

Determination of the Yeast THI80 Crystal Structure

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Beamline(s): X12C

Introduction: The yeast THI80 gene product is a homologue of the mammalian enzyme thiamin pyrophosphokinase (TPK). TPK is required for the conversion of vitamin B1 (thiamin) to the active thiaminpyrophosphate (TPP) cofactor by catalyzing the transfer of a pyrophosphate group from ATP to thiamin. TPP is required for carbohydrate and amino acid metabolism. TPP is essential for the enzymatic activity of the pyruvate, alpha-ketoglutarate and branched chain alpha-keto acid dehydrogenase complexes and is also required by the enzymes pyruvate decarboxylase and transketolase. TPP functions as a carrier of activated aldehyde units during the formation of acetyl-CoA from pyruvate, during the carbohydrate interconversions of the pentose phosphate pathway and during catabolism of branched chain amino acids.

Methods and Materials: Recombinant THI80 containing selenomethionine was produced in *E. coli* and was crystallized from sitting drops using the vapor diffusion method. A THI80 crystal diffracting to 2.0 Angstrom was used for a multiwavelength anomalous diffraction (MAD) experiment conducted at X12C using X-ray energies corresponding to the inflection point and peak of the Se K edge and at a remote energy of 13.3 keV. The partial structure of four Se atoms was determined using the program SOLVE, but proved inadequate for calculating interpretable electron density for the 76 kDa dimer present in the asymmetric unit of the crystals. The Se MAD data were used along with data collected at the APS beamline 19ID from a native crystal soaked with 0.5 M NaBr to obtain a 2.0 Angstrom experimental electron density map used to solve the THI80 crystal structure.

Results: The THI80 crystal structure reveals a subunit formed from a central ten stranded mixed beta-sheet. One half of the central beta-sheet is sandwiched by alpha-helices forming a Rossmann fold, while the other half forms a beta-sandwich structure containing a jelly roll motif. The THI80 subunits associate as a dimer primarily through contacts between the beta-sandwich structures. The active site of the enzyme was identified from the position of symmetrically placed thiamin molecules bound in the interface between the subunits.

Conclusions: THI80 represents the second pyrophosphokinase structure to be determined. The THI80 structure differs from the monomeric hydroxymethyl dihydropterin pyrophosphokinase, which catalyzes a similar pyrophosphate transfer reaction in folate metabolism. The THI80 structure will enable the mechanism of TPK and pyrophosphokinase reactions to be studied at the molecular level.