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Purification, crystallization and preliminary X-ray analysis of catabolic ornithine carbamoyltransferase from *Pseudomonas aeruginosa*

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The catabolic ornithine carbamoyltransferase (OTCase) from *Pseudomonas aeruginosa* exhibits allosteric behaviour, with two conformational states of the molecule: an active R form and an inactive T form. The enzyme is a dodecamer with a molecular mass of 455 700 Da. Three crystal forms have been obtained. Crystals of allosteric state T are rhombohedral, belonging to the *R*3 space group, with hexagonal unit-cell parameters $a = b = 180.6$, $c = 122.0$ Å. They diffract to a resolution of 4.5 Å. Two crystal forms for allosteric state R have been obtained, with hexagonal and cubic symmetries. Hexagonal crystals, which diffract to a resolution of 3.4 Å, belong to the space group $P6_3$ with unit-cell parameters $a = b = 140.8$, $c = 145.6$ Å. The cubic crystals belong to space group *I*23, with unit-cell parameter $a = 134.32$ Å and diffract to a resolution better than 2.5 Å. In all crystal forms, the dodecamer exhibits a 23 point-group symmetry.

1. Introduction

Pseudomonas aeruginosa is an arginine prototrophic organism which possesses two distinct ornithine carbamoyltransferases (OTCases): an anabolic (aOTCase) and a catabolic (cOTCase) enzyme. The aOTCase is an ubiquitous and essential metabolic enzyme which catalyzes the production of L-citrulline and phosphate from L-ornithine and carbamoylphosphate (CP). This protein, which is involved in the arginine biosynthesis pathway, is generally a functional homotrimer with a subunit molecular mass of about 35–40 kDa (Tricot *et al.*, 1989; Jin *et al.*, 1997). The anabolic enzyme displays Michaelis–Menten kinetics (Itoh *et al.*, 1988; Stalon *et al.*, 1977). The cOTCase is involved in the anaerobically inducible arginine deiminase pathway. This protein promotes the synthesis of ornithine and CP from the two substrates phosphate and citrulline (Stalon *et al.*, 1976; Haas *et al.*, 1979; Mercenier *et al.*, 1980; Vander Wauven *et al.*, 1984). The catabolic enzyme displays a marked cooperativity towards CP (Haas *et al.*, 1979; Baur *et al.*, 1990). It is heterotropically activated by phosphate and nucleoside monophosphates (AMP, GMP, CMP, UMP), designated positive effectors, and is inhibited by polyamines (such as spermidine, spermine or putrescine), designated negative effectors (Tricot *et al.*, 1993).

Preliminary crystallographic studies have shown that the cOTCase is formed of 12 38 kDa subunits organized in four trimers (Marcq *et al.*, 1991) with a 23 point-group symmetry. The two allosteric forms, the active R ('relaxed') form and the inactive T ('tense') form, coexist in solution. The addition of positive and of negative effectors in the protein solution promotes the R and T states, respectively. The OTCase structures (R and T states) will give some understanding of the allosteric transition.

In this paper, we report the purification, the crystallization and a preliminary X-ray analysis of the two allosteric states of the cOTCase protein.

2. Materials and methods

2.1. Purification

The wild-type enzyme was genetically overexpressed in *P. aeruginosa* and purified using a modified version of the method described by Baur *et al.* (1987). Initially, the foreign proteins were thermoprecipitated at 338 K for 10 min in the presence of 100 m*M* ornithine and phosphate; this was followed by an ammonium sulfate precipitation step. After dialysis against 10 m*M* potassium phosphate buffer (KH_2PO_4/K_2HPO_4 pH 7.5), the protein solutions were deposited on an arginine-Sepharose Pharmacia column (2.5 × 35 cm) pre-equilibrated with the same buffer. The enzyme was then eluted with a linear gradient of KCl (0–200 m*M*, 400 ml) in the same buffer. The most active fractions were pooled and then concentrated by ultrafiltration. The molecular mass was determined using a Sepharose 4B column (Marcq *et al.*, 1991). The enzyme was stored in 10 m*M* potassium

phosphate buffer pH 7.5 at 277 K. Mass spectra were obtained on a API III+ triple-quadrupole mass spectrometer (Perkin–Elmer) equipped with a nebulizer-assisted electrospray source (ion spray) operating at atmospheric pressure. For this analysis, the protein sample (5 mg ml^{-1}) was dialyzed against 20 m*M* ammonium acetate buffer. The monomer molecular mass observed is 37977 Da, in agreement with the theoretical value of 37978 Da obtained from the sequence.

