Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Preliminary X-ray analysis of a new crystal form of recombinant pig kidney DOPA decarboxylase

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Received 3 March 1998
Accepted 30 April 1998

DOPA decarboxylase is responsible for the synthesis of the key neurotransmitters dopamine and serotonin *via* decarboxylation of L-3,4-dihydroxyphenylalanine (L-DOPA) and L-5-hydroxytryptophan, respectively. The crystals of recombinant DOPA decarboxylase differ from those previously reported for the enzyme purified from pig kidney. They belong to space group *P*622 with unit-cell dimensions $a = b = 302.6$, $c = 178.1$ Å. Both the self-rotation function and the good diffraction quality of these crystals (2.5 Å on a synchrotron source) suggest that there should be at least three protein dimers in the asymmetric unit. Diffraction data sets have been collected for the native enzyme and a heavy-atom derivative.

1. Introduction

DOPA decarboxylase (DDC; E.C. 4.1.1.28) is a pyridoxal-5′-phosphate (PLP)-dependent enzyme which catalyses the decarboxylation of L-3,4-dihydroxyphenylalanine (L-DOPA) and L-5-hydroxytryptophan (L-5HTP) to produce dopamine and serotonin, major neurotransmitters in the mammalian nervous system. DDC has been implicated in a number of clinic disorders, including Parkinson's disease and hypertension. Peripheral inhibitors of DDC, DOPA hydrazide and α-methylDOPA, are currently used to treat Parkinson's disease and hypertension, respectively (Marsden *et al.*, 1973; Sjoerdsma, 1982). Rational design of new drugs with better pharmacological characteristics has not been possible because of the lack of knowledge of the crystal structure of the enzyme. Elucidation of the DDC crystal structure would, therefore, strongly enhance efforts in this direction.

The enzyme from pig kidney exists as a homodimer with 486 amino-acid residues per chain and a monomer molecular mass of 53 936 Da. It was isolated with one molecule of PLP per dimer (Borri Voltattorni *et al.*, 1979). The enzyme can be purified from pig kidney in large quantities (Borri Voltattorni *et al.*, 1979) and has been used to initiate structural studies (Malashkevich *et al.*, 1992). Despite significant improvements in the purification protocol (Dominici *et al.*, 1993), the crystallization behaviour of the enzyme varies from batch to batch, strictly limiting the number of crystals available for a heavy-atom-derivative search and hampering further progress.

Recently, pig kidney DDC has been expressed in *Escherichia coli* in sufficient amounts for structural studies; during its characterization it was observed to possess two active-site PLP molecules per dimer (Moore *et al.*, 1996). Based on comparative kinetic and spectral studies it was suggested that the natural enzyme contains one functioning PLP molecule per dimer, while the second PLP probably exists as an inactive irreversibly bound coenzyme derivative. Rat liver DDC has also been cloned and expressed (Hayashi *et al.*, 1993). In order to identify residues involved in catalysis and substrate/inhibitor binding, some DDC mutants have been constructed and characterized (Ishii *et al.*, 1996; Nishino *et al.*, 1997; Dominici *et al.*, 1997). In the absence of the three-dimensional structure, the rationale guiding mutagenesis experiments has been substitution of invariant residues, derived by multiple alignment of the group-II decarboxylases (Sandmeier *et al.*, 1994), or of residues previously identified by chemical means as crucial to the enzyme's catalytic activity.

