s8a.m1.p29 **Crystal Structure of *Pseudomonas fluorescens* 4-hydroxyphenyl-pyruvate dioxygenase involved in the degrading pathway of tyrosine.**
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In plants as well as in photosynthetic bacteria, tyrosine degradation is a crucial pathway since homogentisate, a tyrosine degradation product, is a precursor for biosynthesis of photosynthetic pigments. The homogentisate biosynthesis includes a decarboxylation, a dioxygenation and a rearrangement of the pyruvate side chain. That complex reaction is carried out by one single enzyme, the 4-hydroxyphenyl-pyruvate dioxygenase (HPPD), a non-heme-iron-dependent enzyme active as a homotetramer in bacteria and as a homodimer in plants. Moreover, in humans, a HPPD deficiency is found related to tyrosinemia, a rare hereditary disorder of the tyrosine catabolism.

The crystal structure of Pseudomonas fluorescens HPPD has been refined at 2.4 Å resolution (R-free: 27.6%, R-factor: 21.9%)[1]. The general topology of the protein consists of two barrel-shaped domains and is similar to the structures of two extradiol ring cleavage dioxygenases: Pseudomonas 2,3-dihydroxybiphenyl dioxygenase [3,4] and Pseudomonas putida catechol 2,3-dioxygenase [2]. Each structural domain contains two repeated ?????? modules. There is one non-heme iron atom per monomer liganded to the side chains of H161, H240, E322 and one acetate molecule.

The analysis of the HPPD structure and its superposition with the structures of the 2,3-dihydroxybiphenyl dioxygenase and catechol 2,3-dioxygenase highlight some important differences in the active site of these enzymes which could be correlated to their specific activities. These comparisons suggest also that the pyruvate part of 4-hydroxyphenyl-pyruvate and the O_2 molecule would occupy the three free coordination sites of the catalytic iron atom.

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s8a.m1.p30 **The structure and function of a proficient enzyme: orotidine 5'-monophosphate decarboxylase**
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Orotidine 5'-monophosphate decarboxylase (ODCase) catalyses the decarboxylation of orotidine 5'-monohosphate to uridine 5'-monophosphate (UMP) the last step in the *de novo* synthesis of UMP. ODCase enhances the rate of reaction by a factor of 10^{17}[1] at ambient temperatures. Considering the extreme proficiency, the mechanism of ODCase is unique, because no cofactor is required for the decarboxylation.

We have determined the structure of the ODCase from *E. coli* crystallised with and without the inhibitor 1-(5'-phospho-β-D-ribofuranosyl)barbituric acid (BMP)[2]. The structure of the ODCase:BMP complex was solved from a 3.0 Å MAD data set collected at beam-line BM14 at the ESRF. A change in symmetry was seen in the crystals when methionine was substituted with selenomethionine - additional weak diffraction spots was observed. By ignoring these additional spots the structure was solved and it was refined to 2.5 Å using a data set collected on BL-7-11 at MAXLAB on the native ODCase:BMP crystals. The structure solution of the uncomplexed enzyme was hampered by twinned crystals. Several data sets have been collected both at BW7B at the EMBL outstation in Hamburg and at BL-7-11 at MAXLAB. We have, finally, succeeded in solving and refining this crystal form to 2.5 Å.

ODCase folds as an α/β-barrel. The ODCase:BMP complex is a closely packed homodimer with a BMP molecule tightly bound in the C-terminal end of each barrel. The structural results showed that all previously suggested catalytic mechanisms are unlikely. From the position of the highly converved residues in the uncomplexed and complexed enzymes, respectively, we have suggested a mechanism that does not involve large conformational changes in the active site.

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