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Crystallization of Biosynthetic Arginine Decarboxylase from *Escherchia coli*

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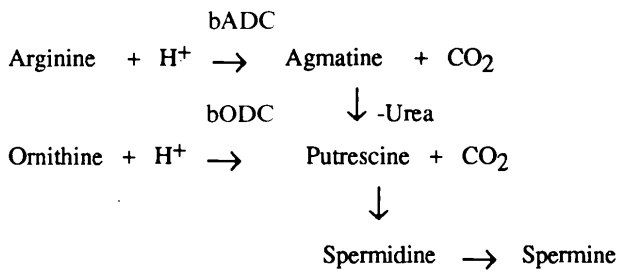
Abstract

Putrescine is the immediate precursor for the synthesis of polyamines and is normally generated by the action of ornithine decarboxylase. However, putrescine can also be produced by the conversion of arginine to agmatine by arginine decarboxylase (bADC) followed by the release of urea by agmatine ureohydrolase. Amino-acid sequence homology with the eukaryotic ornithine decarboxylases suggests that bADC may be a model for this group of decarboxylases. We report here the crystallization of arginine decarboxylase from *E. coli*. Crystals up to 1 mm in size are grown by vapor equilibration using Li_2SO_4 and polyethylene glycols as precipitants. The crystals exhibit diffraction maxima beyond 3 Å resolution and belong to space group $P4_{1(3)}2_12$ with a = 192.4 and c = 121.0 Å. These unit-cell dimensions together with the estimated density of the crystals suggest the presence of one tetramer of bADC (71 kDa subunit^{-1}) per asymmetric unit (V_m = 2.0 Å^3 Da^{-1}).

Decarboxylases are involved in the synthesis of polyamines and neurotransmitters. Some decarboxylases are pyruvoyl dependent, while others are pyridoxal-5′-phosphate (PLP) dependent. To date, X-ray structures for only two decarboxylases have been reported, namely the pyruvoyl-dependent histidine decarboxylase (HDC) (Gallagher, Snell & Hackert, 1989) and the PLP-dependent biodegradative ornithine decarboxylase (dODC) from *Lactobacillus* 30a (Momany, Ernst & Hackert, 1992).‡ Some decarboxylases have two forms, biodegradative and biosynthetic, which differ in function. The biodegradative form is thought to function as a pH regulator by neutralizing acidic end products of carbohydrate fermentation (Gale, 1946), while the biosynthetic form is involved in polyamine or neurotransmitter synthesis. The latter is the case for biosynthetic arginine decarboxylase (bADC) and ornithine decarboxylase (bODC) (Moore & Boyle, 1990).

Putrescine (diaminobutane), cadaverine (diaminopentane), spermidine and spermine are ubiquitous polyamines. Putrescine is made by the action of ornithine decarboxylase and cadaverine by the action of lysine decarboxylase. Putrescine is the starting material for the synthesis of spermidine and spermine which are required for the growth of all cells.



Most eukaryotes use only bODC and lack dODC. However, in bacteria and plants, there is a second pathway which uses bADC to produce agmatine which is then converted to putrescine. In *E. coli*, the ADC pathway ensures that putrescine is produced as nutritional conditions change (Morris, Wu, Applebaum & Koffron, 1970). Cyclic AMP, putrescine and spermidine are transcriptional regulators of the gene (*speA*) that encodes arginine decarboxylase (Moore & Boyle, 1991).

The amino-acid sequence of dODC from *L.* 30a (Hackert *et al.*, 1993) can be aligned with many other biodegradative decarboxylases, but not so well with eukaryotic ODC's (Momany & Hackert, 1993), which are also usually smaller (~50 *versus* ~82 kDa subunit^{-1}). Instead, one can identify amino-acid sequence homology between bADC and eukaryotic ODC's, such as seen in the 43 amino-acid region shown for trypanosomal ODC (TbODC) (Fig. 1).

