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Preliminary Crystallographic Study of Cyclohexadienyl Dehydratase from *Pseudomonas aeruginosa*

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Single crystals of cyclohexadienyl dehydratase from *Pseudomonas aeruginosa* have been obtained by vapour diffusion from ammonium sulphate solution (pH 6·0) at 4°C. The crystals belong to the tetragonal space group $P4_32_12$ or $P4_12_12$ with $a=b=105{\cdot}5$ Å and $c=165{\cdot}0$ Å. The asymmetric unit contains at least one dimeric protein molecule with $M_r=72$ kDa. The crystals diffract to 3 Å resolution and are suitable for an X-ray analysis.

Keywords: cyclohexadienyl dehydratase; *Pseudomonas aeruginosa*; purification; crystallization; X-ray analysis

The presence of dual pathways for phenylalanine biosynthesis, first discovered in *Pseudomonas aeruginosa* (Patel *et al.*, 1977), is now known to be typical of the entire phylogenetic group of Gram-negative bacteria denoted as superfamily B (Jensen, 1985). In one of the pathways, chorismate is converted to phenylpyruvate by the bifunctional P-protein which possesses two catalytic domains: chorismate mutase and prephenate dehydratase (Fig. 1). Phenylpyruvate is then transaminated to L-phenyl-alanine. A monofunctional enzyme of chorismate mutase generates molecules of prephenate that have alternative fates of transamination to L-arogenate catalysed by prephenate aminotransferase or of conversion to phenylpyruvate *via* cyclohexadienyl dehydratase (CDT‡). CDT can also convert L-arogenate to L-phenylalanine. The CDT-catalysed reactions (prephenate dehydratase and arogenate dehydratase) are analogous in being decarboxylative dehydrations in which aromatization of a cyclohexadienyl substrate occurs. Thus, in contrast to the P-protein, CDT is a monofunctional enzyme with broad substrate specificity. Both monofunctional chorismate mutase and CDT are unrestrained by allosteric control leading to the overflow character of the pathway.

Pseudomonas aeruginosa CDT exists as a homodimer with a molecular mass of 72 kDa. The gene encoding the protein has been cloned and expressed in *Escherichia coli* and the nucleotide sequence has been determined (Zhao *et al.*, 1992). In order to determine the three-dimensional structure of CDT we obtained crystals of the protein suitable for an X-ray analysis.

The cloned CDT was purified as described by Zhao *et al.* (1992). SDS/polyacrylamide gel electrophoresis using silver staining showed only one band. The protein has been crystallized by vapour diffusion from 40 to 45% ammonium sulphate solution in 0·1 M Mes buffer with 1 mM dithiothreitol and 3% (v/v) 2-methyl-2,4-pentanediol (pH 6·0) at 4°C. Within two weeks the crystals reached a size of 0·2 mm. All attempts to grow larger crystals using macroseeding were unsuccessful, possibly because of the microheterogeneity of the protein. Isoelectric focusing (Fig 2) indicated several bands which could be separated by FPLC (Pharmacia). The protein solution was applied to a Mono-Q HR 5/5 column equilibrated in 10 mM phosphate buffer (pH 7·0) at a flow rate of 0·5 ml/min. The column was washed using a linear gradient from 0 to 0·14 M NaCl in the same buffer (total volume 15 ml). The enzyme was

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‡ Abbreviation: CDT, cyclohexadienyl dehydratase.

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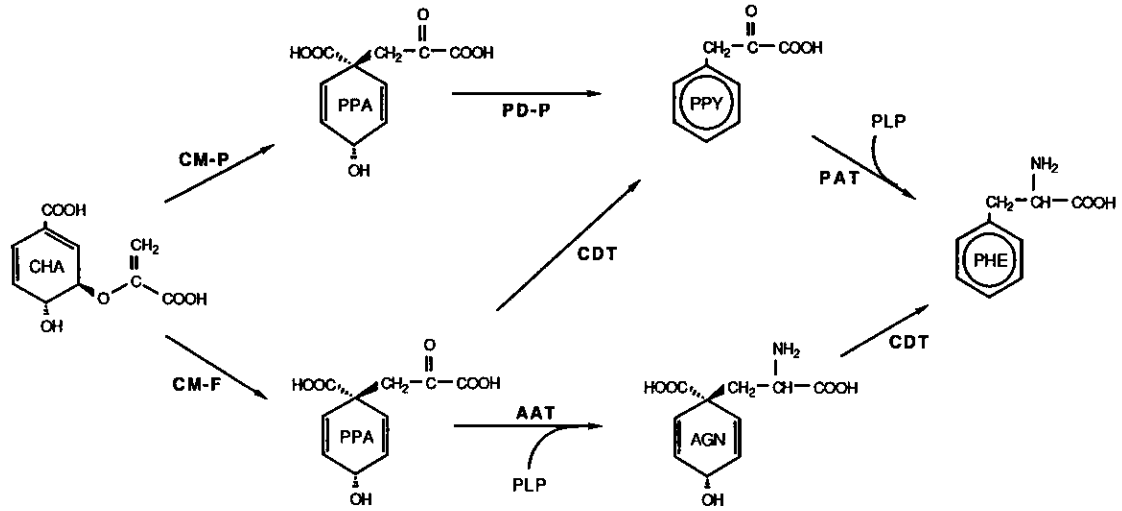

