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Crystallization and preliminary X-ray diffraction analysis of recombinant hydrolase domain of 10-formyltetrahydrofolate dehydrogenase

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10-Formyltetrahydrofolate dehydrogenase (FDH) is an abundant enzyme in liver cytosol. It is important for the regulation of 10-formyltetrahydrofolate/tetrahydrofolate pools, for *de novo* purine biosynthesis and for the removal of formate in the form of CO_2. The enzyme is a natural fusion of two unrelated genes and consists of two functional catalytic domains. Here, the crystallization of the N-terminal domain of FDH is reported. This domain binds folate and functions as a 10-formyltetrahydrofolate hydrolase. The crystals grow as either spear-shaped needles or large plates, with the largest crystals reaching dimensions of 1.2 × 0.2 × 0.05 mm. Diffraction analysis revealed the space group to be $P2_12_12$, with unit-cell parameters a = 100.00, b = 64.63, c = 64.59 Å. Based on the estimated solvent content, there is one 34 kDa molecule in the asymmetric unit. A native data set extending to 2.3 Å resolution has been collected with good merging statistics.

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1. Introduction

10-Formyltetrahydrofolate dehydrogenase (FDH) is a cytosolic enzyme that converts 10-formyltetrahydrofolate (10-formyl-THF) to tetrahydrofolate in an $NADP^+$-dependent dehydrogenase reaction (Cook *et al.*, 1991). An essential role for FDH is supported by its abundance in a number of tissues including liver (Krupenko & Oleinik, 2002), where it comprises about 1% of the total cytosolic protein (Kisliuk, 1999). One possible role for FDH is to channel excess 10-formyl-THF from the *de novo* purine pathway back to the THF pool, thus making folate available for other single-carbon reactions (Wagner, 1995). Another role might be to protect the cell from formate toxicity by removing formate in the form of CO_2 (Tephly, 1991).

FDH is a natural fusion of two unrelated genes and is comprised of two functional domains that are connected by a 100-residue linker (Cook *et al.*, 1991; Krupenko, Wagner & Cook, 1997*a*,*b*). The domain structure of the enzyme suggests a complicated catalytic mechanism. The binding site for folate is located in the N-terminal region of the enzyme (termed N_t-FDH, residues 1–310; Krupenko, Wagner & Cook, 1997*b*). This domain shows sequence similarity with folate-binding domains in other proteins such as methionyl-tRNA-formyltransferase (FMT) and glycinamide ribonucleotide formyltransferase (GART; Cook *et al.*, 1991; Krupenko, Wagner & Cook, 1997*b*). When expressed separately, it functions as a 10-formyl-THF hydrolase (Krupenko, Wagner & Cook, 1997*b*). The dehydrogenase reaction is catalyzed only by the full-length FDH (Krupenko, Wagner & Cook, 1997*a*,*b*), implying that the hydrolase reaction is a necessary component of the dehydrogenase mechanism. *In vitro*, the hydrolase reaction requires high concentrations of 2-mercaptoethanol (Krupenko *et al.*, 1995; Krupenko, Wagner & Cook, 1997*b*), making it unlikely that this reaction occurs *in vivo* without coupling to the overall dehydrogenase reaction of FDH. The molecular nature of this coupling, however, remains unclear. As a first step toward deciphering the catalytic mechanism of FDH, we are pursuing structural studies of this enzyme. In this paper, we report the crystallization of N_t-FDH and its preliminary analysis by X-ray diffraction.

2. Materials and methods

2.1. Protein expression and purification

N_t-FDH was expressed in insect cells using a baculovirus expression system as described previously (Krupenko, Wagner & Cook, 1997*b*). The protein was purified from culture medium by affinity chromatography on 5-formyltetrahydrofolate-Sepharose followed by chromatography on a Mono Q column using an FPLC system (Amersham-Pharmacia) (Krupenko, Wagner & Cook, 1997*b*). Further purification was performed using size-exclusion chromatography on a Sephacryl S-300 (Amersham-Pharmacia) column (1.5 × 100 cm) in 20 m*M* Tris–HCl buffer pH 7.5. All

solutions used in the purification steps contained 10 mM 2-mercaptoethanol. SDS–PAGE analysis (Laemmli, 1970) of the purified protein showed only a single band corresponding to the expected molecular weight of 34 kDa. The hydrolase activity of the purified recombinant enzyme was assayed as described previously (Krupenko, Wagner & Cook, 1997*b*).

