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Cloning, purification, and crystallization of *Escherichia coli* cystathionine β-lyase

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Abstract **The *metC* gene coding for cystathionine β-lyase of *Escherichia coli* has been cloned and used to construct an overproducing *E. coli* strain. An efficient purification scheme has been developed and the purified enzyme has been crystallized by the hanging drop vapour diffusion method using either ammonium sulfate or polyethyleneglycol 400 as precipitating agent. The crystals belong to the orthorombic space group $C222_1$. Their unit cell parameters are $a = 60.9$ Å, $b = 154.7$ Å and $c = 152.7$ Å. Consideration of the possible values of V_M accounts for the presence of one dimer per asymmetric unit. The crystals are suitable for X-ray analysis and a complete native date set to 1.83 Å resolution has been collected using synchrotron radiation.**

Key words: Protein crystallization; X-Ray crystallography; Cystathionin β-lyase; Cystathionase; Methionine biosynthesis; Pyridoxal 5′-phosphate; *Escherichia coli*

1. Introduction

Cystathionine-β-lyase (CL; EC 4.4.1.8; also commonly refered to as β-cystathionase) catalyses the penultimate step in microbial and plant methionine biosynthesis, the pyridoxal 5′-phosphate (PLP) dependent cleavage of L-cystathionine to L-homocysteine, pyruvate, and ammonia. The *E. coli* enzyme is composed of four identical subunits of 395 amino acids (MW 43,032) each [1], encoded by the *metC* gene. Each subunit contains one molecule of PLP as a cofactor, transiently bound at the active site through a Schiff base with lysine 210 [2]. CL has been shown to share extensive homology with cystathionine γ-synthase, which catalyzes step two in methionine biosynthesis, i.e. formation of cystathionine from *O*-succinylhomoserine and cysteine, and a common evolutional origin of the two enzymes has been suggested [1,3].

PLP-dependent enzymes are used in all living organisms to synthesize, degrade and interconvert amino acids by a variety of different chemical reactions which include α-amino acid-keto acid and ω-amine-aldehyde transamination; α-, and β-amino acid decarboxylation; α-amino acid racemisation; β-hydroxy amino acid retro-aldol cleavage; α-amino acid deamination, and elimination and replacement reactions at C^{β} and C^{γ}. A comprehensive comparision of the amino acid sequences of PLP-dependent enzymes has shown that most of them can be assigned to one of three different families, termed the α-, β-, and γ-family, of homologous proteins [4]. Despite their important functions in metabolism and the host of reactions catalyzed, the three-dimensional structure of only a limited number of PLP-dependent enzymes has been determined yet. Most of them, i.e. aspartate aminotransferase [5], ω-amino acid:pyruvate aminotransferase [6], tyrosine phenol-lyase [7], and dialkylglycine decarboxylase [8], belong to the α-family and resemble each other very strongly in their three-dimensional structures [9]. D-Amino acid aminotransferase, which is only very distantly related to the members of the α-family, has a structural organisation completely different from those of any of the enzymes that utilize PLP [10]. Furthermore, the β-subunit of tryptophan synthase, which catalyzes the PLP-dependent part of the reaction, is a member of the β-family and has a tertiary structure [11] that bears no resemblance to that of the enzymes of the α-family.

In the present paper we report the crystallization of the first member of the γ-family of PLP-dependent enzymes, namely CL, and present preliminary crystallographic data.

2. Experimental

2.1. Gene cloning

The *metC* gene was cloned by PCR [12] from genomic DNA of *E. coli* K 12, using primers synthesized according to the published DNA sequence [1], but with an extra *Bam*HI site at the 5′-ends (Primer 1: 5′-TAC TCA GGA TCC ATG GCG GAC AAA AAG CTT GAT ACT CAA CTG G-3′; Primer 2: 5′-GCG TGA GGA TCC TTA TAC AAT TCG CGC AAA ACC GGC-3′; *Bam*HI-sites and start-codon underlined). The 1,1 kb DNA-fragment was isolated after *Bam*HI treatment by preparative agarose gel electrophoresis and subsequently ligated into the *Bam*HI-site of the *tac*-promoter vector pDR540. Transformation of *E. coli* DH5 gave *E. coli* pCS1 containing the *metC* gene under the transcriptional control of the *tac*-promoter.

2.2. Protein purification

CL activity was assayed as described previously [13]. Protein was determined by the method of Bradford [14] with bovine serum albumin as the standard.

E. coli pCS1 was grown at 37°C in 10 l of LB-medium [15] containing ampicillin (100 μg/ml). At $OD_{600} = 0.8$ the cells were induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside, cultivated for 8 hours, and harvested by centrifugation. The following steps were done at 4°C using Pharmacia FPLC equipment. The bacteria were resuspended in buffer A (50 mM KP_i, pH 7.0, 1 mM EDTA, 10 μM PLP), disrupted (IMA-Disintegrator S glass-bead disintegrator), and subsequently subjected to $(NH_4)_2SO_4$ (70% saturation) precipitation. Pro-

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Abbreviations: CL, cystathionin β-lyase; FPLC, fast performance liquid chromatography; MES, 2-(*N*-morpholino)propanesulfonic acid; PCR, polymerase chain reaction; PEG, polyethylenglycol; PLP, pyridoxal 5′-phosphate; V_M, Matthews-parameter (protein volume per mass unit in the crystal).