Figure 1. Dual biosynthetic routes to L-phenylalanine in *Pseudomonas aeruginosa*. Abbreviations: CM-P and PD-P are chorismate mutase and prephenate dehydratase domains of the bifunctional P-protein, respectively; CM-F, monofunctional chorismate mutase; CDT, cyclohexadienyl dehydratase; PAT, phenylpyruvate aminotransferase; AAT, L-arogenate aminotransferase; PLP, pyridoxal-5′-phosphate; CHA, chorismate; PPA, prephenate; PPY, phenylpyruvate; PHE, L-phenylalanine; AGN, L-arogenate.

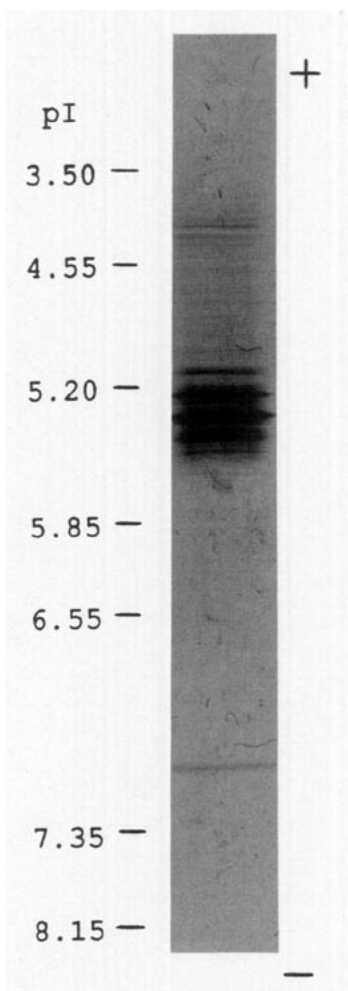

Figure 2. Isoelectric focusing of CDT in a polyacrylamide gel at pH 3 to 9.

eluted in 0·14 M NaCl (Fig. 3). One of the two main fractions used for macoseeding resulted in large well-shaped isometric crystals of 0·5 mm size.

The crystals diffract to 3·0 Å resolution on a synchrotron X-ray source and are stable for at least 12 hours. They belong to the tetragonal space group $P4_32_12$ or $P4_12_12$ with unit cell parameters: $a=b=105{\cdot}5$ Å, $c=165{\cdot}0$ Å. Assuming a dimer of CDT in the asymmetric part, a solvent content of 61% can be calculated ($V_m=3{\cdot}2$ Å^3/Da). Diffraction data were collected from one of these crystals using synchrotron beam line X31 (EMBL, Hamburg) and a MAR Research imaging plate scanner. The data contains 19,146 unique reflections, 99% complete to 3·0 Å resolution. A search for heavy-atom derivatives is in progress.

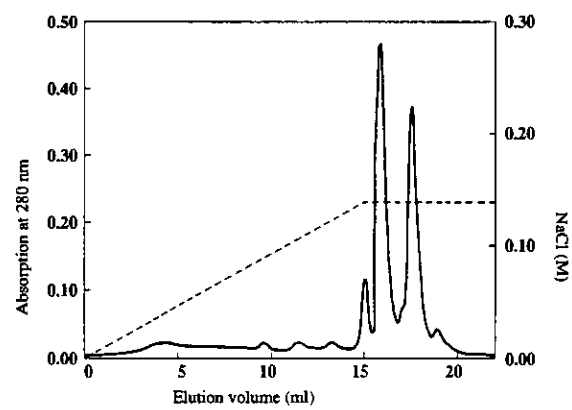

Figure 3. The FPLC elution profile. Absorption at 280 nm is shown as a continuous line; NaCl concentration is shown as a broken line. The flow rate was 0·5 ml/min.

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