Molecular Mechanism of ADP-ribose Pyrophosphatase from *Thermus thermophilus* HB8

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The nudix pyrophosphatases share a highly conserved amino acid sequence, called the "*nudix motif*". Recent genome analyses have revealed a wide distribution of nudix proteins from phage to human, although as yet their function has not been fully established. To elucidate a detailed molecular function we have analyzed nudix proteins from *Thermus thermophilus* HB8. Here, we report the structural and mutational study of ADP-ribose pyrophosphatase (*Tt*ADPRase), a major group within the nudix protein family.

We have determined the crystal structure of *Tt*ADPRase with its ligands [1]. To further investigate the substrate recognition and catalytic mechanism in detail, we performed a mutational analysis based on the structural data. From kinetic studies, we identified several residues involved in recognition of ADP-ribose. In addition, the results strongly suggested that *Tt*ADPRase employs a *two-metal ion mechanism*, in which a water molecular is coordinated by two cations and activated to be a nucleophile. This catalytic mechanism differs from that of other nudix proteins, including 8-oxo-dGTPase (MutT). These results also reveal the diversity in molecular function of nudix proteins both in terms of substrate recognition and catalysis.

[1] Yoshiba S., et al., J. Biol. Chem., 2004, 279, 37163-37174.

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