Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray crystallographic studies on recombinant rat choline acetyltransferase

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Received 14 October 2003
Accepted 8 December 2003

Choline acetyltransferase (ChAT) catalyzes the biosynthesis of the neurotransmitter acetylcholine from acetyl-CoA and choline in cholinergic neurons. Rat ChAT (rChAT) was overexpressed in *Escherichia coli*, purified by affinity chromatography and crystallized. Diffraction data were collected from a single crystal under cryoconditions at the F1 beamline at the Cornell High Energy Synchrotron Source, with a maximal useful diffraction pattern to 1.55 Å resolution. The crystals were shown to belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 138.97, b = 77.67, c = 59.67 Å and a scaling $R_{\rm sym}$ of 0.054 for 72 446 unique reflections. Packing considerations indicate there to be one molecule per asymmetric unit. It is expected that in the near future the structure of rChAT will be obtained using molecular-replacement methods. Elucidation of the structure of rChAT will aid in the development of therapeutic agents for Alzheimer's disease.

1. Introduction

Choline acetyltransferase (EC 2.3.1.6; ChAT) catalyzes the transfer of an acetyl group from acetyl coenzyme A (acetyl-CoA) to choline to form the neurotransmitter acetylcholine (Oda, 1999). Acetylcholine functions in the peripheral nervous system to stimulate muscle contraction and functions in the central nervous system primarily to facilitate learning and short-term memory formation. Cholinergic neurons have been implicated in the pathophysiology of several diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease, schizophrenia and sudden infant death syndrome (Oda, 1999).

ChAT is localized in the cholinergic nerve terminals and exists as soluble and membrane-bound forms. Approximately 80–90% of ChAT activity is attributed to the soluble form (Smith & Carroll, 1980). A phosphorylated form appears to be responsible for membrane association (Dobransky *et al.*, 2001). ChAT is thought to exist as an extremely low abundance monomeric globular protein in nature, with its mechanism of action still unknown. Several inactivation studies with chemical reagents suggest the presence of histidine, arginine and cysteine residues at or near the active site (Roskoski, 1974*a*,*b*; Malthe-Sorenssen, 1976; Roskoski *et al.*, 1975; Hersh *et al.*, 1979; Mautner *et al.*, 1981; Carbini *et al.*, 1990). Furthermore, it has been hypothesized that the histidine residue in the active site serves as an acid–base catalyst and that an arginine residue may bind to the phosphate group of the CoA/acetyl-CoA substrate (Currier & Mautner, 1974; Mautner *et al.*, 1981; Wu & Hersh, 1995).

Recently, the crystal structures of human and mouse carnitine acetyltransferase (hpCAT and mCAT) have been reported (Wu *et al.*, 2003; Jogl & Tong, 2003). These enzymes show significant amino-acid sequence homology (~40% identity) with ChAT and can therefore be used for the molecular-replacement solution of the structure of ChAT. In our efforts to determine the crystal structure of ChAT, we report the purification, crystallization and preliminary X-ray diffraction studies of recombinant rat ChAT (rChAT).

2. Materials and methods

2.1. Purification

Newly transformed bacterial cells B834 (Novagen) harboring recombinant rChAT expression plasmid pQE-rChAT (pQE31, Qiagen) were grown at 303 K to 0.5 OD at 600 nm; 1 m*M* IPTG was then added and the cells were induced for 2 h. Bacterial cells were then harvested by centrifugation and frozen at 193 K until ready for further use. The purification procedure involves application of the *Escherichia coli* extract prepared in 20 m*M* sodium phosphate buffer pH 7.6 to a column of Ni–nitriloacetate–agarose (Qiagen) equilibrated with loading buffer. The column was washed batchwise with buffer containing increasing concentrations of NaCl from 0 to 2 *M* and lastly with loading buffer containing 5 m*M* imidazole pH 7.4. The enzyme, eluted with an imidazole gradient from 5 to 150 m*M*,

was applied to a Mono S column (Pharmacia) previously equilibrated with 20 m*M* sodium phosphate buffer pH 7.6. After washing the column with equilibration buffer, the enzyme was eluted with a linear salt gradient from 0 to 1 *M* and concentrated/dialyzed using Ultrafree Tangential membranes (Millipore). The purified enzyme was either used immediately or stored at 193 K in 20 m*M* sodium phosphate buffer pH 7.6 containing 40% glycerol.

2.2. Crystallization

Prior to crystallization trials, the purified recombinant rChAT was concentrated using Ultrafree Tangential membranes (Millipore) with a molecular-weight cutoff of 30 kDa in 20 m*M* sodium phosphate buffer pH 7.6, 50 m*M* NaCl. Initial crystallization experiments were carried out by the hanging-drop vapor-diffusion method in a 24-well tissue-culture Linbro plate (ICN Inc.) at three different temperatures: 277, 289 and 295 K. Each drop was formed by mixing equal volumes (3 µl) of 10 mg ml^{-1} enzyme solution and the reservoir solution. A sparse-matrix crystallization screen based on the original report by Jancarik & Kim (1991) was used and promising conditions were further optimized with respect to pH and precipitants. The optimal condition was found to consist of 50 m*M* MES buffer pH 6.0, 100 m*M* NaCl and 8–10% PEG 8000 at 277 K.

