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FOR THE RECORD

Purification and preliminary X-ray crystallographic studies of recombinant L-ribulose-5-phosphate 4-epimerase from *Escherichia coli*

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Abstract: The *araD* gene from *Escherichia coli*, coding for L-ribulose-5-phosphate 4-epimerase, was overexpressed and the resulting enzyme was purified to homogeneity. Crystals of L-ribulose-5-phosphate 4-epimerase, obtained with 4.0 M sodium formate as precipitant, belong to space group $P42_12$ with unit cell dimensions $a = b = 107.8$ Å and $c = 281.4$ Å and diffract to at least 2.2 Å resolution. Density measurements of these crystals are consistent with eight subunits in the asymmetric unit.

Keywords: *araBAD* operon; crystallization; L-ribulose-5-phosphate 4-epimerase; X-ray crystallography

Some bacteria can utilize L-arabinose as carbon source by converting L-arabinose into D-xylulose-5-phosphate, a metabolic intermediate of the pentose phosphate pathway. The necessary set of enzymes for the L-arabinose pathway—L-arabinose isomerase, L-ribulokinase, and L-ribulose-5-phosphate 4-epimerase—is encoded by the *araBAD* operon. The nucleotide sequence of the whole *araBAD* operon from *Escherichia coli* has been determined (Lee et al., 1986).

L-Ribulose-5-phosphate 4-epimerase from *E. coli* has a molecular weight of 100 kDa (Lee et al., 1968). The enzyme subunit consists of 231 amino acids (Lee et al., 1986), and four of these subunits assemble to form the holoenzyme. The enzyme does not require the addition of any cofactors for catalytic activity (Lee et al., 1968). L-Ribulose-5-phosphate 4-epimerase catalyzes the last step in the L-arabinose pathway, epimerization at the C4 carbon atom of L-ribulose-5-phosphate, resulting in D-xylulose-5-phosphate.

Metabolically unrelated to L-ribulose-5-phosphate 4-epimerase is the enzyme D-ribulose-5-phosphate 3-epimerase, found in the pentose phosphate pathway. This epimerase converts D-ribulose-5-phosphate into D-xylulose-5-phosphate. The two enzymes represent an interesting case of stereospecific recognition by converting two stereoisomers of the substrate, D- and L-ribulose-5-phosphate, respectively, into the same product, D-xylulose-5-phosphate. The molecular basis for the substrate specificity of these epimerases is of considerable interest for the design of useful enzymes in stereospecific organic synthesis. We have therefore initiated crystallographic studies of these enzymes to reveal the structural basis for this difference in substrate specificity. In this paper, we report on purification and preliminary crystallographic studies of recombinant L-ribulose-5-phosphate 4-epimerase.

Expression and purification: The *araD* gene (Mineno et al., 1990) was amplified by sticky end PCR (Saiki et al., 1985; Scharf et al., 1986) with *Taq* polymerase (STRATAGEN) using the following oligonucleotides, 5′-TTCGCGGGTACCCACGAAGGAGTCAACATGTTAG-3′ and 5′-GCAAACTTCGAATGTGGTTTTATACAGTCATTAC-3′. The PCR product was digested with *Kpn* I and *Hin*d III (Pharmacia; underlined sites in the primer sequences) and ligated into the pUC18 vector (Pharmacia) digested with the same restriction enzymes, resulting in the plasmid pAA1. The nucleotide sequence of the insert was checked by dideoxy sequencing (Sanger et al., 1977).

The *E. coli* strain JM109 was transformed with pAA1 and these cells were grown for 5 h in 4,000 mL 2xYT medium. Expression was induced when cells reached log phase by addition of 960 mg/L isopropylthio-β-D-galactoside. After 5.5 h the cells were harvested by centrifugation. The pellet (14.6 g) was suspended in 50 mL of 50 mM Tris, pH 8.1, and the suspension was sonicated at 10-s intervals for a total of 1 min. The broken cells were centrifuged and $MnCl_2$ was added to the supernatant to a final concentration of 50 mM. After centrifugation, solid ammonium sulfate was added to the supernatant to 40% saturation and pH was kept at 7.6 with 1 M KOH. This solution was centrifuged for 20 min at 15,000 rpm and 4 °C. The pellet was resuspended in a minimum volume of 10 mM potassium phosphate buffer, pH 7.6, and then dialyzed overnight against the same buffer, which was changed three times during dialysis. The material was applied to a Mono Q HR10/10 anion-exchange col-

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umn (Pharmacia) and bound protein was eluted with a NaCl gradient (0–0.5 M, 50 mM Tris, pH 8.0). Active fractions were pooled and the protein was flash frozen in liquid N_2 and stored at −80 °C. A Coomassie blue-stained SDS-polyacrylamide gel (Fig. 1) showed that the purified enzyme was electrophoretically pure. The yield was estimated to be about 30% of the total amount of protein and from 4,000 mL medium, 180 mg pure recombinant L-ribulose-5-phosphate 4-epimerase were isolated.

