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Crystallization and preliminary crystallographic analysis of human alanine:glyoxylate aminotransferase and its polymorphic variants

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The human hereditary disease primary hyperoxaluria type 1 is caused by a deficiency of the liver-specific peroxisomal enzyme alanine:glyoxylate aminotransferase (AGT). In this study, the crystallization and preliminary crystallographic analysis of C-terminal His-tagged human AGT expressed in *Escherichia coli* is reported. At least two crystal forms were obtained using similar conditions for three different polymorphic variants, namely AGT, AGT[P11L] and AGT[P11L, I340M]. Complete data have been collected for all three AGT variants. The crystals of AGT[P11L] belong to space group $P4_12_12$ (or its enantiomorph), with unit-cell parameters $a = b = 90.81$, $c = 142.62$ Å, and diffract to a resolution of 2.8 Å.

1. Introduction

Primary hyperoxaluria type 1 (PH1) is a rare autosomal recessive disorder of glyoxylate metabolism in which there is excessive synthesis and urinary excretion of oxalate. This leads to the cumulative deposition of calcium oxalate stones in the kidney and urinary tract and eventually to kidney failure (Danpure, 2001). PH1 is caused by a deficiency of the liver-specific intermediary metabolic enzyme alanine:glyoxylate aminotransferase (AGT; Danpure & Jennings, 1986). AGT is a pyridoxal phosphate-dependent homodimeric protein. Each subunit contains 392 amino acids and has a molecular mass of 43 kDa. AGT catalyses the transamination/detoxification of glyoxylate to glycine, using alanine as the amino donor, but its deficiency in PH1 allows glyoxylate to be oxidized to oxalate instead. Although most patients have a complete absence of AGT protein and/or catalytic activity, about one third have the disease because AGT, which is normally peroxisomal, is mistargeted to the mitochondria (Danpure *et al.*, 1989).

AGT exists in two main polymorphic variants, one encoded by the major *AGXT* allele, with an allelic frequency of ~80%, and one encoded by the minor *AGXT* allele, with a frequency of ~20%. The latter contains two amino-acid substitutions: Pro11Leu and Ile340Met (Purdue *et al.*, 1990). The Ile340Met polymorphism appears to be without functional consequence, but the Pro11Leu polymorphism leads to several changes in the properties of AGT. It decreases the specific activity of AGT by two-thirds (Lumb & Danpure, 2000), decreases the rate of dimerization under certain conditions (Lumb *et al.*, 1999) and sensitizes the protein to the effects of at least four different mutations found in PH1 (Lumb & Danpure, 2000). More particularly, the Pro11Leu polymorphism acts synergistically with the PH1-specific Gly170Arg mutation to bring about the peroxisome-to-mitochondrion mistargeting of AGT (Purdue *et al.*, 1990; Motley *et al.*, 1995; Leiper *et al.*, 1996).

Our understanding of the mechanism of action of the Pro11Leu polymorphism is hampered by our ignorance of the three-dimensional structure of AGT and especially how it folds, dimerizes and acquires functional (catalytic) activity. In order to rectify this situation, we have expressed various polymorphic forms of AGT (AGT, AGT[P11L], AGT[P11L, I340M]) as C-terminal His-tagged recombinant proteins in *E. coli* and obtained crystals that diffracted to resolutions of between 2.2 and 2.8 Å.

2. Materials and methods

Cloning, expression and purification of the various AGT constructs is described elsewhere (Lumb & Danpure, 2000). Large-scale preparations of proteins were obtained from 1 m*M* isopropyl-β-D-thiogalactopyranoside (IPTG) induced cultures of *E. coli* JM109 and were purified using the Xpress Protein Purification System (Invitrogen) according to the manufacturer's instructions. Fractions containing AGT were concentrated to a final concentration of 15 mg ml^{-1} using ultrafiltration (Ultrafree, Waters).

