Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray crystallographic studies of wild-type human ornithine transcarbamylase and two naturally occurring mutants at position 277

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Received 7 October 2000
Accepted 9 February 2001

Wild-type human ornithine transcarbamylase (OTCase) and two mutants (R277Q and R277W) that cause 'late-onset' hyperammonemia were crystallized and a preliminary structure determination was carried out. The unliganded wild-type enzyme crystallizes in the cubic space group $I23$, with unit-cell parameters $a = b = c = 203.4$ Å. R277Q crystallizes in two crystal forms under the same crystallization conditions. One crystal form is isomorphous to that of unliganded wild-type crystals, with unit-cell parameters $a = b = c = 202.2$ Å. The second form also belongs to a cubic space group, $P4_332$, but has unit-cell parameters $a = b = c = 139.8$ Å. R277W crystals are isomorphous to the second crystal form of R277Q, with unit-cell parameters $a = b = c = 138.7$ Å. None of these crystal forms is isomorphous to other crystal forms of OTCase that have been studied. The structures in both crystal forms have been solved using molecular replacement. In the first crystal form there are two monomers in the asymmetric unit, corresponding to a solvent content of 75%. Because of its high molecular and crystal symmetry and the presence of non-crystallographic symmetry, this structure could not be solved with *AMoRe* or *X-PLOR*, but was solved successfully with *COMO*. There is only one monomer in the asymmetric unit in the second crystal form, corresponding to a solvent content of 62%. This structure was successfully solved with *AMoRe*.

1. Introduction

The mammalian urea cycle is the main chemical pathway for the 'detoxification' of ammonia by conversion to urea, which can be efficiently eliminated in the urine. Ornithine transcarbamylase (OTCase) is an enzyme of the urea cycle that catalyses the formation of citrulline and inorganic phosphate from carbamoyl phosphate and L-ornithine (Snodgrass, 1968). Defects in OTCase give rise to an inherited urea-cycle disorder that causes hyperammonemia and leads to mental retardation or death. More than 160 mutations causing OTCase deficiency have been identified (Tuchman *et al.*, 1998).

We have recently reported the crystal structures of human OTCase complexed with the bisubstrate analogue *N*-phosphonacetyl-L-ornithine (PALO) and complexed with carbamoyl phosphate and L-norvaline (Shi *et al.*, 1998, 2000). Catalytically active human OTCase consists of a trimer with a quaternary structure similar to those of other OTCases (Allewell *et al.*, 1999; Villeret *et al.*, 1995, 1998; Ha *et al.*, 1997; Jin *et al.*, 1997) and the catalytic trimer of ATCase (Lipscomb, 1994; Endrizzi *et al.*, 2000). The carbamoyl phosphate-binding domain is close to the molecular threefold axis and the second substrate-binding domain for L-ornithine or L-aspartate is at the periphery of the molecule. The active site is located in the cleft between the two domains and is shared by two adjacent subunits. In *Escherichia coli* OTCase and ATCase (Ha *et al.*, 1997; Lipscomb, 1994; Endrizzi *et al.*, 2000) binding of substrates induces conformational changes and domain movements. In order to precisely determine the specific substrate-induced conformational changes and domain movements for human OTCase, we have crystallized the unliganded wild-type human OTCase and two mutants that cause 'late-onset' hyperammonemia.

2. Experimental

Human OTCase and R277Q and R277W mutants have been cloned, overexpresssed and purified to homogeneity as described by Morizono *et al.* (1997). Each of the purified proteins was concentrated by centrifugation using Centricon tubes at 5000 rev min^{-1}. Protein concentration was determined with a dye-binding protein assay (Bio-Rad) using bovine serum albumin as a standard. The concentrated protein (3 mg ml^{-1}) was used for

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Table 1
Data-collection statistics of wild-type OTCase and R277Q, R277W.

