Scaling up Protein Production for Functional Genomics: Study of new anti-fungal target proteins

Partners: Protein Expert - Structural and Genomic information laboratory

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Given the private status of this project we wish these results to remain confidential

The CA1462 Structure

Description: This Profun target corresponds to a 326 residues long protein predicted as a thiamin pyrophosphokinase (TPK, EC 2.7.6.2). This enzyme catalyses the transfer of a pyrophosphate group from ATP to vitamin B1 (thiamin) to form the coenzyme thiamin pyrophosphate (TPP). TPP is essential for enzymes in carbohydrate metabolism, it is required for the biosynthesis of isoleucine and valine, and is also used in amino acid catabolism (Hohmann & Meacock, 1998). *C. albicans* TPK has 2 homologues in the PDB: 1IG0, TPK from *S. cerevisiae* (Baker *et al.*, 2001) and 1IG3, TPK from mouse (Timm *et al.* 2001).

The crystal structure of *S. cerevisiae* TPK in complex with thiamin has been determined to 1.8 Å resolution. TPK is a homodimer and each subunit consists in two domains. One domain resembles a Rossman fold with four α helices on each side of a six-strand parallels β barrel. The other domain has one 4 strand and one 6 strand antiparallel β sheet which form a flattened sandwich structure containing a jellyroll topology. Both domains contribute to the dimeric association. A groove is formed at the dimer interface with one thiamin bound at each end between the α/β domain of one subunit and the β sandwich of the opposite subunit. The site for ATP binding is likely to occur near the thiamin hydroxyethyl tail.

We used AMP-PNP in co-crystallization experiments (the oxygen atom connecting the β and γ-phosphate groups in the ATP molecule is replaced by a nitrogen) in order to visualize the ATP binding site. This inhibitor works by preventing transfer of the γ-phosphate to the substrate peptide. We thus were able to confirm the ATP binding site location in the dimer structure.

Altered thiamin activity is also found in chronic alcoholism and suggests an inhibitory role for ethanol (Rindi *et al.*, 1986). Nevertheless a direct interaction between ethanol and TPK has not been proven.

Data collection:

Space group P1; a=50.9 b=60.8 c=65.3 α=66.4 β=90.1 γ=65.3
2 molecules in the AU.

A- ID29 ESRF (Grenoble, France)
Resolution: 1.97 to 20Å.
Structure of the CA1462 dimer in complex with thiamin (Fig 1A).
R_{work} 0.23 - R_{free} 0.266.

B- FIP ESRF (Grenoble, France)
Resolution: 1.8 to 20Å.
Structure of the CA1462 dimer in complex with thiamin and AMP-PNP (Fig 1B-C).
R_{work} 0.233 - R_{free} 0.27.

Structure determination:

The CA1462 structure was solved using our CaspR server (Claude et al., 2004; http://www.igs.cnrs-mrs.fr/Caspr/index.cgi) to perform molecular replacement using homology modeling of the available structures 1IG0 and 1IG3.

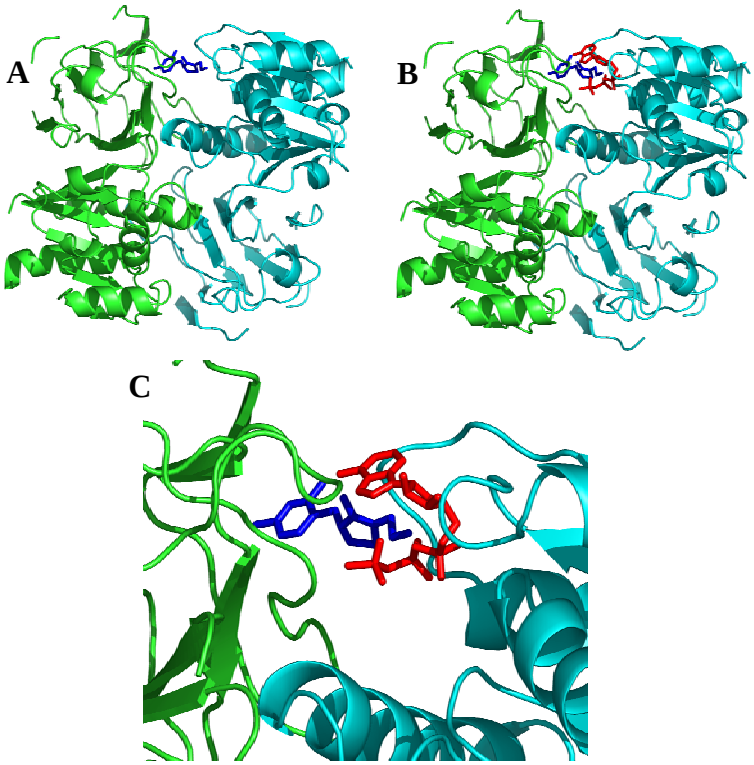
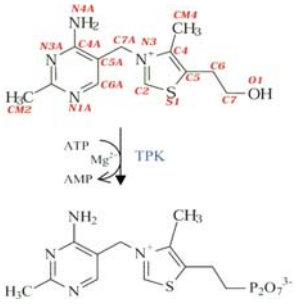


Fig. 1 Structure of CA1462. The dimer with thiamin alone (A) or thiamine and AMPPNP (B-C).

Enzymatic Assay:

Specific activity: 0.367 IU/mg (thiamin consumed at 25°C, pH 7.5)



References:

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Hohmann S, Meacock PA (1998)**.** Thiamin metabolism and thiamin diphosphate-dependent enzymes in the yeast Saccharomyces cerevisiae: genetic regulation. *Biochim. Biophys. Acta.* **1385**:201-19.