Expression of selenomethionine (SeMet) substituted AGT was performed using the method described by Hendrickson *et al.* (1990) using a methionine auxotroph strain, *E. coli*

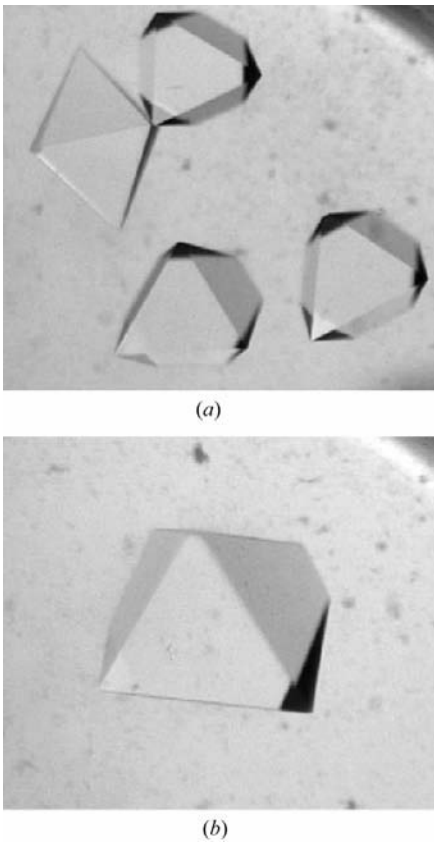

Figure 1
Photomicrographs of single crystals of AGT[P11L]. (*a*) Type 1 crystals; (*b*) type 2 crystal.

B834(DE3). Cultures were grown at 310 K in LeMaster medium containing 40 mg l^{-1} L-selenomethionine (Sigma). When the A_{600} reached the value of 0.5, the cultures were induced with 500 m*M* IPTG for 16–20 h. Purification of the selenomethionine protein was performed as described above; however, all buffers contained 0.2 m*M* β-mercaptoethanol to prevent oxidation of the selenium.

3. Results and discussion

3.1. Crystallization of AGT

Single crystals of AGT, AGT[P11L] and AGT[P11L, I340M] were obtained at 293 K using the under-oil microbatch method (Chayen *et al.*, 1990). A preliminary screen was carried out using the Hampton Research sparse-matrix crystallization screening kit. Single crystals generally appeared overnight when 1 µl of a 15 mg ml^{-1} protein solution was added to 1 µl of 10% PEG 4000, 0.1 *M* Na HEPES pH 7.5. One AGT[P11L] crystal, with dimensions of 0.2 × 0.2 × 0.3 mm (crystal type 1; Fig. 1*a*), was used in diffraction studies (Table 1).

Larger crystals (0.3 × 0.4 × 0.5 mm; crystal type 2) were obtained at 283 K under conditions close to those used for obtaining type 1 crystals: in the presence of 2.5%(*v*/*v*) 2-propanol, 10% PEG 4000, 0.1 *M* Na HEPES pH 7.5. These crystals generally appeared after 2 d (Fig. 1*b*).

3.2. Diffraction and data collection

The diffraction data shown in Table 1 to 2.8 Å were collected at 100 K from a single crystal on beamline 9.6 (λ = 0.870 Å) at the Synchrotron Radiation Source CLRC, Daresbury Laboratory and recorded on an ADSC Quantum 4 CCD detector. Diffraction images were integrated using *DENZO* and merged and reduced using *SCALEPACK* and other programs from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994).

Type 1 crystals of AGT[P11L] are tetragonal, with unit-cell parameters $a = b = 90.81$, $c = 142.62$ Å and systematic absences consistent with space group $P4_12_12$ or $P4_32_12$. With two molecules in the asymmetric unit, the Matthews coefficient V_M is 2.39 $Å^3$ Da^{-1} and the solvent content is 58.1% (Matthews, 1968). The diffraction data have an R_{sym} of 7.7% and a completeness of 96.6%. Data-collection statistics are summarized in Table 1. Type 2 crystals are orthorhombic, with unit-cell parameters $a = 128.12$, $b = 141.45$, $c = 255.95$ Å. The observed pattern of systematic absence for two reciprocal axes indicate a space group of $P2_12_12$ or $P2_12_12_1$.

Attempts to solve the structure using molecular replacement have been unsuccessful owing to the low homology of AGT to other proteins of known structure. We have expressed SeMet-labelled AGT[P11L] in order to perform MAD experiments at the Se edge. AGT[P11L] provides a promising one Se per 5 kDa, which may give useful phasing information.

Table 1
Crystal parameters and data-reduction statistics of AGT[P11L].

Values in parentheses refer to the highest resolution shell.

Space group	$P4_12_12$ or $P4_32_12$
Unit-cell parameters (Å)	$a = b = 90.81$, $c = 142.62$
Beamline	SRS 9.6 (λ = 0.870 Å)
Resolution (Å)	8.85–2.8 (2.95–2.8)
R_{sym} (%)	7.7 (30.9)
$I/\sigma(I)$	7.8 (2.3)
Completeness (%)	96.6 (96.9)
Multiplicity	3.0 (3.0)

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