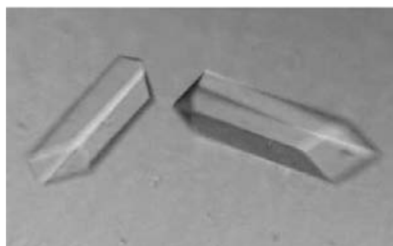

Figure 1
Photograph of recombinant rat choline acetyltransferase crystals (approximate dimensions 0.4 × 0.2 × 0.2 mm).

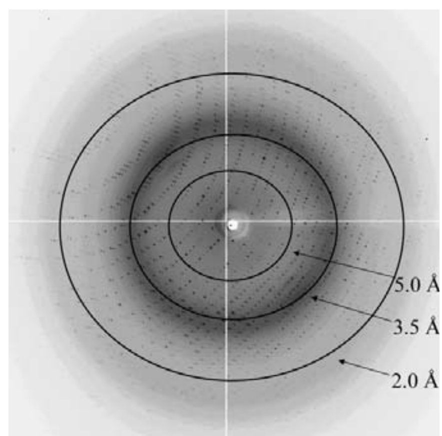

Figure 2
X-ray diffraction pattern of a rat choline acetyltransferase crystal. Data were collected on the F1 beamline (λ = 0.9504 Å) with an ADSC Quantum 4 CCD detector system at the Cornell High Energy Synchrotron Source. This data was collected with a crystal-to-detector distance of 150 mm, an exposure time of 120 s per image and an oscillation range of 1.0° at 100 K. The black concentric rings show the demarcation of the 5.0, 3.5, 2.0 Å resolution diffraction shells. The highest observed diffraction extended beyond 1.55 Å resolution.

Table 1
Summary of data-collection and processing parameters.

Data statistics in the outer resolution shell (1.61–1.55 Å) are given in parentheses.

Crystal dimensions (mm)	0.4 × 0.2 × 0.2
Temperature (K)	100
Wavelength (Å)	0.9504
Resolution range (Å)	30–1.55
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	*a* = 138.97, *b* = 77.67, *c* = 59.67
Total No. reflections	429316
No. unique reflections	72446
R_{sym}†	0.054 (0.337)
Completeness (%)	77.5 (65.6)
Average $I/\sigma(I)$	10.5 (3.2)
Solvent content (%)	45
V_M (Å^3 Da^{-1})	2.3

† R_{sym} is defined as $\sum|(I - \langle I \rangle)|/\sum I$, where *I* is the intensity of an individual reflection and $\langle I \rangle$ is the average intensity of this reflection.

2.3. X-ray data collection and processing

A single crystal was cryoprotected by a 60 s soak in a reservoir solution containing 15% PEG 8000 and 40% glycerol. The crystal was then mounted in a thin fiber nylon loop (Hampton Research) and flash-frozen prior to data collection at 100 K. The diffraction data were collected at the Cornell High Energy Synchrotron Source (CHESS) F-1 beamline (λ = 0.9504 Å) using the ADSC Quantum 4 CCD detector system. Data were collected using a 0.2 mm collimator, a crystal-to-detector distance of 150 mm and a 1.0° oscillation angle with an exposure time of 120 s for each image. Reflection intensities were indexed and evaluated with *DENZO* and scaled using *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results and analysis

RChAT was overexpressed in *E. coli*, purified by affinity chromatography and crystallized, as shown in Fig. 1. A total of 70° of data were collected (70 images) from a single crystal. Fig. 2 shows a typical 1.0° oscillation diffraction image. The data were initially processed as the Laue orthogonal system *P*222, with unit-cell parameters *a* = 138.97, *b* = 77.67, *c* = 59.67 Å. The data set was merged and scaled to consist of 429 316 total observations (of 72 446 independent reflections) with an overall R_{sym} of 5.4% and 77.5% completeness (30–1.55 Å) with 65.6% completeness in the outer resolution shell (1.61–1.55 Å). On examination of the intensity of the (*h*00), (0*k*0) and (00*l*) reflections, it can be inferred from the pattern of systematic absences that the space group is $P2_12_12_1$. The calculated Matthews coefficient gives a V_M of 2.3 Å^3 Da^{-1} (Matthews, 1968) consistent with one rChAT present in the asymmetric unit. The data-collection, reduction and processing parameters are summarized in Table 1.

The structure of rChAT should be determined easily by molecular-replacement methods (Rossmann, 1990) using the structure of hpCAT (Wu *et al.*, 2003) as a phasing model using the *CNS* program suite (Brünger *et al.*, 1998).

This research is supported in part by NIH grant GM58197 (DHW) and the University of Florida, College of Medicine start-up funds (RM). TK and BP are supported by a University of Florida Alumni Fellowship.

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