2.2. Crystallization

Crystallization trials were performed for both cOTCase allosteric forms. After numerous trials varying critical factors such as temperature, drop size and crystallization techniques, the best results for both R and T forms of cOTCase were obtained using the hanging-drop method at 294 K with droplets consisting of 3.0 µl of 10 mg ml^{-1} protein solution mixed with an equal volume of buffered precipitating solution.

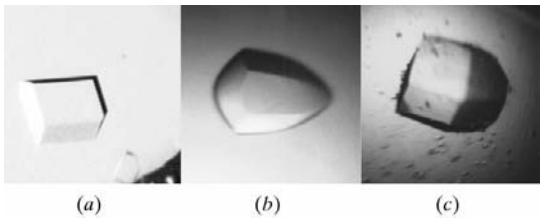

Figure 1
Crystals of cOTCase: (*a*) T form, (*b*) hexagonal R form, (*c*) cubic R form.

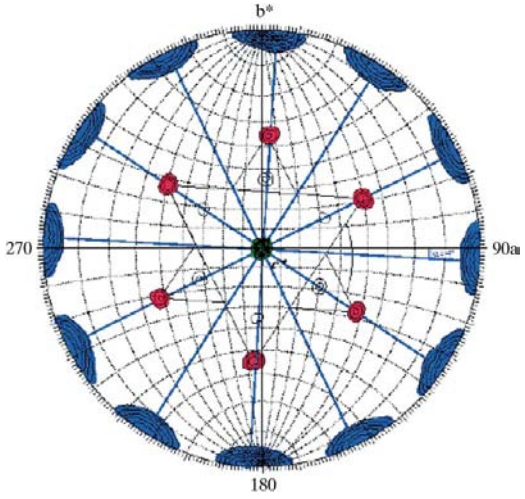

Figure 2
The $\kappa = 180^\circ$ section of the self-rotation function for the cOTCase R form hexagonal crystal, using data from 10 to 6 Å, calculated and drawn using *GLRF*. The orthogonalization was chosen to place *x* along *a*, *y* along *b** and *z* along *c*. Spherical polar angles are defined as φ, the angle from the Cartesian *x* axis to the projection of the rotation axis onto the *zx* plane; ψ, the angle from the *z* axis to the rotation axis; κ, the rotation angle around the axis. The map-contour level varies by 1σ with a starting value of 2σ. The direction of the three true twofold non-crystallographic axes are indicated by the red peaks. Blue peaks arise from the crystal twinning: blue lines are the planes containing the *z* axis and the twofold axes arising from the twinning.

3. Results and discussion

3.1. Crystallization of both allosteric states of the cOTCase

For the T allosteric form, crystals formed in 4–6 months. The best crystals were obtained by vapour diffusion against a reservoir solution containing 100 m*M* glycylglycine (pH 9), 12%(*w*/*v*) polyethylene glycol (PEG) 6000 and 1 m*M* dithiothreitol (DTT); a 10 m*M* concentration of the negative effector spermidine was added to displace the equilibrium in solution towards a monodisperse T-form solution. The biggest T crystal reached dimensions of 0.6 × 0.5 × 0.2 mm (Fig. 1*a*) and diffracted to at least 4.5 Å resolution. The crystals belong to rhombohedral space group *R*3, with hexagonal unit-cell parameters of $a = b = 180.6$, $c = 122.0$ Å. Trials are under way to improve the quality of these crystals.

