Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary crystallographic analysis of formyl-CoA tranferase from *Oxalobacter formigenes*

Stefano Ricagno,[a] Stefan Jonsson,[b] Nigel Richards[b] and Ylva Lindqvist[a]*

[a]Molecular Structural Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-17177 Stockholm, Sweden, and [b]Department of Chemistry, University of Florida, Gainesville, FL 32611-7200, USA

Correspondence e-mail: ylva@alfa.mbb.ki.se

Received 3 February 2003
Accepted 30 April 2003

Formyl-CoA transferase from *Oxalobacter formigenes* has been expressed as a recombinant protein in *Escherichia coli* and purified to homogeneity. Crystals of formyl-CoA transferase were grown at 293 K using polyethylene glycol 4000 as a precipitant. The diffraction pattern of flash-frozen crystals at 100 K extends to 2.2 Å resolution with synchrotron radiation ($\lambda = 0.933$ nm). The crystals are tetragonal and belong to space group $I4$, with unit-cell parameters $a = b = 151.44$, $c = 99.49$ Å. The asymmetric unit contains one dimer and the solvent content is 53%. Formyl-CoA transferase was crystallized both as the apoenzyme and as its complex with coenzyme A.

1. Introduction

Oxalate is a highly oxidized compound that is introduced into man not only as a dietary component, but also as a byproduct of pathways in normal cellular metabolism. The accumulation of oxalate in the body gives rise to several disorders including urolithiasis, renal failure and cardiac conductance disorders (Rodby *et al.*, 1991; Williams & Smith, 1968). Mammals do not appear to have an oxalate-degradation pathway; instead, oxalate is eliminated in the kidneys and in the intestines by excretion (Hatch *et al.*, 1994). In this regard, a remarkable correlation has been observed between the absence of an oxalate-dependent bacterium, *Oxalobacter formigenes* (Allison *et al.*, 1985), in the human intestine and the propensity of individuals to form calcium oxalate stones in the kidney (Sidhu *et al.*, 1998). It has therefore been proposed that *O. formigenes* may play an important role in the mammalian intestinal flora, degrading oxalate excreted into the intestinal lumen and preventing reabsorption of oxalate in the lower tract of the intestine (Sidhu *et al.*, 1999), thus keeping the transepithelial gradient favourable for further oxalate excretion. While the involvement of *O. formigenes* in regulating oxalate homeostasis remains a controversial hypothesis (Trinchieri *et al.*, 1991), it suggests a novel therapeutic/prophylactic approach for treating oxalate-related illness involving the introduction of oxalate-degrading enzymes into the human gastrointestinal tract (Sidhu *et al.*, 1999). Clinical investigations of the ability of oxalate-degrading enzymes to impact on urolithiasis and other oxalate-related diseases in man are hindered, however, by a significant lack of information concerning the structures, biochemical properties and catalytic mechanisms of these enzymes.

Formyl-CoA transferase (FRC) from *O. formigenes* catalyses the reversible transfer of CoA from formate to oxalate (Baetz & Allison, 1990). This activates the oxalyl moiety for decarboxylation, catalysed by the second enzyme in the pathway, oxalyl-CoA decarboxylase, releasing formyl-CoA and carbon dioxide (Baetz & Allison, 1989). By this degradation pathway, the bacterium produces the proton gradient necessary for ATP synthesis (Ruan *et al.*, 1992). FRC is reported to be a monomer consisting of 428 amino acids and has a molecular weight of 47 000 Da (Baetz & Allison, 1990). The enzyme belongs to a recently proposed new family of CoA-transferases (Heider, 2001), for which no three-dimensional structures are available.

2. Materials and methods

2.1. Expression and purification

Formyl-CoA transferase has been expressed as recombinant protein in *Escherichia coli*, cloned in pET9a (Novagen) and transformed in BL21 (DE3). The average yield is 9 mg ml^{-1}. The protein has been subsequently purified by four steps of chromatography: DEAE Sepharose, Blue Sepharose Fast Flow and a desalting column (G25), after which the sample was applied onto a Q Sepharose HP column, from which the protein was eluted in a highly pure state.

