ESRF	**Experiment title:** Time resolved studies of an ATP-dependent carboxylase : dethiobiotin synthetase	**Experiment number:** LS834
Beamline: ID09, BM2	**Date of experiment:** 971016-971019 (ID09), 971213-971214(BM2)	**Date of report:** 980902
Shifts: 9(ID9) 5(BM2)	**Local contact(s):** Michael Wulff, Richard Kahn	*Received at ESRF:*

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Report:

Results from data collection on this project at the ESRF have given rise to the following publication : Kack H, Gibson KJ, Lindqvist Y and Schneider G, Proc. Natl. Acad. Sci. USA 95:5495-5500, 1998

Abstract: The ATP-dependent enzyme dethiobiotin synthetase from *Escherichia coli* catalyses the formation of dethiobiotin from CO2 and 7,8-diaminopelargonic acid. The reaction is initiated by the formation of a carbamate and proceeds through a phosphorylated intermediate, a mixed carbamic phosphoric anhydride. Here, we report the crystal structures at 1.9- and 1.6-Å resolution, respectively, of the enzyme-MgATP-diaminopelargonic acid and enzyme-MgADP-carbamic-phosphoric acid anhydride complexes, observed by kinetic crystallography. Reaction initiation by addition of either NaHC03 or diaminopelargonic acid to crystals already containing co-substrates resulted in the accumulation of the phosphorylated intermediate at the active site. The phosphory1 transfer step shows inversion of the configuration at the phosphorous atom, consistent with an in-line attack by carbamate oxygen onto the phosphorous atom of ATP. A key feature in the structure of the complex of the enzyme with reaction intermediate is two magnesium ions, bridging the phosphates at the cleavage site. These magnesium ions

compensate the negative charges at both phosphate groups after phosphoryl transfer and contribute to the stabilisation of the reaction intermediate.

Following these successful experiments attempts have been made to capture a second postulated intermediate in the reaction pathway of dethiobiotin synthetase. The phosphorylated intermediate breaks down into the products, dethiobiotin and inorganic phosphate in a step that most likely goes through a tetrahedral intermediate. Although breakdown of the carbamic-phosphoric acid anhydride is the rate-limiting step at physiological conditions in solution, changes in pH and temperature can result in an accumulation of an intermediate at a later stage of the reaction pathway.

The experimental approach to trap this intermediate by cryo crystallography were as follows: (1) Crystallised dethiobiotin synthetase was supplemented with substrates in a manner so that the phosphorylated intermediate accumulates at the active site (se reference above). (2) Conditions were changed to those appropriate for accumulation of the second intermediate (pH 9.0 at 4 °C). (3) At various times after pH and temperature jump a crystal was flash frozen in a nitrogen stream at 100 K and data was collected using a CCD camera to 2 Å resolution (limited by detector size).

The maps calculated from these data were of good quality. The protein chain is well defined and continuous. In the active site ADP is clearly visible, so is the intermediate/product-phosphate which probably is firmly kept in place after the phosphoryl transfer step by a number of interactions. The carbon tail of the substrate/product is also reasonably well defined. Unfortunately the maps are not very informative about the reactive part of the substrate/intermediate molecule. Probably the kinetics for protein in solution is not representative for the crystallised protein in this case, so the electron density maps represent a mixture of several catalytic states. There is no spectroscopic signal which can be followed in this reaction system, to confirm the reaction coordinates for a particular data set. These have to be judged based on only the resulting electron density maps. In a case like this, when the density is not well determined the interpretation is ambiguous. To date we do not want to draw conclusions based on these experiments.

At ID09, 4-5 shifts were used to adjust the experimental setup.