Regulation of *Sulfolobus solfataricus* Uracil Phosphoribosyl-transferase

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UPRTase is a salvage enzyme that catalyzes the formation of UMP from PRPP (5-phosphoribosyl-1-α-diphosphate) and uracil.

CTP and UMP can independently bind to *Ss*UPRTase and simultaneous binding of CTP and UMP strongly inhibits the enzyme. A structure to 2.8 Å resolution of *Ss*UPRTase-CTP-UMP has already been determined [1].

GTP causes a 20-fold increase in the turnover number k_{cat} and raises K_M for PRPP and uracil by 2- and >10-fold, respectively [2]. In order to make an *Ss*UPRTase-GTP-PRPP complex, the enzyme must be depleted for the product UMP. Co-purified UMP binds so strongly to *Ss*UPRTase that an unfolding and refolding procedure was necessary to remove it. UMP-depleted *Ss*UPRTase (5 mg/mL) with 5 mM GTP and 5 mM PRPP was crystallized by vapour diffusion with PEG8000 at pH 6.5. Synchrotron data to 2.8 Å resolution has been recorded ($P6_422$, a=b=122.2 Å, c=62.2 Å) and the structure determined by molecular replacement.

*Ss*UPRTase in solution as well as in crystals is tetrameric with 222 symmetry. The allosteric binding sites for CTP/GTP are situated in the middle of the tetramer ca. 24 Å from the active sites. Transformation from inhibited to activated structure involves structural changes in the quaternary structure along with major active site movements.

[1] Arent S., Harris P., Jensen K.F., Larsen S., *Biochemistry*, 2005, **44**, 883. [2] Jensen K.F., Arent S., Larsen S., Schack L., *FEBS Journal*, 2005, **272**, 1440.

Keywords: nucleotide metabolism, protein regulation, enzyme catalysis