For allosteric form R, the protein solution was dialyzed overnight against 10 m*M* HEPES buffer pH 7.2. Crystallization trials using ammonium sulfate leading to the R form of the molecule gave two crystal forms: one with hexagonal symmetry and the other with cubic symmetry. Shell-like crystals with hexagonal symmetry were obtained against a reservoir solution containing 1.9 *M* ammonium sulfate, 50 m*M* HEPES pH 7.2, 1 m*M* DTT, 1 m*M* ethylenediaminetetraacetic acid (EDTA), 3% PEG 400 and 10 m*M* spermidine (Fig. 1*b*). The unit-cell parameters of this crystal form are $a = b = 140.8$, $c = 145.6$ Å and the space group is $P6_3$. Four monomers in the asymmetric unit would correspond to a Matthews coefficient, V_m (Matthews, 1968), of 2.8 Å^3 Da^{-1} and a solvent content of approximately 50%. The cubic crystals were obtained against a reservoir solution containing 1.85 *M* ammonium sulfate, 50 m*M* HEPES pH 7.2, 1 m*M* DTT, 1 m*M* EDTA, 2% PEG 400 and 10 m*M* spermidine (Fig. 1*c*). The space group is *I*23 with unit-cell parameter $a = 134.21$ Å. Assuming one monomer per asymmetric unit, V_m is 2.7 Å^3 Da^{-1}, corresponding to a solvent content of 47.5%. The high concentration of sulfate (~1.9 *M*), which mimics the positive effector phosphate, displaces the equilibrium toward the R form, despite the presence of 10 m*M* spermidine. For both R forms, the spermidine was used as an additive to improve the crystal quality.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell.

	Hexagonal	Cubic
Space group	$P6_3$	*I*23
Resolution range (Å)	15–3.2	48–2.48
Highest resolution shell (Å)	3.36–3.20	2.62–2.48
Asymmetric unit contents	4 monomers	1 monomer
V_m (Å^3 Da^{-1})	2.8	2.7
No. of observed reflections	168715	80597
No. of unique reflections	26910	13154
Completeness (%)	99.0 (100)	94.9 (65.5)
$R_{\rm merge\ (all)}$† (%)	6.7 (33.3)	7.5 (25.8)
I/σ_I	8.0 (2.3)	6.0 (2.1)

† $R_{\rm merge\ (all)} = \sum |I - \langle I\rangle| / \sum \langle I\rangle$.

3.2. X-ray analysis of both cOTCase R state crystal forms

For the hexagonal form, data were collected using synchrotron radiation from beamline BW7B at DESY (Hamburg) with a MAR Research image-plate detector. The experiment was performed at 100 K with a crystal-to-detector distance of 239.8 mm and oscillation frames of 1° over an exposure time of 90 s at the wavelength $\lambda = 0.8815$ Å. The data set was processed using *DENZO* (Otwinowski & Minor, 1997). The results of the processing are summarized in Table 1.

A self-rotation function (*GLRF*; Tong & Rossmann, 1990, 1997) was calculated in order to identify the positions of the non-crystallographic threefold and twofold axes relative to the crystal symmetry. The self-rotation function was calculated for different resolution ranges. One of the threefold axes of the molecule coincides with the crystallographic threefold axis: there is one functional trimer plus one monomer from the dodecamer per asymmetric unit. Fig. 2 shows a $\kappa = 180^\circ$ section calculated with intensity data between 10 and 6 Å resolution. On this stereographic projection of the rotation function, the six red peaks indicate the orientations of the non-crystallographic twofold axes of the dodecameric molecule in the unit cell. This projection also shows extra peaks (coloured

blue) corresponding to six twofold axes perpendicular to the *c* axis and rotated by 30° from each other, as in space group $P6_322$. However, they are not aligned with the *a* or *b* axis of the hexagonal unit cell as they should be in this space group: they are 4° away from the *a* or *b* axis. This indicates that the space group is not $P6_322$ and that these crystals are probably twinned. However, intensity statistics do not support the twinning hypothesis.

