

Crystallization and Preliminary Crystallographic Studies of Glucosamine-6-phosphate Deaminase from *Escherichia coli* K12

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Hexameric glucosamine-6-phosphate deaminase from *Escherichia coli* has been crystallized isomorphously with both phosphate and ammonium sulphate as precipitants, over a wide pH range (6.0 to 9.0). The crystals belong to space group *R*32 and the cell parameters in the hexagonal setting are $a=b=125.9$ Å and $c=223.2$ Å. A complete native data set was collected to 2.1 Å resolution. Self-rotation function studies suggest that the hexamers sit on the 3-fold axis and have point group symmetry 32, with a non-crystallographic dyad relating two monomers linked by an interchain disulfide bridge. A possible packing for the unit cell is proposed.

Keywords: deaminase; glucosamine; hexamer; allostery; crystallization

The amino sugars D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) are both potential carbon and nitrogen sources for the bacterium *Escherichia coli*, and its growth in the presence of amino sugars induces the expression of the enzymes necessary for their metabolism (White, 1968). The genes involved in the uptake and utilization of amino sugars were mapped at 15.5 minutes on the bacterial chromosome (White, 1968; Holmes & Russell, 1972; Jones-Mortimer & Kornberg, 1980). There are five genes arranged in two divergent operons, *nagE-nagB,A,C,D*, which have been cloned and sequenced and the control of their expression is well known (Rogers *et al.*, 1988; Plumbridge, 1989; Vogler & Lengeler, 1989). The gene *nagB* encodes the enzyme glucosamine-6-phosphate deaminase (E.C. 5.3.1.10), which catalyzes

the conversion of D-glucosamine-6-phosphate (GlcN6P) into D-fructose 6-phosphate and ammonia. The reaction catalyzed is an aldo-keto isomerization coupled with an amination-deamination, termed an Amadori rearrangement (Hodge, 1955). This enzyme is allosterically activated by N-acetyl-D-glucosamine 6-phosphate (GlcNAc6P), the same metabolite identified as the co-inducer of the *nag* regulon (Plumbridge, 1991). Similar enzymes have been identified in several other microorganisms and animal tissues (Noltmann, 1972).

Glucosamine-6-phosphate deaminase from *E. coli* can be obtained easily from an overproducing strain and purified by allosteric site-affinity chromatography (Altamirano *et al.*, 1991). The enzyme is a hexameric homopolymer with subunits of 29.7 kDa, exhibiting an intense homotropic co-operativity towards GlcN6P, which is modulated by the allosteric activator GlcNAc6P (Calcagno *et al.*, 1984; Altamirano *et al.*, 1987). A search of the OWL

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sequence database version 12.1 (Bleasby & Wootton, 1990; 34,140 entries) has shown no significant homology with any other known protein family. Sequence-based secondary structure prediction, supported by circular dichroic spectroscopy, suggests that the enzyme has a dominant α/β structure (Altamirano *et al.*, 1991). Of the four cysteine residues in the sequence, two are titrable in the native protein, in the absence of ligands. The allosteric T to R transition produces complete loss of reactivity of this thiol pair (Cys118 and Cys239). These thiols are vicinal and participate in a high-affinity Zn-binding site. The two remaining cysteinyl residues are buried. One is titrable when the enzyme is denatured by urea or SDS and the fourth is exposed only after reduction, suggesting the presence of an interchain disulfide bridge (Altamirano *et al.*, 1989, 1992; Altamirano & Calcagno, 1990). This last observation indicates that the subunits are possibly arranged as dimers linked by a disulfide bridge, which then oligomerize as hexamers.

To increase our understanding of the structure and allosteric mechanism of deamination, we have initiated a crystallographic study of glucosamine-6-phosphate deaminase from *E. coli* K12. We report here the preparation of single crystals of the enzyme suitable for high-resolution X-ray structure determination and the results of the preliminary crystallographic study. We discuss the local symmetry of the hexamer and a possible packing for the molecules in the unit cell.