2.2. Crystallization

Formyl-CoA transferase was dialysed against 10% glycerol, 25 m*M* MES pH 6.2 in order to remove the elution buffer from the last step of the purification (25 m*M* sodium phosphate, 10% glycerol, 0.3 *M* NaCl).

Crystallization trials were performed using the hanging-drop technique at 291 K with a

protein concentration of 7.5 mg ml^{-1}. For the initial screen, Hampton Research Crystal Screen Kits I and II were used. The drop volume was typically 2 µl of protein solution and 2 µl of mother liquor.

The optimized conditions for obtaining crystals were 26% polyethylene glycol 4000, 100 m*M* HEPES pH 7.5 and 0.5 *M* $MgCl_2$. The crystals grew to final dimensions of 0.1 × 0.2 × 0.2 mm in one week. In optimization, increasing the $MgCl_2$ concentration helped to change the shape of the crystals from the original very thin sheets to the final shape shown in Fig. 1.

In order to crystallize FRC in the presence of coenzyme A, the crystallization conditions needed to be reoptimized. Indeed, adding coenzyme A increased the nucleation rate several-fold, giving many small crystals in the drops. This problem was overcome by decreasing the protein concentration to 5 mg ml^{-1} and changing the drop volume (to 2 µl mother liquor and 1 µl protein solution).

Table 1
Statistics of the two data collections.

Values in parentheses are calculated using data from the highest resolution shell (2.32–2.20 Å for the apoenzyme and 2.65–2.50 Å for FRC co-crystallized with CoA).

	Native crystal	CoA co-crystal
Data collection	ID14-1 (ESRF)	ID29 (ESRF)
Wavelength (nm)	0.933	0.979
Resolution (Å)	25–2.2	25–2.5
V_M (Å^3 Da^{-1})	3.26	3.26
Total observations	289009	209269
Unique reflections	56425	39007
Completeness (%)	99.0 (99.0)	99.7 (99.7)
Redundancy (%)	5.1 (4.6)	5.4 (3.1)
Average $I/\sigma(I)$	15.3 (4.9)	16.4 (4.2)
Wilson *B* factor (Å^2)	29.52	49.87
R_{sym} (%)	9.8 (23.6)	8.5 (21.9)

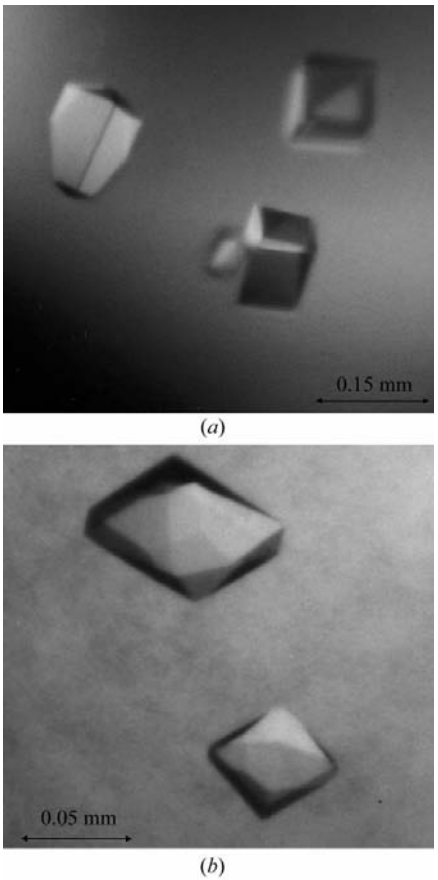

Figure 1
Crystals of formyl-CoA transferase crystallized (*a*) as the apoenzyme and (*b*) in presence of CoA.

2.3. X-ray crystallographic studies

All data were collected in a nitrogen stream at 110 K. The crystals were flash-frozen using the mother liquor as cryoprotectant solution.

Data for the apoenzyme and for FRC co-crystallized with CoA were collected on beamlines ID14-1 and ID29 equipped with an ADSC Quantum 4 at the European Synchrotron Radiation Facility (ESRF), Grenoble, France (Table 1). All data sets were processed using *MOSFLM*/*SCALA* (Collaborative Computational Project, Number 4, 1994) and *DENZO*/*SCALEPACK* (Otwinowski & Minor, 1996).