	Wild-type	R277Q	R277Q	R277W
Shape of crystals	Cubic	Cubic	Bipyramidal	Bipyramidal
No. of crystals	4	1	1	1
Space group	$I23$	$I23$	$P4_332$	$P4_332$
Unit-cell parameters (Å)	203.4	202.2	139.8	138.7
Resolution (Å)	2.6	3.5	3.2	3.2
No. of observations	300506	138078	59145	70062
No. of reflections	42459	17444	7942	7610
Redundancy	7.1	7.9	7.4	10.3
Completeness (%)	98.7	98.3	94.2	91.5
$R_{\rm merge}$ (%)	10.8	9.0	12.4	17.9
$\langle I/\sigma(I)\rangle$	6.8	6.0	6.9	3.6
Unit-cell volume (Å^3)	8414975	8266915	2732257	2665902

crystallization screening. Crystallization conditions were analyzed using the Hampton Crystallization Screen Kits I and II, which were based on the sparse-matrix sampling method (Jancarik & Kim, 1991). The screening was carried out at 290 K using vapor diffusion in a hanging-drop method (McPherson, 1982). The drop contained equal amounts (3 µl) of the reservoir and protein solutions. The best crystals of wild-type human OTCase were obtained by mixing 8 µl of the protein solution (6 mg ml^{-1}) with 8 µl of reservoir solution consisting of 16% polyethelene glycol 8000 and 0.1 *M* MES buffer pH 6.5. These crystals were grown over a period of 1–2 weeks to dimensions of 0.4 × 0.4 × 0.3 mm, as shown in Fig. 1(*a*). Crystals of R277Q and R277W were also grown using the hanging-drop method in crystallization conditions similar to those of wild-type OTCase. Two crystal forms appear in the same drop for R277Q. The second crystal form is bipyramidal, as shown in Fig. 1(*b*).

3. Data collection and preliminary crystallographic studies

Crystals were mounted in a thin-walled glass capillary that contained a small amount of mother liquor at the end to prevent crystal dehydration. The wild-type crystal data were collected on a MAR Research 30 cm image-plate detector using a crystal-to-detector distance of 30.0 cm at beamline X12B at the National Synchrotron Light Source. Four crystals were used in data collection owing to the rapid decay of diffraction with synchrotron radiation. The final data set to 2.6 Å consisted of 71 1.0° oscillation images. Each image was exposed for 200 s. The data were indexed, integrated and scaled using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The data for R277Q and R277W were collected on a Siemens area detector with a rotating-anode X-ray source operated at 50 kV and 200 mA. The detector was positioned 14.0 cm from the crystal, with the 2θ angle set at 15°. Data collection utilized a φ scan with a step size of 0.25°.

Data-collection statistics are summarized in Table 1. Analysis of diffraction data for the wild-type crystals indicated that they belong to the space group $I23$ or $I2_13$, with unit-cell parameters $a = b = c = 203.4$ Å. Human OTCase is a trimer with 321 amino acids per monomer and a molecular weight of approximately 36 kDa per polypeptide chain (Kalousek *et al.*, 1978). Assuming one monomer per asymmetric unit, the Matthews coefficient was calculated to be 9.7 Å^3 Da^{-1}, well outside the normal range of values (1.7–3.5 Å^3 Da^{-1}; Matthews, 1968). Therefore, there are at least two monomers in the asymmetric unit. In order to determine the non-crystallographic symmetry elements, a self-rotation analysis was performed using the program *POLARRFN* (written by W. Kabsch) from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994) and *GLRF* (Tong & Rossmann, 1990, 1997). However, the self-rotation analysis was inconclusive as no large non-crystallographic peaks were observed on the $\kappa = 90$, 120 or 180° sections. This suggests that the non-crystallographic symmetry is either close to or parallel to the crystallographic threefold or twofold axes.