2. Materials and methods

Expression and purification of recombinant DDC were carried out as described by Moore *et al.* (1996). Crystallization was performed using the hanging-drop vapour-diffusion method (McPherson, 1982). Initial crystallization trial experiments were carried out with the Crystal Screens I (Jancarik & Kim, 1991) and II (Cudney *et al.*, 1994) from Hampton Research. Diffraction data were collected with a MarResearch imaging plate (MarResearch, Hamburg, Germany) using both the in-house modified (E. Bratschi, unpublished work) rotating-anode Elliott GX20 X-ray generator (Cu $K\alpha$ radiation, $\lambda = 1.5418$ Å) and synchrotron radiation at the X11 beamline ($\lambda = 1.0$ Å) at the EMBL Outstation (DESY, Hamburg) and the DW32 beamline ($\lambda = 0.9$ Å) at LURE (Paris Sud). Diffraction intensities were integrated with *DENZO*, scaled with *SCALE-*

Table 1
List of 00l reflections in the native DDC data set.

l	I	σI	$I/\sigma I$
3	132	142	0.9
4	−87	137	−0.6
5	−105	187	−0.6
7	−26	222	−0.1
8	298	301	1.0
9	320	74	4.3
10	88	55	1.6
11	−124	68	−1.8
12	783 142	18 743	41.8
13	−89	74	−1.2
14	225	81	2.8
15	624	80	7.8
16	122	90	1.4
17	−98	77	−1.3
18	480 995	10 776	44.6
19	−182	108	−1.7
20	782	107	7.3
21	3114	367	8.5
22	−500	152	−3.3
23	817	271	3.0
24	354 778	6953	51.0
25	−280	154	−1.8
26	217	115	1.9
27	1716	166	10.3
28	59	120	0.5
29	−260	221	−1.2
32	−326	215	−1.5
33	−6	217	−0.0
34	−234	136	−1.7
35	836	253	3.3
37	−122	168	−0.7
38	−122	278	−0.4
39	38 731	1690	22.9
40	3358	423	7.9
41	19 907	1255	15.9
42	29 180	1665	17.5
43	−87	341	−0.3
44	1393	395	3.5
45	12 355	565	21.9
46	868	394	2.2
47	644	319	2.0
48	13 686	766	17.9

PACK (Otwinowski, 1993) and further processed with the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994).

3. Results

Despite otherwise similar physico-chemical characteristics, recombinant DDC resisted crystallization under the conditions previously established for the enzyme isolated from pig kidney (Malashkevich *et al.*, 1992). Small crystals of recombinant DDC of various shapes were initially obtained with the sparse-matrix crystallization kits (Jancarik & Kim, 1991; Cudney *et al.*, 1994). Further refinement of the most favourable conditions produced large diamond-shaped crystals suitable for diffraction analysis. The final crystallization protocol was as follows: 3 µl drops of a reservoir solution containing 19–24% polyethylene glycol monomethyl ester (PEG MME) 5000, 0.5 *M* 2-morpholinoethanesulfonic acid (MES)-KOH at pH 6.5 and 200 m*M* ammonium sulfate (AS) were mixed on a siliconized cover slip with an equal amount of 30 mg ml^{-1} DDC in 100 m*M* potassium phosphate, pH 6.8. 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 0.8 × 0.8 × 0.6 mm in 1–2 weeks. Before data collection, the crystals were transferred into a cryoprotectant solution containing 25% PEG MME 5000, 25% ethylene glycol, 100 m*M* MES-KOH at pH 6.5 and 200 m*M* AS. Diffraction data from a native crystal were collected at 100 K, initially on the in-house MAR Research imaging plate, and later on the X11 beamline (EMBL Outstation, DESY Hamburg). Diffraction data from a Ta_6Br_{12} derivative (a kind gift from Professor Robert Huber, Martinsried) at four different wavelengths were collected on the BW6 beamline (MPG-ASMB, DESY, Hamburg) with an R_{merge} of 10.6% and a completeness of 83.3% at a resolution of 2.5 Å. These DDC crystals belong to space group *P*622 with unit-cell dimensions $a = b = 302.6$, $c = 178.1$ Å, and diffract to 2.5 Å resolution in the synchrotron beam. Determination of the space group was not straightforward. The diffraction pattern demonstrated 622 Laue symmetry. Appearance of very strong reflections along the 00*l* axis with $l = 6n$ suggested a screw component for the crystallographic sixfold axis. Further analysis of 00*l* reflections indicated, however, that this periodicity disappears at higher resolution and relatively strong reflections with index l odd are present (Table 1). Therefore, the correct space group is *P*622. Systematic pseudo absences throughout the diffraction pattern (including the 00*l* axis) and the appearance of strong peaks on the $(u, v, 0)$ and $(u, v, \frac{1}{2})$ sections of the native and difference Patterson maps are apparently due to special positions of the DDC dimers in the asymmetric unit. The self-rotation function reveals no peaks other than those originating from the crystal symmetry. This fact and the arrangement of four strong peaks on the native Patterson map suggest that the non-crystallographic twofold axes of the DDC dimers are parallel to the sixfold crystallographic *c* axis and that all dimers have the same orientation. A modelled crystal packing with four dimers in the asymmetric unit shows the same features on the native Patterson map. The good diffraction quality and the mechanical strength of these crystals indicate that the asymmetric unit can accommodate from three to five DDC dimers, giving rise to V_M values (Matthews, 1968) between 3.62 and 2.17 Å^3 Da^{-1} and a solvent content between 66.0 and 43.0%, respectively.