Since bADC is more stable and yielded more suitable crystals than we have obtained from eukaryotic ODC's, we have elected to undertake a crystallographic study of bADC.

bADC protein was obtained by overexpression of the *speA* gene (plasmid, pRM90, gift of Dr Stephen Boyle) in *E. coli*. The protein was isolated essentially as described by Morris & Boeker (1983) except that a hydroxyapatite column was unnecessary. From approximately 80 g of cells, about 125 mg of protein judged pure by SDS-gel electrophoresis were obtained. Crystals

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‡ *Note added in proof*: Recently, the structure of dialkyl glycine decarboxylase has been reported (Toney *et al.*, 1993).

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bADC : 271FHLGSQMANIRDIATGVRESARFYVEL-HKLGVNIQCFDVGGGL313
TbODC: 216FHVGSGSTDASTFAQAISDS-RFVFDMGTELGFNMHILDIGGGF258
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Fig. 1. The amino-acid sequences of bADC and trypanosomal ODC (TbODC).

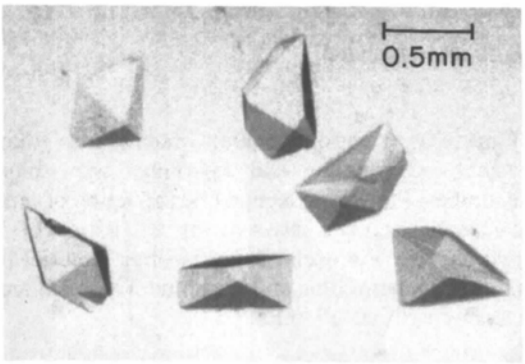
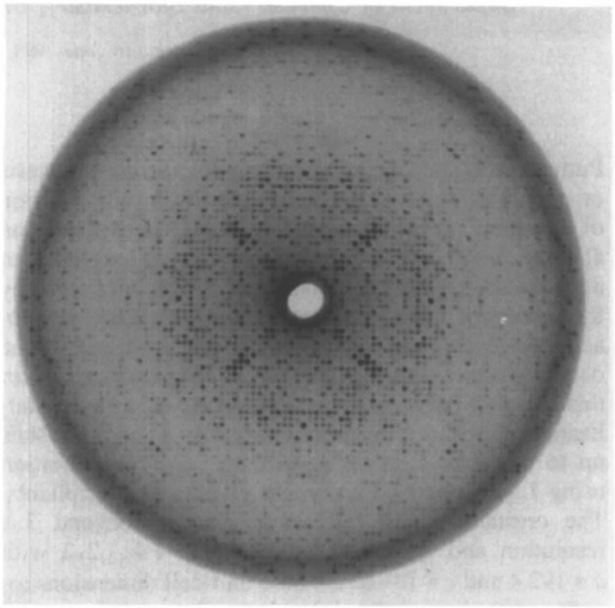


Fig. 2. Crystals of biosynthetic arginine decarboxylase grown from PEG and salt solutions by vapor diffusion.

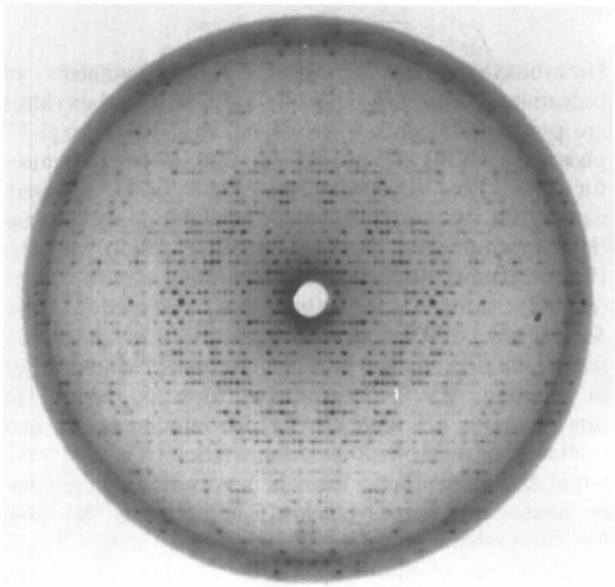
were grown using the hanging-drop method (McPherson, 1976). The protein droplet was made up of 10 µl of the protein solution [bADC at 25 mg ml^{-1} in 50 m*M* potassium phosphate buffer (pH 7.0) containing 1 m*M* dithiothreitol, 5 m*M* magnesium sulfate and 40 m*M* PLP] mixed with 10 µl of the well solution (15% PEG8000 and 0.50 *M* lithium sulfate). Yellowish crystals with a truncated octahedral habit (Fig. 2) appeared after about a week at room temperature. More symmetrical crystals were obtained when the crystals were grown at 277 K with lower concentrations of PEG (5.0% PEG8000 and 0.7 *M* lithium sulfate). After about a month, the crystals grew to about 0.7–1.0 mm in length and 0.5 mm in width.