Analysis of the diffraction data for the cubic crystal form of R277Q indicated that it is isomorphous to that of wild-type human OTCase and has similar unit-cell parameters $a = b = c = 202.2$ Å. Self-rotation function analysis also indicates that it might have similar packing, as the self-rotation function maps are also similar. However, the intensity pattern of the bipyramidal crystal form of R277Q indicated that its space group was either $P4_132$ or $P4_332$, with unit-cell parameters $a = b = c = 139.8$ Å. The volume of the unit cell for this crystal form is three times smaller than that for the cubic crystal form, implying that there is only one monomer per asymmetric unit. The Matthews coefficient (Matthews, 1968) was calculated to be 3.15 Å Da^{-3}, corresponding to a solvent content of 61%. Thus, the biologically active trimer must be generated by the crystallographic threefold axis in this crystal form.

The R277W crystals have unit-cell parameters $a = b = c = 138.7$ Å. Analysis of the diffraction data indicated that it is isomorphous to the bipyramidal crystal form of R277Q crystals.

Structure determination was originally sought *via* molecular-replacement techniques using the *Pseudomonas aeruginosa* OTCase (PDB code 1ort; Villeret *et al.*, 1995) and the PALO-liganded human OTCase (PDB code 1oth; Shi *et al.*, 1998) structures as search models. No reasonable solution was found with the programs *AMoRe* (Navaza, 1994) and *X-PLOR* (Brünger, 1992) for the cubic crystal form even with the refined PALO-liganded human OTCase model, probably owing to the large number of crystallographic symmetry elements in this highly symmetric space group and the presence of non-crystallographic symmetry. Others have shown that it is difficult to solve structures using molecular replacement in a similar space group in the presence of non-crystallographic symmetry (Harrop *et al.*, 1996). The structure was eventually solved using a combined molecular-replacement protocol (Tong, 1993, 1996) with the program *COMO* (Tong, unpublished program) and the PALO-liganded human OTCase structure as a search model. This combined molecular-replacement protocol examines all rotation-function grid points that are above a given threshold level. In addition, the packing of the atomic models in the crystal unit cell is checked automatically and those molecular-replacement

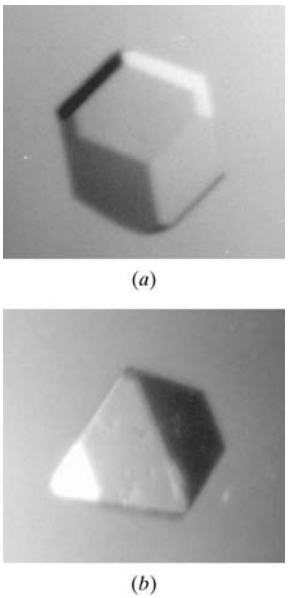

Figure 1
(*a*) Typical cubic crystals of wild-type human OTCase with crystal dimensions of approximately 0.4 × 0.4 × 0.3 mm. (*b*) The second crystal form of R277Q in a typical bipyramidal shape with crystal dimensions of 0.4 × 0.4 × 0.3 mm.

solutions that have obvious packing problems are removed. A reasonable solution could only be found in the space group $I23$. It is interesting to note that there are two monomers in the asymmetric unit. The catalytically active trimers are created by the same crystallographic threefold symmetry and are packed against each other by their concave and convex surfaces along the diagonal direction. The packing of molecules in the unit cell is consistent with the results from the self-rotation function search. Four trimers are arranged at the apices of a tetrahedron with their concave faces pointing inward. No contacts are found between these apical trimers; instead, three trimers are bridged by an additional trimer located at each of the four trigonal faces in the tetrahedral arrangement described above. These bridging trimers have their concave faces pointing outward. These 24-mers form the basic unit and are packed into a cubic I crystal lattice. Adjacent 24-mers along the cubic diagonal are held together by contacts between a bridging trimer and an apical trimer. The bridging trimer and the apical trimer are eclipsed by about 20° (Fig. 2*b*). Rigid-body refinement resulted in a conventional R value of 35% for 10.0–3.0 Å resolution data. In the rigid-body refinement, each domain was treated as a rigid body. The relative orientation of the two domains was not found to be significantly different (less than 1°) from the original model.