Table 2
Final data statistics.

$R_{merge} = (\sum_h \sum_i |\langle I_h\rangle - I_{hi}|)/(\sum_h \sum_i \langle I_h\rangle)$ for all reflections. Overall redundancy for this data set was 5.9.

Resolution (Å)	R_{merge}	Available data (%)	No. unique reflections
25.0–8.11	0.051	97.3	5028
8.11–5.73	0.070	99.9	9043
5.73–4.68	0.068	95.3	10 928
4.68–4.05	0.053	94.2	12 701
4.05–3.62	0.058	97.7	14 925
3.62–3.31	0.065	99.6	16 777
3.31–3.06	0.080	100.0	18 258
3.06–2.87	0.100	99.8	19 525
2.87–2.70	0.130	99.7	20 725
2.70–2.56	0.163	91.9	20 179
All *hkl*	0.068	97.5	148 089

4. Discussion

It has been commonly observed in the recent past that proteins produced by recombinant techniques provide better opportunities for structural studies. Recombinant DDC crystallizes readily with a high yield of well diffracting crystals. The significantly larger unit cell of these crystals as compared with the previously reported crystal form ($P6_2$ or $P6_4$, $a = b = 155.9$, $c = 87.7$ Å) makes data collection, reduction and interpretation more difficult, but the high non-crystallographic symmetry provides a unique opportunity for phase improvement and extension through non-crystallographic symmetry averaging and density modification. Numerous soaking experiments with the previous crystal form did not yield a useful heavy-atom derivative. The original crystals of DDC (Malashkevich *et al.*, 1992) are extremely sensitive to mercurials and many other commonly used heavy-atom compounds, probably because of the presence of 12 cysteine residues per chain (Moore *et al.*, 1996). The use of large heavy-atom clusters which bind at the interface between protein molecules in the crystal lattice proved to be useful in many cases (Knäblein *et al.*, 1997). We are pursuing work in the direction of initial low-resolution phasing with the strongly diffracting $Ta_6Br_{12}^{2+}$ cluster and further phase extension *via* density-modification methods. Diffraction data have been collected for ligand-free DDC at 2.56 Å resolution in the new crystal form (Table 2) and for the complex of DDC with the anti-Parkinson drug carbidopa at 2.27 Å resolution in the original crystal form ($R_{sym} = 0.075$, 96.8% complete).

This study was supported by the Swiss National Science Foundation grant 31-36432.92 to JNJ and MURST grants to CBV. We are grateful to the staff of the EMBL Outstation and the MPG-ASMB (DESY, Hamburg) and LURE (Paris Sud) for their help with synchrotron data collection, and to Professor R. Huber, Max-Planck Institut für Biochemie, Martinsried, Germany, for his kind gift of the tantalum cluster.

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