Cubic crystals were mounted and sealed in a quartz capillary tube. Unit-cell dimensions and space group were determined using an Enraf–Nonius *FAST* system with a Cu rotating-anode generator (FR5H). These crystals are very sensitive to X-rays. Cryocooling trials have been performed in order to decrease radiation damage, but were unsuccessful despite numerous and different cryoprotecting solution tests. The crystal-to-detector distance was 100 mm. Data collection took place at room temperature and with an exposure time per frame (0.1°) of 90 s (see Table 1). Two data sets were collected on the same crystal at high and low resolution in two passes. The completeness of the merged data set is 95%. The data were processed using the *MADNES* program (Messerschmidt & Pflugrath, 1987). For further processing, the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994) was used.

For the cubic crystal form, the 23 symmetry of the dodecameric cOTCase merges with the crystallographic symmetry of the space group *I*23, and there is one monomer per asymmetric unit. The structure was solved by molecular-replacement methods using *AMoRe* (Navaza, 1994). The structure of one monomer of the Glu105→Gly cOTCase mutant from the same organism (Villeret *et al.*, 1995) was used as a search polyalanine model. Reflections in the 15–3.5 Å resolution range (5153 reflections) were used in a first step, but a better contrast between solutions was obtained using reflections in the 10–6 Å resolution range (831 reflections). After rigid-body refinement, the solution gave a correlation coefficient of 68.0% and an *R* factor of 38.6%. The packing of the molecular-replacement solution was examined with the program *O* (Jones *et al.*, 1991). No significant unfavourable contacts were observed. Refinement using *X-PLOR* (Brünger, 1992) is under way.

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References

Baur, H., Stalon, V., Falmagne, P., Lüthi, E. & Haas, D. (1987). *Eur. J. Biochem.* **166**, 111–117.
Baur, H., Tricot, C., Stalon, V. & Haas, D. (1990). *J. Biol. Chem.* **265**, 14728–14731.
Brünger, A. T. (1992). *X-PLOR Version* 3.1. *A System for X-ray Crystallography and NMR.* New Haven, CT: Yale University Press.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Haas, D., Evans, R., Mercenier, A., Simon, J. P. & Stalon, V. (1979). *J. Bacteriol.* **139**, 713–720.
Itoh, Y., Soldati, L., Stalon, V., Falmagne, P., Terawaki, Y., Leisinger, T. & Haas, D. (1988). *J. Bacteriol.* **170**, 3225–3234.
Jin, L., Seaton, B. A. & Head, J. F. (1997). *Nature Struct. Biol.* **4**, 622–625.
Jones, T. A., Zou, J. Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.
Marcq, S., Diaz-Ruano, A., Charlier, P., Dideberg, O., Tricot, C., Piérard, N. & Stalon, V. (1991). *J. Mol. Biol.* **220**, 9–12.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mercenier, A., Simon, J. P., Vander Wauven, C., Haas, D. & Stalon, V. (1980) *J. Bacteriol.* **144**, 159–163.
Messerschmidt, A. & Pflugrath, J. W. (1987). *J. Appl. Cryst.* **20**, 306–315.
Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Stalon, V., Ramos, F., Piérard, A. & Wiame, J. M. (1976). *Biochim. Biophys. Acta*, **139**, 91–97.
Stalon, V., Legrain, C. & Wiame, J. M. (1977). *Eur. J. Biochem.* **74**, 319–327.
Tong, L. & Rossmann, M. G. (1990). *Acta Cryst.* A**46**, 783–792.
Tong, L. & Rossmann, M. G. (1997). *Methods Enzymol.* **276**, 594–611.
Tricot, C., Decoen, J.-L., Momin, P., Falmagne, P. & Stalon, V. (1989). *J. Gen. Microbiol.* **135**, 2453–2464.
Tricot, C., Nguyen, V. T. & Stalon, V. (1993). *Eur. J. Biochem.* **215**, 833–839.
Vander Wauven, C., Piérard, A., Kley-Reymann, M. & Haas, D. (1984). *J. Bacteriol.* **160**, 928–934.
Villeret, V., Tricot, C., Stalon, V. & Dideberg, O. (1995). *Proc. Natl Acad. Sci. USA*, **92**, 10762–10766.