Glucosamine-6-phosphate deaminase from *E. coli* K12 was purified as described (Altamirano *et al.*, 1991), and stored precipitated in 2.5 M-ammonium sulfate with 5 mM-disodium EDTA. The enzyme suspension was dialyzed against a low ionic strength buffer (10 mM-Hepes/NaOH, pH 7.4) and concentrated to approximately 10 mg/ml. Isomorphous crystals were obtained using the vapor diffusion technique in hanging drops (McPherson, 1982) at 20°C under the following conditions: 1.4 M,

1.37 M and 1.33 M-Na/K phosphate buffer (pH 6.0, 7.0 and 8.0, respectively); 42% (w/v) ammonium sulfate in 50 mM-Tris·HCl (pH 8.0 and 9.0); and 42% ammonium sulfate in 50 mM-Hepes/NaOH (pH 7.0). The drops contained equal amounts (3 to 5 μ l) of the dialyzed protein stock (10 mg/ml) and reservoir solutions. Crystal growth is visible after five days and usually complete within 21 days. The crystals are multifaceted and show variable morphology, growing up to 1.0 mm \times 0.6 mm \times 0.3 mm. SDS/polyacrylamide gel electrophoresis of the crystalline material in the presence of β -mercaptoethanol confirmed that the enzyme monomers were intact and migrated identically with those of glucosamine-6-phosphate deaminase before crystallization. The crystals belong to the rhombohedral system with space group *R*32. The unit cell dimensions of the crystals grown at 1.4 M-Na/K phosphate were measured from precession photographs obtained with a Rigaku RU200B rotating anode generator operating at 50 kV and 190 mA, and are $a=b=125.9$ Å, $c=223.2$ Å (1 Å=0.1 nm) for the hexagonal setting. The unit cell volume of 3.064×10^6 Å³ is compatible with two monomers in the asymmetric unit, yielding a calculated V_m value (Matthews, 1968) for these crystals of 2.87 Å²/dalton. Assuming a protein partial specific volume of 0.737 cm³/g, estimated from the amino acid composition (Calcagno *et al.*, 1984), the solvent content of the crystal is approximately 60%. Rotation photographs taken on a high-brilliance synchrotron radiation source show that the crystals diffract to at least 2 Å resolution.

Complete native data to a resolution of 2.1 Å were collected from one crystal on the EMBL Protein Crystallography Station X31 in HASYLAB at DESY, Hamburg, using an image-plate detector. The crystals are very sensitive to CuK α radiation ($\lambda=1.54$ Å) but proved to be stable for over 24 hours during the synchrotron data collection performed using radiation of wavelength 1.0 Å. Processing of the data was performed with the

Table 1
Data collection statistics for the 2.1 Å native dataset of glucosamine-6-phosphate deaminase

Resolution shell lower limit (Å)	R_{sym}^\dagger	R_{cum}	Reflections measured	Independent reflections	Percentage of completeness	Cumulative % of reflections with $I > 3\sigma(I)$
5.01	0.060	0.060	14,864	2586	87.1	99.2
3.60	0.089	0.080	35,515	4912	99.3	97.4
2.95	0.116	0.091	41,184	6231	99.3	93.2
2.56	0.110	0.093	32,555	7270	99.2	85.6
2.30	0.153	0.097	36,491	8232	99.4	78.2
2.10	0.201	0.101	40,083	9137	99.6	68.5
Totals	0.101	0.101	200,692	38,368	97.4	81.6

$$\dagger R_{sym} = \frac{\sum_h \sum_i |I_{hi} - \langle I_h \rangle|}{\sum_h \langle I_h \rangle}$$

where I_{hi} is the scaled intensity of the i th measurement of reflection h or its equivalent and $\langle I_h \rangle$ is the average intensity of reflection h .

