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Crystallization and preliminary X-ray analysis of the β-isoform of glutamate decarboxylase from *Escherichia coli*

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Abstract

Glutamate decarboxylase (GAD) is a vitamin B_6 enzyme which catalyzes the α-decarboxylation of L-glutamate to γ-aminobutyric acid (GABA). *Escherichia coli* cells coexpress two highly homologous enzyme isoforms, GADα and GADβ. Well diffracting crystals of GADβ were obtained by taking advantage of the possibility of expressing each isoform separately. They belong to space group $P3_1$ or $P3_2$ with the unit-cell dimensions $a = b = 115.6$ and $c = 206.6$ Å and contain one GAD hexamer in the asymmetric unit. High-resolution synchrotron data were collected at 100 K for the native protein and a potential heavy-atom derivative.

1. Introduction

Pyridoxal-5′-phosphate (PLP) dependent decarboxylases constitute a large class of ubiquitous enzymes involved in the biosynthesis of a number of physiologically important amines. Although these enzymes have been classified into four groups, which appear unrelated on the basis of comparative analysis of their sequences (Sandmeier *et al.*, 1994), the recently determined tertiary structure of ornithine decarboxylase suggests relatedness among some of these groups (Momany *et al.*, 1995). Obviously, such a hypothesis could be verified if more three-dimensional structures of representative enzymes of this class were available. Glutamate decarboxylase (GAD, E.C. 4.1.1.15) has an important function in the mammalian central nervous system since it catalyzes the α-decarboxylation of L-glutamate to GABA. Instead, GAD expression in enteric bacteria is probably induced for the maintenance of physiological pH under acidic conditions (Gale, 1946) such as low-pH stress in the natural 'non-host' environment as well as during passage through the stomach *en route* to the intestine (Gorden & Small, 1993). GAD was purified from *Escherichia coli* in several laboratories and characterized biochemically (Fonda, 1972; Sukhareva *et al.*, 1989). The importance of the role played by GAD activity in bacteria is confirmed by the finding in *E. coli* of two structural genes, conserved in diverse strains and designated *gad*A and *gad*B (Smith *et al.*, 1992). Each gene encodes a 466 amino-acid polypeptide, named, respectively, GADα and GADβ, which differ in only five amino-acid residues. The conservation of two homologous coding segments among diverse strains of *E. coli* suggests that both gene products play a role in the bacteria. Recently, GADα and GADβ were expressed and purified individually and proven to possess almost identical kinetic and physico-chemical characteristics (De Biase *et al.*, 1996).

On the basis of electron-microscopy studies it was proposed that GAD from *E. coli* is a hexamer with dihedral symmetry (D_3), and that the subunits are packed in an octahedral arrangement (To, 1971). Attempts to crystallize this enzyme and to determine its spatial structure have a long history (Sukhareva *et al.*, 1989; Markovic-Housley *et al.*, 1987). Various crystal forms have been grown before in our laboratory (V. N. Malashkevich, Z. Markovic-Housley, R. Müller & J. N. Jansonius, unpublished data), but despite the large size of some of those crystals, they either diffracted poorly or displayed significant disorder in their diffraction patterns. The only crystal form for which the space-group determination was reported (Markovic-Housley *et al.*, 1987) could never be reproduced. Assuming that the crystallization problems with GAD from *E. coli* arose mainly from the heterogeneity of the protein, we performed crystallization experiments with the pure GADβ isoform.

2. Materials and methods

Expression and purification of recombinant GADs were carried out as described before (De Biase *et al.*, 1996). Apo GADβ (10 mg ml^{-1}) was prepared as described (De Biase *et al.*, 1991), reconstituted with a fivefold molar excess of phosphopyridoxyl-L-glutamate for 1 h at room temperature and purified on a Sephadex G-25 column equilibrated with 100 m*M* sodium acetate, pH 4.6, containing 0.1 m*M* dithiothreitol. Crystallization was performed using the hanging-drop vapour-diffusion method (McPherson, 1982). Initial crystallization trial experiments were carried out with the Crystal Screens I and II from Hampton Research (Jancarik & Kim, 1991; Cudney *et al.*, 1994). Diffraction data were collected with a Mar Research imaging plate (Mar Research, Hamburg, Germany) using both the in-house modified (E. Bratschi, unpublished) rotating-anode Elliott GX20 X-ray generator (Cu $K\alpha$ radiation, $\lambda = 1.5418$ Å) and synchrotron radiation from the BW7B beamline ($\lambda = 0.94$ Å) at the EMBL Outstation (DESY, Hamburg). Diffraction intensities were integrated with *DENZO*, scaled with *SCALEPACK* (Otwinowski, 1993) and processed further with the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994).

