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Report:

Dethiobiotin synthetase converts 7,8-diaminopelargonic acid (DAPA) to dethiobiotin in a reaction where Mg-ATP and carbondioxide are used as additional substrates. The reaction occurs in at least four distinct steps over three reaction intermediates (1,3); DAPA carbamate, a mixed carbamic phosphoric anhydride and a tetrahedal intermediate.

Our experiments, which have been done with the ambition to crystallographically capture these intermediate have given us pictures of the first two reaction steps. The first intermediate, N7-DAPA carbamate, which can be seen using conventional methods as it does not demand presence of ATP has earlier been reported (2,4), while we during our experiments at BL3, ID9 have managed to capture the second reaction intermediate, the carbamic phosphoric anbydride.

The experiments were all carried out using a monochromatic beam (0.899 Å) and

the CCD detector at the beamline. The data which was collected to 1.8 A was of reasonable quality with R_{merge} ranging from 4 to 6 %. A general strategy followed in all experiments included reaction initiation followed by a time delay to allow for the reaction to proceed. The crystal was subsequently mounted in a cryo stream at 100 K and data collected. The time delay varied from a few seconds up to 40 minutes.

The reaction initiation which in this experiment is the crucial step has been done in three different ways :

1. Soaking of substrates for a few hours at a pH where the enzyme is inactive (pH 4.9, the reaction was then started by transferring the crystal to a solution with a pH in which the protein is .
2. A different approach is to use caged ATP, a non hydrolysable ATP analogue which is activated, as the caging dinitrophenyl group is cleaved off by photolysis upon exposure to a laser pulse at 350 nm. Crystals were soaked with this compound together with the other substrates and later exposed to a series of laser pulses (4-5 mJ/pulse) providing a totally energy input of 20-25 mJ to release ATP and induce the reaction.
3. A third way of reaction initiation used was by diffusion of carbondioxide into crystals that had been grown and soaked with ATP and DAPA in the absence of carbondioxide.

The result of experiments (1) and (2) are yet ambiguous, however using approach (3) we have been able to trap one of the catalytic intermediates, the mixed carbamic phosphoric anhydride.

This is the first direct observation of such a high energy intermediate in an enzymatic reaction. It is of general biological significance, since the generation of phosphorylated intermediates in enzymatic reactions is a strategy used by a large number of different enzymes.

References :
1. Huang et al (1994), Structure 2,407-414
2. Huang et al (1995), Biochemistry 34,10985-10995
3. Gibson et al (1995), Biochemistry 34, 10976-10984
4. Alexeev et al (1995), Structure 3,1207-1215