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The YDR353W Structure

Description : This Profun target corresponds to a 319 residues long protein predicted to belong to the oxydoreductases group acting on a sulfur group of donors with NAD^+ or $NADP^+$ as acceptor (EC 1.8.1). These enzymes catalyze the transfer of electrons from a pyridine nucleotide *via* a flavin carrier to disulfide-containing substrates.

The structural family defined by sequence similarity includes at least 2 thioredoxin reductase (EC 1.8.1.9): 1TDE from *Escherichia coli* (Waksman *et al.* 1994) and 1VDC from *Arabidopsis thaliana* (Dai *et al.* 1996) and 2 glutathione transferase (EC 1.8.1.7): 1GET from *Escherichia coli (*Mittle *et al.* 1994) and 1XAN from *Human* (Savvides & Karplus 1996). All solved structures form a dimer. Each subunit is divided into two domains linked by a beta sheet, one binding the FAD and the other binding the NADP.

Two noncompetitive inhibitors of the glutathione reductase are known: 3,7-diamino-2,8-dimethyl-5-phenyl-phenazinium chloride (safranin) and 6-hydroxy-3-oxo-3H-xanthene-9-propionic acid (XAN) they occupy the same site in the reductase structure (Savvides & Karplus 1996) corresponding to a large cavity at the dimer interface. The stoichiometry is one inhibitor molecule / dimer. Because it was impossible to get XAN we have used a chemical neighbor: AH6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid).

As expected, the multiple alignment of YDR353W, 1VDC (60% of sequence identity) and 1TDE (49% Id) highlights a strict conservation of the residues involved in the FAD binding (10-14, 34, 45, 49, 53-54, 64, 120, 140, 146, 287-288, 296), the NADP binding (124, 161-163, 166, 185, 187, 190, 248-249, 295), and the active site (142-143, 145-147, 168-169). The residues defining the active site, are located in the NADPH domain and contain 2 extremely conserved cystein residues, in the various crystal structures they can be either oxidized in a disulfide bridge conformation or reduced.

The thioredoxin reductases are inhibited by 1-chloro-2,4-dinitrobenzene (Arner *et al.* 1995), gold salts are also used to treat rheumatoid arthritis (Gromer *et al.* 1998) and the 1-methyl-1-propyl-2-imidazolyl disulfide (Smart *et al.* 2004), a molecule undergoing phase I trials in cancer research (ProlX Pharmaceuticals).

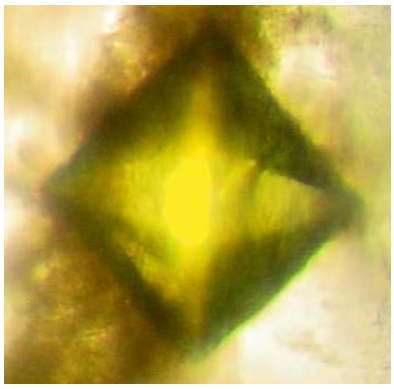
Data collection:

Space group $P2_12_12$; a=140.3 b=70.4 c=60.8
2 molecules in the AU.

FIP-BM30A ESRF (Grenoble, France)
Resolution: 3.0 to 20Å.
Structure of the YDR353W dimer in complex with FAD (Fig 2).
Still in the process of refinement

Structure Determination:

The structure was solved by molecular replacement based on homology modelling of the available PDB structures 1VDC, 1CL0, 1TDE, 1F6M, 1TRB and 1FL2 on our CaspR web-server (Claude et al., 2004; http:// www.igs.cnrs-mrs.fr/Caspr/index.cgi). (AMoRe correlation: 48%, R=42)



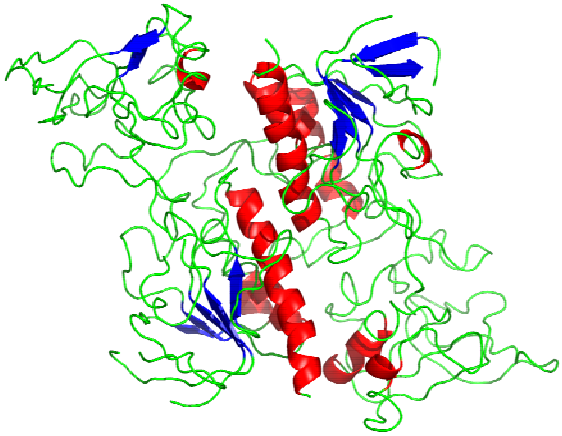
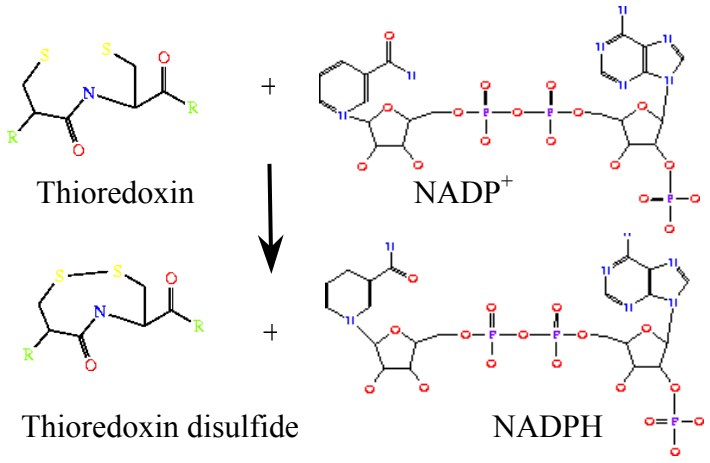


Fig. 2 Pre-refined structure of YDR353W.

Enzymatic Assay:



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