Amino acid sequencing (Applied Biosystems 477A protein sequencer) of the recombinant L-ribulose-5-phosphate 4-epimerase showed that the first eight amino acid residues were identical to the amino acids predicted by the DNA sequence. The specific activity of the purified enzyme was 17.3 U/mg (1 U = 1 μmol D-xylulose-5-phosphate formed per minute) as determined using a modified spectrophotometric method previously described (Wood, 1979). This value is slightly higher than that obtained for the enzyme from *Aerobacter aerogenes* 12.5 U/mg (Wolin et al., 1958) and similar to the value obtained for the enzyme from *Lactobacillus plantarum* 16.5 U/mg (Burma & Horecker, 1958).

Crystallization and preliminary crystallographic analysis: Crystals were grown using the hanging drop vapor diffusion technique, by mixing equal volumes (3 μL) of protein solution (15 mg/mL in water) and well solution. Initially, 48 different conditions for crystallization (Jancarik & Kim, 1991) were tested at 4 °C and at 25 °C. Crystals were obtained with 4.0 M sodium formate in the presence of 0–0.1% β-octyl glucoside and had a size of 0.4 × 0.4 × 0.5 mm (Fig. 2). The addition of β-octyl glucoside gave fewer and bigger crystals.

Space group and cell dimensions were determined from precession photographs recorded with a Huber camera mounted on a Rigaku rotating anode running at 50 kV and 90 mA. The crystals are tetragonal, space group $P42_12$, with cell dimensions $a = b = 107.8$ Å, $c = 281.4$ Å.

The crystal density was measured using a modified gradient method previously described (Bode & Schirmer, 1985) and was consistent with eight polypeptide chains in the asymmetric unit. This number of subunits per asymmetric unit corresponds to a packing density of 2.0 Å^3/Da (Matthews, 1968).

A native data set to 2.5 Å resolution was collected at station 9.6 at SRS Daresbury on a Raxis imaging plate detector in oscillations of 1.2°. The wavelength used was 0.89 Å. Data frames were processed using Denzo (Otwinowski, 1993) and the CCP4 program suite (Collaborative Computational Project Number 4, 1994) was used for all further processing. This data set was 94.7% complete, with an R_{merge} of 8.1%.

The native Patterson function, calculated from data in the resolution range 5–20 Å showed one significant peak (7.3% of the origin peak, 10σ) at fractional coordinates 0.5, 0.5, 0.043, indicating that some subunits are related by this translation.

Using the program AMORE (Navaza, 1992), a self-rotation function was calculated using different resolution shells and varying the radius of integration. One significant peak (71% of origin, 13σ) with Eulerian angles $\alpha = 0°$, $\beta = 180°$, $\gamma = 50.5°$ was found, which corresponds to a rotation around an axis parallel to the crystallographic fourfold axis.

In conclusion, the crystals are suited for a three-dimensional structure determination by protein crystallography, and a search for heavy atom derivatives is well under way.

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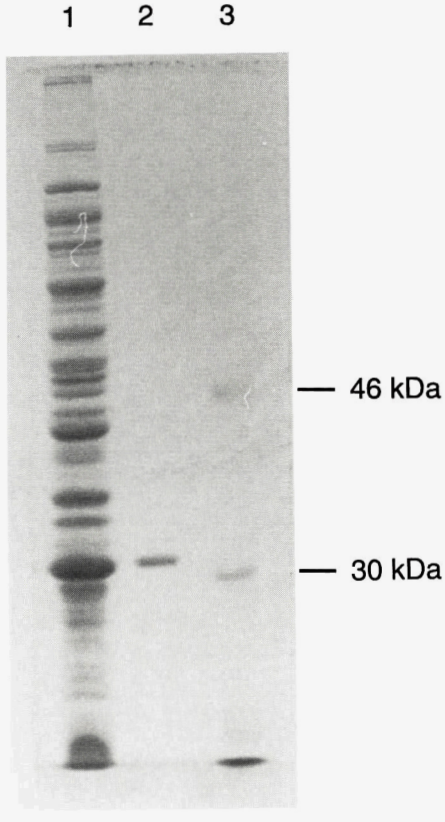

Fig. 1. 12% SDS-PAGE of crude cell extract (lane 1), purified enzyme (lane 2), and low molecular weight markers (lane 3).

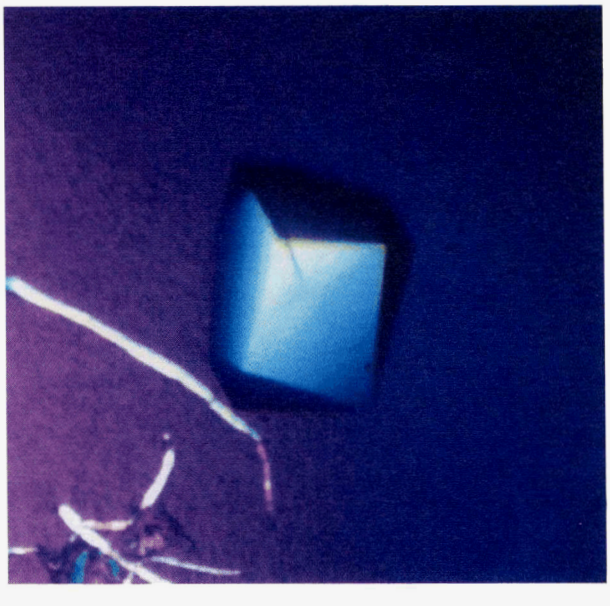

Fig. 2. Crystal of L-ribulose-5-phosphate 4-epimerase.

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