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ESRF

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Time resolved crystallographic studies on dethiobiotin synthetase	LS 968

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Report:

In previous experiments at the ESRF, we had been able to trap one of the catalytic intermediates in the reaction catalyzed by dethiobiotin synthase (see Käck et al. (1998) PNAS 95, 5495-5500). The planned experiment aimed at trapping the next catalytic intermediate in the reaction. However we had not yet been able to identify the conditions under which this intermediate accumulates in sufficient amounts in the crystal. After discussions with Dr. A. Thompson and subsequent approval by the safety group, we switched the project to a MAD experiment aiming at the structure determination of diaminopelargonic acid synthase. This vitamin B_6 dependent enzyme is found in the same metabolic pathway as dethiobiotin synthase. The experiment was very successful and resulted in a beautiful electron density map. The structure has been refined to 1.9 Å resolution and a manuscript is in preparation.

Multiple anomalous difference data was collected on a ETMS soaked crystal with cell dimensions a=58.50 Å, b=56.63 Å, c=121.7 Å and =96.14°, space group $P2_1$.

Data was collected at three different wavelengths, λ1 (peak) = 0.9918 Å, λ2 (inflection point) = 1.007 Å and λ3 (high energy remote) = 0.8265 Å to 2.0 Å resolution. Eight mercury sites were identified by visual inspection of the difference Patterson maps and using difference Fourier methods.

A map calculated after refinement of the parameters for the mercury ions was of excellent quality and backbone tracing and sequence assignment was straighforward for the whole protein. The protein model has been refined to a crystallographic R-value of 17.5% (R-free 22.6%).

The enzyme subunit consists of two domains, a large domain (residues 50-329) containing a seven stranded predominantly parallel β-sheet, surrounded by α-helices. The small domain comprises residues 1-49 and 330-429. Two subunits are related by a non-crystallographic diad and form the homodimeric molecule. The cofactor pyridoxal-5-phosphate is bound in a cleft formed by the small domain from one subunit and the large domain of the second subunit. The cofactor is anchored to the enzyme by a covalent linkage to the side chain of the invariant residue Lys274. The phosphate group interacts with main-chain nitrogen atoms and the side chain of Ser113, located at the N-terminus of an α-helix. The pyridine nitrogen forms a hydrogen bond to the side chain of the invariant residue Asp245. Electron density corresponding to a metal ion, most likely a monovalent Na^+ ion, was found in a tight turn at the surface of the enzyme. The structure analysis reveals that diaminopelargonic acid synthase belongs to the family of vitamin B_6 dependent aminotransferases with the same fold as originally observed in asparatate aminotransferase. A multiple structure alignment of enzymes in this family revealed that they form at least six different subfamilies. Striking differerences in the fold of the N-terminal part of the polypeptide chain are one of the hallmarks of these subclasses.