechanism of the aldose-ketose isomerase reactions. *Adv Enzymol Relat Areas Mol Biol 43*:491–517.

Teplyakov A, Obmolova G, Badet-Denisot MA, Badet B, Polikarpov I. 1998. 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. *Structure 6*:1047–1055.

Traxinger RR, Marshall S. 1991. Coordinated regulation of glutamine:fructose-6-phosphate amidotransferase activity by insulin, glucose, and glutamine. *J Biol Chem 266*:10148–10154.

Zalkin H. 1993. The amidotransferases. *Adv Enzymol Relat Areas Mol Biol 66*:203–309.

Zalkin H, Smith JL. 1998. Enzymes utilizing glutamine as an amide donor. *Adv Enzymol Relat Areas Mol Biol 72*:87–144.