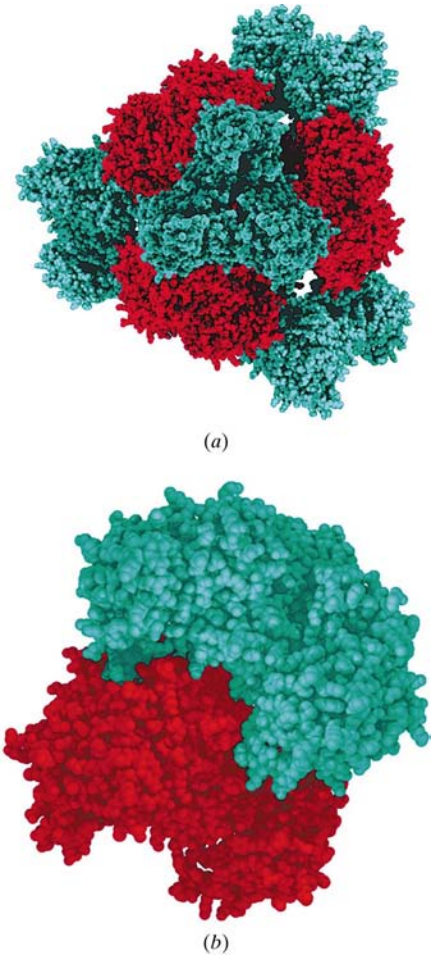

Figure 2
(*a*) Molecular packing in the cubic crystals. An inner tetrahedral arrangement of four trimers, shown in red, is held in place by an outer tetrahedron of four trimers, shown in blue. (*b*) The two trimers, the inner trimer in red and outer trimer in blue, form the contacts between adjacent 24-mers. These figures were generated with *SwissPDBViewer* (Guex & Peitsch, 1997).

In contrast to the difficulty met in solving the structure of the cubic crystal form, a significant molecular-replacement solution could readily be found for the bipyramidal crystal form of R277Q even with the *P. aeruginosa* OTCase structure as the search model. The structure was solved using the program *AMoRe* (Navaza, 1994). The cross-rotation function was calculated with data in the resolution range 15.0–4.0 Å and an integration radius in Patterson space of 25.0 Å. The top 20 peaks from the cross-rotation function search were used for the translation search. The best solution after the translation search had a correlation coefficient of 40.0 and an R factor of 46.8%, indicating that the solution was likely to be correct, compared with values of 36.8 and 50.1% for the next best solution in the space group $P4_332$. There were no significant solutions for the translation search using the space group $P4_132$, with the best solution having a correlation coefficient of 36.9 and an R factor of 51.1%, establishing that the correct space group is $P4_332$.

We are confident that the structures in both crystal forms have been solved. Firstly, the packing is reasonable with no short contacts between various trimers in the unit cell. Secondly, the biologically active trimers can be assembled correctly from the monomers by threefold crystallographic symmetry. Finally, the electron density is easily interpreted. Model building and refinement are currently in progress. Meanwhile, we are testing cryocooling conditions in order to obtain better quality and higher resolution data.

We thank Dr M. Capel for his assistance during data collection at beamline X12B at the National Synchrotron Light Source at Brookhaven National Laboratory. This facility is supported by the United States Department of Energy Offices of Health and Environmental Research and of Basic Energy Sciences and by the National Science Foundation. We also thank Dr L. Banaszak for facilitating our use of the diffraction equipment in the Kahlert Center for Structure Biology at the University of Minnesota. This work was supported by Public Health Service Grant DK-47870 from the National Institute of Diabetes, Digestive and Kidney Diseases (to MT and NMA).

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