UNIVERSITY OF Southampton
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TDH - an Enzyme Involved in Metabolising Threonine to Glycine

A novel threonine dehydrogenase from the hyperthermophile Thermococcus kodakaraensis

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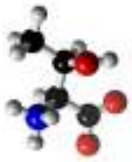
The preliminary structure of L-threonine dehydrogenase has been solved to 2.4Å by X-ray crystallography, which should allow us to hypothesise how this enzyme functions as a biological catalyst at the molecular level.

What does TDH do?

Although amino acids are essential for cellular growth, repair, and maintenance, some higher organisms are unable to synthesise all the ones they need. These must therefore be absorbed through their diet.



Threonine is an essential amino acid. It is synthesised by plants and microorganisms and can be metabolised to glycine.

L-threonine is an indispensable amino acid and under normal conditions is synthesised by microbes from oxaloacetate. Threonine degradation occurs via two major pathways:

- either it is converted by **TDH** to 2-amino-3-ketobutyrate, which by the action of 2-amino-3-oxobutyrate CoA ligase forms glycine and acetyl CoA
- or alternatively, L-serine/threonine dehydratase converts threonine to NH_4^+ and 2-ketobutyrate and the latter is further metabolized to glycine by way of acetyl CoA.

How Does TDH Work?

How Tk-TDH functions at the molecular level is a question we are hoping to answer upon the completion of this structure. The reaction this enzyme catalyses is shown below:

Methods

Hyperthermophiles thrive above 90°C and proteins from these organisms are known to be extremely thermostable. They therefore attract much attention for potential use in industrial processes and technologies. As hyperthermophilic archaea represent organisms closest to the last common ancestor of all life, they are also of scientific interest to understand the origin of life and its early evolution.

Electron micrograph of the hyperthermophilic archaeon *Thermococcus kodakaraensis* (KOD1), isolated from a solfatara on Kodakara Island, Kagoshima, Japan.

Expression, Purification and Crystallisation

Tk-TDH, from the gene TK0916, was cloned into the *E.coli* BL21 (DE3) expression system, using a recombinant plasmid (from pET-8c), and soluble protein expression induced with IPTG at 37°C. The protein was purified via sonication, a heat treatment step and anion exchange and hydrophobic interaction column chromatography. Purified Tk-TDH has a mass-spectrometric weight of 38,016Da.

Crystals grew in 0.05M sodium citrate pH 5.6, 2.4M ammonium sulphate, 0.1M sodium / potassium tartrate and 5% glycerol. They were taken to the ESRF in France, where data was collected to 2.4Å resolution.

Data Processing

The data were processed using programs in the CCP4 suite, however the predicted spacegroup was ambiguous and took time to determine correctly. P4 was found to generate the best predictions in MOSFLM and the data was subsequently processed and scaled, giving an R_{merge} of 0.154. It was later processed in all space groups of the 422 point group and the final choice was $P4_32_12$ with an R_{merge} of 0.146. The unit cell dimensions are a=b=124.48Å, c=271.14Å.

It was estimated that 4 monomers per asymmetric unit would give a solvent content of 64%. Molecular replacement was performed with a monomeric search model using PHASER, which gave a LLG 15485.

Results

We have recently determined the preliminary three-dimensional structure of threonine dehydrogenase (Tk-TDH) from the hyperthermophillic archaeon *Thermococcus kodakaraensis*.

This enzyme exists as a homotetramer with 1 structural Zn^{2+} ion per monomer stabilising the quaternary structure. Without the zinc this flexible loop region is highly disordered. It is still unclear whether a second Zn^{2+} is required for catalytic activity at the active site, as in many alcohol dehydrogenases.

The structural zinc ions appear to stabilise an interaction between 2 short helices (one from each monomer i.e. 1+2 and 3+4) to form a dimer. The monomers also interact (i.e. 1+3 and 2+4) to form an extended β sheet along the width of the protein.

Further Work…

Structural studies are continuing on this enzyme, with further refinement using PHENIX (with NCS) and model building in COOT. Crystals have also been grown in similar conditions in the presence of the essential co-factor zinc, and further screens have been set up in the presence of NAD^+. It is hoped that higher resolution data can be obtained from these crystals and that the presence of one or other of these co-factors will stabilise the structure for better refinement.

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