The Crystal Structure of Yeast Thiamin Pyrophosphokinase

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Summary

Thiamin pyrophosphokinase (TPK) catalyses the transfer of a pyrophosphate group from ATP to vitamin B_1 (thiamin) to form the coenzyme thiamine pyrophosphate (TPP). Thus, TPK is important for the formation of a coenzyme required for central metabolic functions. TPK has no sequence homologues in the Protein Data Bank and functions by an unknown mechanism. The TPK structure has been determined as a significant step towards elucidating its catalytic action. The crystal structure of *Saccharomyces cerevisiae* TPK complexed with thiamin has been determined at 1.8 Å resolution by MAD phasing from the anomalous signal of bromine, added to the crystal as a short soak in sodium bromide. A three-wavelength MAD experiment was performed at Structural Biology Center beamline 19-ID. TPK is a homodimer, and each subunit consists of two domains (Fig. 1). One domain resembles a Rossman fold with 4 α-helices on each side of a 6-stranded parallel β-sheet. The other domain has a 4-stranded and a 6-stranded antiparallel β-sheet, which form a flattened sandwich structure containing a jelly-roll topology. The active site is located in a cleft at the dimer interface and is formed from residues from domains of both subunits. The TPK dimer contains two compound active sites at the subunit interface. The structure of TPK with one substrate bound identifies the location of the thiamin binding site and probable catalytic residues. The structure also suggests a likely binding site for ATP. These findings are further supported by TPK sequence homologies. Although possessing no significant sequence homology with other pyrophosphokinases, thiamin pyrophosphokinase may operate by a mechanism of pyrophosphoryl transfer similar to those described for pyrophosphokinases functioning in nucleotide biosynthesis. A manuscript describing this structure is now "in press" in the journal *Structure*.

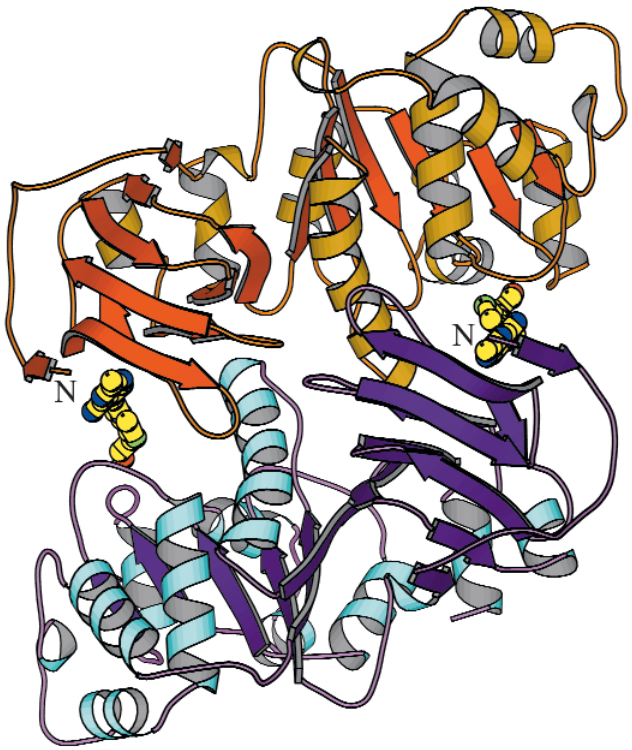

FIG. 1. A ribbons diagram of the structure of yeast thiamin pyrophosphokinase viewed down the local dimer axis.

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