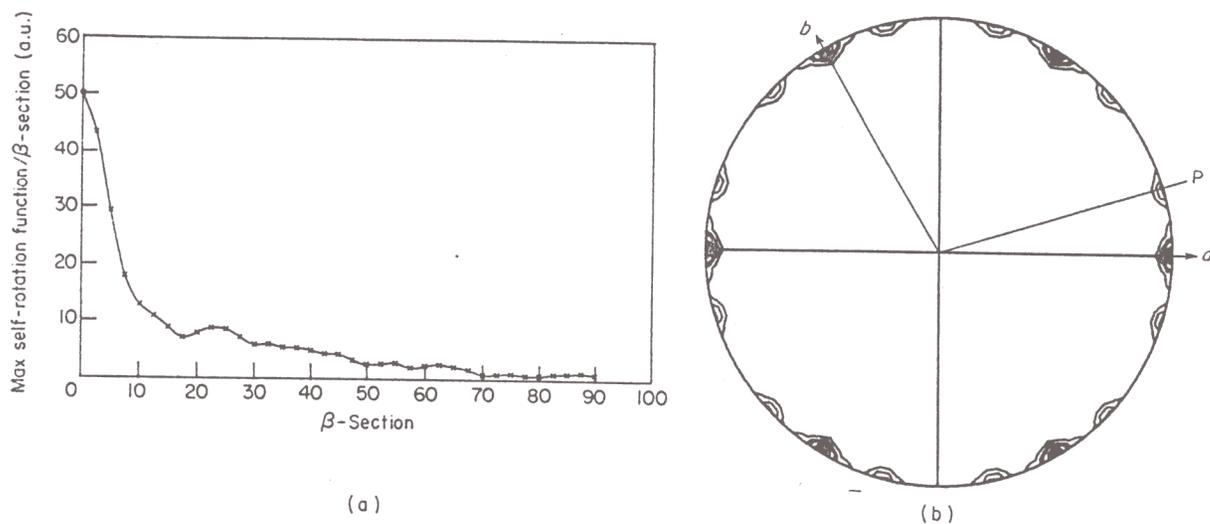


Figure 1. Self-rotation function of glucosamine-6-phosphate deaminase. The resolution range is 1.5 to 2.5 Å, with a radius of integration of 2 to 20 Å, using normalized structure factors. The rotations are assumed to be γ about the crystal c -axis, β about the reciprocal a^* -axis and α again about the c -axis. (a) Maximum value of the self-rotation function for each β -section, in arbitrary units (a.u.) normalized to a value of 50 for the origin peak, with a map root-mean-square value of 6 a.u.; (b) stereographic projection of the diagonal cross-section ($\alpha + \gamma = 180^\circ = \chi$) of the self-rotation map down the crystal c -axis, contoured at 2.5, 3.5, 4.5, 5.5, 6.5 and 7.5 times the map root-mean-square value, showing the only significant peak apart from the origin, which is at section $\beta = 0^\circ$ with a height of 26 a.u., and corresponds to a non-crystallographic 2-fold axis (P) parallel to the crystal ab -plane, 15° away from the a -axis.

MOSFLM (Wonacott *et al.*, 1985) package and the merging of the 200,692 intensity measurements, resulted in 38,368 independent reflections with an overall R_{sym} of 10% to 2.1 Å. Data collection statistics are given in Table 1.

To determine the non-crystallographic symmetry relation between the two independent monomers in the asymmetric unit, a self-rotation function was calculated using the Fast Rotation Function of Crowther (1972) as implemented in the program ALMN (CCP4, 1985). The data included were in the resolution range of 20 to 2.5 Å and the outer radius of integration was varied between 15 Å and 25 Å, and all of them produced similar results. Figure 1(b) is the stereographic projection of the diagonal cross-section ($\alpha + \gamma = 180^\circ = \chi$) of the self-rotation function map, showing a single peak (P) corresponding to a non-crystallographic dyad perpendicular to the crystallographic 3-fold, and displaced from the nearest crystallographic 2-fold by 15° . This result indicates that the hexamer has 32 point group symmetry, centered on the 3-fold axis and necessarily displaced from the origin. This is compatible with the titration experiments that suggest the presence of an interchain disulfide bridge (Altamirano *et al.*, 1992), which can be achieved only if the hexamer subunits are arranged as dimers with the local 2-fold passing through the center of the interchain disulfide bridge and further associated by the crystallographic 3-fold axis to produce the hexamer. The offset of 15° between the non-crystallographic and crystallographic 2-folds could allow for two hexamers related by the crystallographic 2-fold to pack face-to-face in an optimal staggered configuration. In such a way, each monomer from a given

hexamer would lie between two monomers of the 2-fold related hexamer. On the basis of preliminary electron microscopy experiments that suggest a disk-like configuration for the hexamer, and assuming a spherical shape for each monomer with approximate radius of 20 Å to give the correct partial specific volume for the protein molecule, an optimal model for the packing of the hexamers in the unit cell can be achieved, with no superposition of the spheres. A search for suitable heavy-atom derivatives in in progress.

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