3. Results

Initial crystallization trials with recombinant GADβ yielded similarly disordered crystals as observed before. In an attempt to eliminate some of other possible sources of heterogeneity, *e.g.* those resulting from the accumulation of the amino form of the coenzyme due to an abortive transamination, or incomplete binding of inhibitors, the apo GADβ was reconstituted with the reduced coenzyme–substrate adducts phosphopyr-

idoxyl-L-aspartate (PPD), or phosphopyridoxyl-L-glutamate (PPE). These two complexes and the holoenzyme with a reduced aldimine bond between the coenzyme and the active-site lysine were subjected to crystallization under various conditions using the sparse-matrix approach (Jancarik & Kim, 1991; Cudney *et al.*, 1994). All three enzyme forms yielded large crystals shaped as trigonal prisms, as well as smaller crystals with different morphologies. The crystallization conditions were as follows: 3 µl drops of a reservoir solution containing 1.9–2.1 *M* ammonium sulfate (AS), and 0.1 *M* Tris–HCl, pH 7.6 were mixed on a siliconized cover slip with an equal volume of 20 mg ml^{-1} GADβ in 100 m*M* sodium acetate, pH 4.6. Each drop was equilibrated against 1 ml of the reservoir solution at ambient temperature using the hanging-drop technique (McPherson, 1982). Crystals grew to a maximum size of 0.2 × 0.2 × 0.6 mm in 3–4 weeks. Before data collection, the crystals were transferred into a cryoprotectant solution containing 2.1 *M* AS, 28% glycerol, 0.1 *M* Tris–HCl at pH 7.6. Diffraction data from the crystal of GADβ reconstituted with PPE and also from the crystal of a possible heavy-atom derivative were initially collected at 100 K on the in-house Mar Research imaging plate and subsequently on the BW7B synchrotron beamline at the EMBL Outstation, DESY, Hamburg (Fig. 1; Table 1). GADβ crystals belong to space group $P3_1$ or $P3_2$ with unit-cell dimensions $a = b = 115.6$ and $c = 206.6$ Å, and diffract to at least 1.9 Å resolution in the synchrotron beam. The asymmetric unit contains one GAD hexamer giving rise to a V_M of 2.5 Å^3 Da^{-1} and a solvent content of 51% (Matthews, 1968).

4. Discussion

Protein homogeneity is one of the most critical factors affecting crystallization (McPherson, 1982). Impurities which are similar in structure to the target macromolecule can incorporate into the growing crystal and thus inhibit its growth or cause severe crystal imperfections. We succeeded in the elimination of some sources of GAD heterogeneity which had not been recognized in the earlier crystallization experiments: the coexpression of the two very close enzyme subforms (Smith *et al.*, 1992), the heterogeneity arising from slow PLP modifications due to the known side reactions in the active site (Sukhareva *et al.*, 1989), and the ensuing uneven binding of the inhibitors possibly caused by the two above-mentioned factors. Although GADβ reconstituted with PPE or PPD shows a tendency towards time-dependent aggregation, it crystallizes reproducibly under the conditions established in the current work. The crystals diffract well to high resolution, and provide good opportunities for phase improvement and extension using noncrystallographic averaging and density-modification techniques. The self-rotation function and the observation of only low peaks on the native Patterson map (1/35 of the origin peak or 7.5σ) suggest that the threefold noncrystallographic axis of the GAD hexamer is almost parallel to the crystallographic threefold axis, with the noncrystallographic twofold axes being only about 5° away from the crystallographic dyads in the *xy* plane. We have detected binding of several heavy-atom compounds to GADβ, and phase determination by the multiple isomorphous replacement method is in progress.

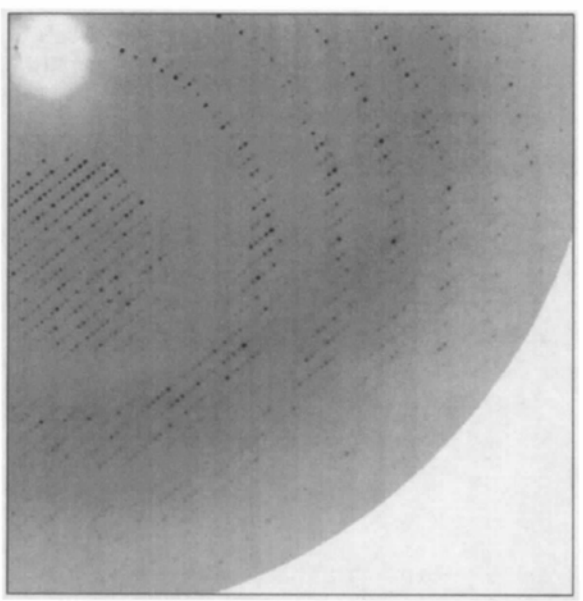

Fig. 1. Oscillation image from a crystal of the PPE complex of GADβ. The image was recorded using a 18 cm Mar Research imaging plate, a crystal-to-detector distance of 225 mm, 1° oscillation, and λ = 0.94 Å. The edge of the plate corresponds to 2.4 Å resolution.

Table 1. *Final data statistics*

Resolution (Å)	R_{merge}†	% Available data	No. unique reflections
Native GADβ + PPE			
25.00–5.37	0.036	96.1	10328
5.37–4.27	0.037	98.4	10490
4.27–3.73	0.042	99.1	10572
3.73–3.39	0.055	99.4	10602
3.39–3.15	0.080	99.3	10615
3.15–2.96	0.114	99.5	10548
2.96–2.82	0.145	99.5	10588
2.82–2.69	0.172	99.3	10606
2.69–2.59	0.218	99.3	10672
2.59–2.50	0.235	97.7	10384
All *hkl*	0.071	98.8	105405

† $R_{merge} = (\sum_h \sum_i |\langle I_h \rangle - I_{hi}|)/(\sum_h \sum_i \langle I_h \rangle)$ for all reflections. Overall redundancy for this data set was 2.2. Geometry of data collection at BW7B beamline limited resolution to 2.5 Å.

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