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Table 1
Crystallisation conditions and cell parameters

Crystal type	Space group	Cell (Å)	V_M† (Å³/Da)	N_{au}‡	d§ (Å)	Crystallisation conditions
1	$C222_1$	a = 61.03 b = 154.93 c = 153.19	2.1	2	2.7	Saturated ammonium sulfate (75%), 50 mM MES/KOH, pH 5.1, 10 μM PLP*
2	$C222_1$	a = 60.91 b = 154.75 c = 152.70	2.1	2	1.83	10% PEG 400, 0.15 M Ca_2Cl, 100 mM HEPES, pH 7.2, 10 μM PLP*

†V_M; the volume of the unit cell divided by total molecular weight of the protein in the unit cell [16].
‡N_{au}, number of monomeric molecules per asymmetric unit.
§d, resolution limit.
*Concentration in the reservoir. Crystals were grown by mixing 5 μl reservoir with 5 μl protein solution.

teins were redissolved in buffer A, desalted (Sephadex G-25 column), applied to a DEAE-Sepharose Fast Flow column, and eluted with a linear gradient of 0 to 1 M KCl. Active fractions were made 1 M in $(NH_4)_2SO_4$, applied to a Phenyl-Sepharose HP column equilibrated with buffer A containing 1 M $(NH_4)_2SO_4$, and eluted with a linear gradient from 1 to 0 M $(NH_4)_2SO_4$. Active fractions were desalted (Pharmacia PD-10 column; buffer A), loaded on an Amicon Blue A column and eluted with a linear gradient from 0 to 1 M KCl in buffer A.

2.3. Crystallization and X-ray diffraction analysis

For crystallisation experiments the purified CL (12 mg/ml) was extensively dialysed against 50 mM KP_i, pH 7.0, 10 μM PLP. All crystals were grown at room temperature by the hanging drop vapor diffusion method using Linbro multiwell tissue culture trays. Sodium azide (0.02%) was added to all solutions to prevent microbial growth.

Data were collected to 2.2 Å using a 180 mm MAR-Research image plate detector attached to a Rigaku RU 200 rotating Cu-anode X-ray generator and subsequently, synchroton diffraction data were collected to 1.8 Å resolution using the HASYLAB BW6 beamline at the DORIS storage ring, DESY, Hamburg.

3. Results and discussion

To obtain large amounts of *E. coli* CL we have cloned the gene coding for CL (*metC*) and constructed an overproducing *E. coli* strain, which contained the *metC* gene under control of the strong, inducible *tac*-promoter. Starting with recombinant bacteria from 10 l culture medium about 150 mg of electrophoretically homogenous CL are typically obtained by the four-step purification procedure. The purified enzyme shows an UV-visible spectrum typically for PLP-dependent enzyme with an absorbance maximum at 426 nm beside the protein absorption at 280 nm and an E_{280}/E_{426} ratio between 6.7 and 7.7.

Fig. 1. Photomocrograph of a typical *type 2* crystal of cystathionine β-lyase. Crystal dimensions are approx. 0.3 × 0.3 × 0.9 mm.

Crystals of native CL have been obtained using the hanging drop vapour diffusion method. A summary of the crystallization experiments is given in Table 1. Crystals of *type 1* were grown by mixing 5 μl of dialysed enzyme solution with 5 μl of 50 mM MES/KOH, pH 5.1, 10 μM PLP in 75% saturated $(NH_4)_2SO_4$ (buffer B) and equilibrated for 10 days against a well containing 1 ml of buffer B. The crystals of *type 2* (Fig. 1) were grown in the presence of different divalent cations in several hours or days depending on pH and PEG 400 concentration. The best crystals with dimensions of 0.3 × 0.3 × 0.9 mm were obtained in two days under conditions listed in Table 1. The crystals of *type 2*, which belong to the orthorombic space group $C222_1$, were used for data collection. Their unit cell parameters are a = 60.9 Å, b = 154.7 Å and c = 152.7 Å, which corresponds to a unit cell volume of 1.41×10^6 Å³. The volume per unit mass (V_M), calculated with the assumption that there is one dimer (M_r 86,000 Da) in the asymmetric unit is 2.1, which is in the range of 1.6–3.6 Å³/Da found in typical protein crystals [16]. Table 2 provides the statistics of the data collection and evaluation.

Structure analysis by crystallographic methods is in progress and some heavy atom derivatives have already been found and analysed. Thus, these crystals should allow the determination of the first high-resolution structure of a member of the γ-family of PLP-dependent enzymes.

Table 2
Data collection statistics

Temperature	Room temperature
Resolution (Å)	1.83
Number of measured reflections	98,783
Number of unique reflections	58,549
R_{merge}*	4.2%
Completeness	
overall (20.3–1.83 Å)	92.55%
last shell (1.91–1.83 Å)	95.28%

*$R_{merge} = \Sigma\Sigma|I(h)i - <I(h)>|/\Sigma <I(h)>$; $I(h)i$ is the observed intensity of the ith measurement of reflection h, and $<I(h)>$ the mean intensity of reflection h, calculated after loading, scaling and merging of Friedel pairs.

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