**MX33: "Substrate complexes of Anthranilate Phosphoribosyltransferase"**

**Status**: 3 structures were completed
**PDB accession codes**: 2GVQ, 1ZXY, and 1ZYK.

Manuscripts related to this work:

**1. Marino M, Deuss M, Svergun DI, Konarev PV, Sterner R, Mayans O. (2006) Structural and mutational analysis of substrate complexation by anthranilate phosphoribosyltransferase from Sulfolobus solfataricus. J Biol Chem. 281(30):21410-21.**

Abstract: The metabolic synthesis and degradation of essential nucleotide compounds are primarily carried out by phosphoribosyltransferases (PRT) and nucleoside phosphorylases (NP), respectively. Despite the resemblance of their reactions, five classes of PRTs and NPs exist, where anthranilate PRT (AnPRT) constitutes the only evolutionary link between synthesis and degradation processes. We have characterized the active site of dimeric AnPRT from Sulfolobus solfataricus by elucidating crystal structures of the wild-type enzyme complexed to its two natural substrates anthranilate and 5-phosphoribosyl-1-pyrophosphate/Mg(2+). These bind into two different domains within each protomer and are brought together during catalysis by rotational domain motions as shown by small angle x-ray scattering data. Steady-state kinetics of mutated AnPRT variants address the role of active site residues in binding and catalysis. Results allow the comparative analysis of PRT and pyrimidine NP families and expose related structural motifs involved in nucleotide/nucleoside recognition by these enzyme families.

**2. Schwab T, Skegro D, Mayans O, Sterner R. (2008) "A rationally designed monomeric variant of anthranilate phosphoribosyltransferase from Sulfolobus solfataricus is as active as the dimeric wild-type enzyme but less thermostable." J Mol Biol. 376(2):506-16.**

Abstract: The anthranilate phosphoribosyltransferase from Sulfolobus solfataricus (ssAnPRT) forms a homodimer with a hydrophobic subunit interface. To elucidate the role of oligomerisation for catalytic activity and thermal stability of the enzyme, we loosened the dimer by replacing two apolar interface residues with negatively charged residues (mutations I36E and M47D). The purified double mutant I36E+M47D formed a monomer with wild-type catalytic activity but reduced thermal stability. The single mutants I36E and M47D were present in a monomer-dimer equilibrium with dissociation constants of about 1 microM and 20 microM, respectively, which were calculated from the concentration-dependence of their heat inactivation kinetics. The monomeric form of M47D, which is populated at low subunit concentrations, was as thermolabile as monomeric I36E+M47D. Likewise, the dimeric form of I36E, which was populated at high subunit concentrations, was as thermostable as dimeric wild-type ssAnPRT. These findings show that the increased stability of wild-type ssAnPRT compared to the I36E+M47D double mutant is not caused by the amino acid exchanges per se but by the higher intrinsic stability of the dimer compared to the monomer. In accordance with the negligible effect of the mutations on catalytic activity and stability, the X-ray structure of M47D contains only minor local perturbations at the dimer interface. We conclude that the monomeric double mutant resembles the individual wild-type subunits, and that ssAnPRT is a dimer for stability but not for activity reasons.