3. Results and discussion

Good diffraction was observed to 2.20 Å for native crystals. The crystals are tetragonal, belonging to the space group *I*4. The unit-cell parameters are $a = b = 151.44$, $c = 99.49$ Å, giving a unit-cell volume of 2.28×10^6 Å^3.

FRC alone and in the presence of coenzyme A crystallized in the same space group with the same unit-cell parameters.

The asymmetric unit contains two subunits with a total weight of 94 000 Da. The calculated solvent content of the crystals is 53% (Matthews, 1968).

The crystals of FRC obtained in the presence of coenzyme A were significantly smaller (0.1 × 0.05 × 0.1) than the apoenzyme crystals and their diffraction extended to 2.5 Å resolution.

A structure determination using selenomethionine-substituted enzyme for MAD phasing is presently under way.

CoA transferases, which are ubiquitous enzymes that catalyse the reversible transfer of coenzyme A from a donor to an acceptor, have traditionally been divided into two families. Enzymes belonging to family I function using a ping-pong mechanism involving enzyme-bound CoA as well as mixed substrate/product anhydrides (Heider, 2001), while family II carry out CoA transfer through a ternary complex intermediate (Heider, 2001). Recent sequence alignments (Elssner *et al.*, 2001) have, however, revealed the existence of a third family of CoA transferases to which FRC belongs (Heider, 2001). Most of the genes belonging to family III are present in anaerobic bacteria, but a few similar genes have also been found in archea and eukarya. Enzymes belonging to family III are involved in the anaerobic metabolism of toluene, carnitine, bile acids, in Stickland fermentation and in oxalate catabolism (Heider, 2001). These CoA transferases are active as homodimers or heterodimers, have a similar mass between 42 and 47 kDa and sequence comparison shows that some regions are quite conserved. No knowledge of the structure or catalytic mechanism of this class of CoA transferases is available.

We gratefully acknowledge the ESRF, Grenoble for provision of synchrotron radiation. We also thank Kjell Ericsson (Amersham Pharmacia Biotech), Harmeet Sidhu (Ixion Biotechnology, Inc.) and Ammon Peck (University of Florida) for information regarding FRC expression and purification. This work was supported by a grant to YL from the Swedish Research Council. We acknowledge partial support for SJ from the National Institutes of Health (DK53556).

References

Allison, M. J., Dawson, K. A., Mayberry, W. R. & Foss, J. G. (1985). *Arch. Microbiol.* **141**, 1–7.
Baetz, A. L. & Allison, M. J. (1989). *J. Bacteriol.* **171**, 2605–2608.
Baetz, A. L. & Allison, M. J. (1990). *J. Bacteriol.* **172**, 3537–3540.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Elssner, T., Engemann, C., Baumgart, K. & Kleber, H. P. (2001). *Biochemistry*, **40**, 11140–11148.
Hatch, M., Freel, R. W. & Vaziri, N. D. (1994). *Pfluegers Arch.* **426**, 101–109.
Heider, J. (2001). *FEBS Lett.* **509**, 345–349.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1996). *Methods Enzymol.* **276**, 307–326.
Rodby, R. A., Tyszka, T. S. & Williams, J. W. (1991). *Am. J. Med.* **90**, 498–504.
Ruan, Z. S., Anantharam, V., Crawford, I. T., Ambudkar, S. V., Rhee, S. Y., Allison, M. J. & Maloney, P. C. (1992). *J. Biol. Chem.* **267**, 10537–10543.
Sidhu, H., Hoppe, B., Hesse, A., Tenbrock, K., Brömme, S., Rietschel, E. & Peck, A. B. (1998). *Lancet*, **352**, 1026–1029.
Sidhu, H., Schmidt, M. E., Cornelius, J. G., Thamilselvan, S., Khan, S. R., Hesse, A. & Peck, A. B. (1999). *J. Am. Soc. Nephrol.* **10** *Suppl.* 14, S334–S340.
Trinchieri, A., Mandressi, A., Luongo, P., Longo, G. & Pisani, E. (1991). *Br. J. Urol.* **67**, 230–236.
Williams, H. E. & Smith, L. H. Jr (1968). *Am. J. Med.* **45**, 715–735.