2.2. Crystallization

Prior to crystallization trials, the protein was concentrated to about 10 mg ml^{-1} using Centricon concentrators (Millipore). The protein concentration was determined by the Bradford assay (Bradford, 1976). A search for crystallization conditions used the sitting-drop vapour-diffusion method, in which the drop volume was 5 µl (2.5 µl of protein solution and 2.5 µl of reservoir solution) and 100 µl of reservoir solution was added to the reservoir of Crystal Clear Strips (Hampton Research). In initial trials, the protein was screened against Crystal Screen and Crystal Screen II sparse-matrix kits (Hampton Research). Crystallization trials were performed at 296, 288 and 277 K.

2.3. X-ray diffraction analysis

The diffraction properties of the resulting crystals were analyzed using a Rigaku RU-H3R rotating-anode generator operating at 50 kV and 100 mA and fitted with Confocal Maxflux optics (Osmic Inc.). Data were recorded on an R-AXIS IV++ imaging-plate system in 0.5° oscillations with an exposure time of 5 min per frame. The crystal-to-image plate distance was 150 mm. A total of 105° of data were collected and these were scaled and reduced using *d*TREK* (Pflugrath, 1999).

3. Results

Initial screening with commercial kits revealed that recombinant N$_t$-FDH expressed in insect cells forms small crystal clusters in 2.0 M ammonium sulfate buffered in 0.1 M sodium acetate pH 4.6 or in 2.0 M ammonium dihydrogen phosphate buffered in 0.1 M Tris–HCl pH 8.5. The first of these conditions was optimized by varying the ammonium sulfate concentration, pH, temperature, protein concentration and use of additives. Temperature was found to be a critical factor for N$_t$-FDH crystallization: at 277 K crystals appeared within a few days, whereas no crystals were observed at 288 or 296 K. Furthermore, it was found that addition of glycerol to final concentrations of 2–10% improved the crystal habit by increasing the size and decreasing the amount of clustering. The best crystals formed over wells containing 1.2–1.4 M ammonium sulfate buffered in the pH range 4.9–5.1 and containing 5% glycerol. The optimal protein concentration was 10 mg ml^{-1}. Typical crystals were spear-shaped needles or plates, with the largest crystals reaching dimensions of 1.2 × 0.2 × 0.05 mm (Fig. 1).

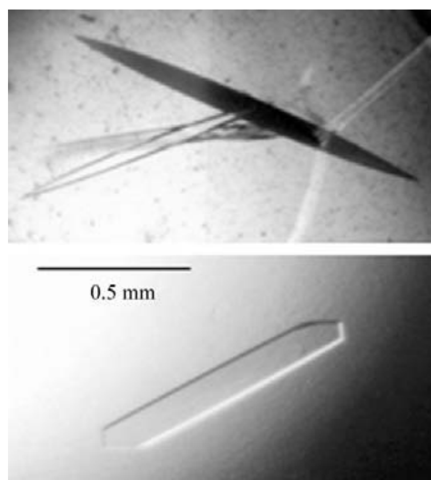

Figure 1
Typical crystals of N$_t$-FDH.

The crystals were passed through a cryoprotectant solution containing 1.3 M ammonium sulfate, 0.1 M sodium acetate, 27% glycerol and then flash-frozen *in situ* in the cryostream. Indexing of the resulting diffraction data revealed that the crystals were orthorhombic, with unit-cell parameters a = 100.00, b = 64.63, c = 64.59 Å. Assuming one 34 kDa molecule in the asymmetric unit, the crystal density V_M is 3.1 Å^3 Da^{-1}, which lies within the normal range for protein crystals (Matthews, 1969). A native data set extending to 2.3 Å resolution was collected which is 99.7% complete (100.0% in the outer shell, 2.38–2.30 Å). A total of 80 514 reflections were collected and these reduced to 19 201 independent reflections with an $R_{\rm merge}$ of 10.0% (34.6%) [where $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$]. Attempts to reduce the data in a tetragonal cell resulted in very high $R_{\rm merge}$ values, confirming that the cell, despite being close to tetragonal, was indeed orthorhombic.

We have also expressed N$_t$-FDH in *Escherichia coli* and purified the protein in the same manner as for the protein expressed in insect cells (Krupenko & Wagner, 1998). However, this protein formed only small crystal clusters using the conditions under which N$_t$-FDH expressed from insect cells formed single and large crystals, indicating some difference in the properties of the two proteins. The small crystals of N$_t$-FDH expressed in *E. coli* were not suitable for diffraction studies and since screening for new crystallization conditions was unsuccessful, the use of selenomethione as a vehicle for phasing is precluded. A search for suitable heavy-atom derivatives to solve the structure of the protein obtained by expression in insect cells by the method of multiple isomorphous replacement is under way.

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