These crystals routinely exhibit X-ray diffraction maxima beyond 3.0 Å resolution. Fig. 3 shows precession photographs (μ = 10°) of (*a*) the *hk*0 zone and (*b*) the *h*0*l* zone, which indicate that the crystal belongs to the tetrahedral space group, $P4_{1(3)}2_12$ with a = 192.4 and c = 121.0 Å.

The 74 kDa, 658-amino acid bADC subunit is known to lose some of its amino-terminal residues during a post-translational modification step. The protein from our crystals shows a single band upon SDS polyacrylamide gel electrophoresis of about 70 kDa. Gas-phase amino-acid sequencing of the first 15 N-terminal amino-acid residues indicated that the crystallized protein was missing the first 27 amino acids. This truncation results in a 71 kDa subunit or native tetramer of 284 kDa. The density of the crystals was measured to be 1.23 g cm^{-3} using a non-linear xylene/bromobenzene gradient (Low & Richards, 1952) with L-leucine (1.165 g cm^{-3}), L-valine (1.23 g cm^{-3}) and L-methionine (1.292 g cm^{-3}) as density markers. If one assumes a partial specific volume of 0.74 cm^3 g^{-1}, the density value implies the presence of 35 subunits or 8.7 tetramers per unit cell. The Matthews



(*a*)



(*b*)

Fig. 3. Precession photographs (μ = 10°) of the (*a*) *hk*0 and (*b*) *h*0*l* zones of the bADC crystals. Photographs were taken for 20 h using graphite-monochromated Cu $K\alpha$ radiation from a Rigaku RU-200 rotating-anode source operating at 100 kV and 50 mA with a crystal-to-film distance of 100 mm.

(1968) parameter based on eight tetramers per unit cell (1 tetramer per asymmetric unit) would be 2.0 $Å^3$ Da^{-1}.

Native data have been collected from bADC crystals using our SDMS area detector and a search for heavy-atom derivatives is in progress. Eventually, we hope to obtain a molecular model of the bADC and compare its structure with that of ornithine decarboxylase and other PLP-dependent enzymes.

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References

GALE, E. F. (1946). *Adv. Enzymol.* **6**, 1-32.

GALLAGHER, T., SNELL, E. E. & HACKERT, M. L. (1989). *J. Biol. Chem.* **264**, 12737-12743.

HACKERT, M. L., CARROLL, D. W., DAVIDSON, L., KIM, S.-O., MOMANY, C., VAALER, G. L. & ZHANG, L. (1993). *J. Biol. Chem.* Submitted.

LOW, B. W. & RICHARDS, F. M. (1952). *J. Am. Chem. Soc.* **74**, 1660-1666.

MCPHERSON, A. (1976). *Methods Biochem. Anal.* **23**, 249-345.

MATTHEWS, B. M. (1968). *J. Mol. Biol.* **33**, 491-497.

MOMANY, C., ERNST, S. & HACKERT, M. L. (1992). *Am. Crystallogr. Assoc. Abstr.* **20**, 44.

MOMANY, C. & HACKERT, M. L. (1993). In preparation.

MOORE, R. C. & BOYLE, S. M. (1990). *J. Bacteriol.* **172**, 4631-4640.

MOORE, R. C. & BOYLE, S. M. (1991). *J. Bacteriol.* **173**, 3615-3621.

MORRIS, D. R. & BOEKER, E. A. (1983). *Methods Enzymol.* **94**, 125-134.

MORRIS, D. R., WU, W. H., APPLEBAUM, D. & KOFFRON, K. L. (1970). *Ann. N. Y. Acad. Sci.* **171**, 968-976.

TONEY, M. D., HOHNESTER, E., COWAN, S. W. & JANSONIUS, J. N. (1993). *Science*, **261**, 756-759.