UNIVERSITY OF Southampton
School of Biological Sciences

TDH - an Enzyme Involved in Metabolising Threonine to Glycine

A novel threonine dehydrogenase from the hyperthermophile *Thermococcus kodakaraensis*

bbsrc

The preliminary structure of L-threonine dehydrogenase has been solved to 2.6Å by X-ray crystallography, allowing us to hypothesise how this enzyme functions as a biological catalyst and what structural factors contribute to its thermal stability.

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.

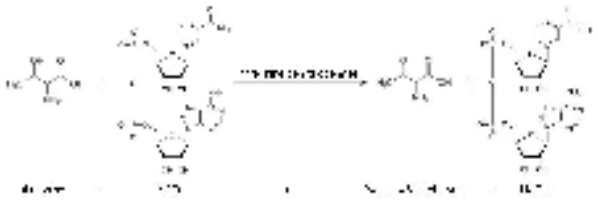
Threonine is an essential amino acid. It is synthesised by plants and microorganisms and can be metabolised to glycine.

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 elucidation 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.

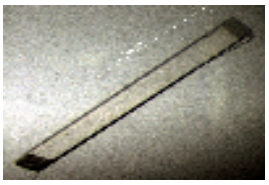
Electron micrograph of the hyperthermophilic archaeon *Thermococcus kodakaraensis* (KOD1). Isolated from water and sediment of a Solfatara at over 100ºC on Kodakara Island, Kagoshima, Japan.

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. Purified TDH has a mass-spectrometric weight of 38,016Da.

Expression, Purification and Crystallisation

Protein was purified by a series of steps including sonication, heating of the cell lysate to 85ºC for 20 minutes to precipitate impurities and anion exchange and hydrophobic interaction column chromatography.

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 were collected to 2.6Å resolution.



Data Processing

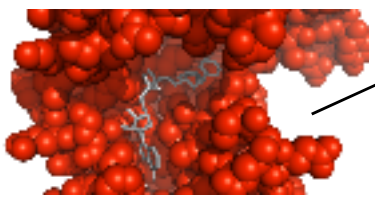
The data were processed in $P4_32_12$ using programs in the CCP4 suite and scaled, giving an R_{merge} of 0.146. The unit cell dimensions are a=b=124.48Å, c=271.14Å.

4 monomers were estimated per asymmetric unit, giving a solvent content of 64%. Molecular replacement was performed with a monomeric search model using PHASER, which gave a LLG 15485 and refinement carried out using CNS, Phenix and Refmac. Coot was used for model building.

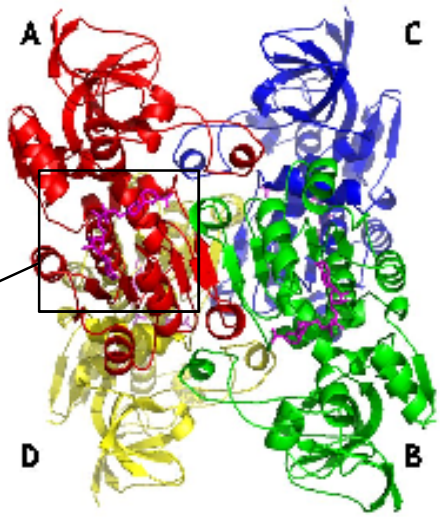
Results and Conclusions

The structure of Tk-TDH was solved to 2.6Å, bound to it's co-factor, NADH. The Rfactor and Rfree are 0.21 and 0.26 respectively.

TDH exhibits a tetrameric structure similar to that of other TDHs and ADHs of lower life forms. It exists as a dimer of dimers (AB and CD), with monomer A forming contacts with each of the other three monomers. The α/β Rossman fold motif of the NADH-binding domain forms an extended beta sheet across the protein that is thought to contribute to the enzyme's high thermal stability (optimal activity at 90ºC).



NADH binds in a pocket at the interface between the catalytic domain and a nicotinamide cofactor (NADH)-binding domain, close to the catalytic residues that are thought to react with threonine.



It is expected that TDHs require a single Zn^{2+} at the small α helix interface between monomers A and C, for structural stability. It is currently ambiguous as to whether a second Zn^{2+} is required for catalytic activity.

Structural studies are continuing to determine the position of metal ions involved and elucidate its mechanism of action.

Trials are continuing to find alternative conditions in which to grow crystals so as to retain any bound zinc; and to crystallise the protein in the holo form to observe any conformational changes that may be induced upon ligand binding.

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