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X-RAY CRYSTALLOGRAPHIC STUDIES OF HUMAN PHOSPHORIBOSYL PYROPHOSPHATE SYNTHETASE

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Introduction

Phosphoribosylpyrophosphate (PRPP) is an important metabolite essential for nucleotide synthesis and PRPP synthetase (PRS) catalyzes synthesis of PRPP from ribose-5-phosphate (R5P) and ATP. Superactivity of PRS is associated with an X chromosome-linked purine metabolic defect in humans. The enzymatic activity of PRS is regulated by phosphate ion, divalent metal cation, and ADP. We determined the crystal structures of wild-type and mutant of human PRS in complexes with sulfate at 2.2 Å, and 2.5 Å resolution, respectively. Human PRS consists of two semblable domains and has a similar architecture as *B. subtilis* PRS. The enzyme forms a hexamer which is proposed to be the minimal active form. The sulfate, an analog of the activator phosphate, is found to bind at both the R5P binding site and the allosteric site defined previously. In addition, an extra sulfate is bound at a new site between the ATP binding site and the allosteric site. Structural and biochemical data show that mutations of the conserved residues at this site influence the binding of sulfate and affect the enzymatic activity. Our results suggest that this new sulfate binding site is a second allosteric site to regulate the enzymatic activity which might also exist in other eukaryotic PRSs, but not bacterial PRSs.

Method and Results

The *hPRS* gene encoding the full-length hPRS protein (318 amino acids) was obtained from the cDNA library of human CD34+ haematopoietic stem/progenitor cells (1). The protein was purified with Ni affinity chromatography method. Sparse-matrix crystallization screening with the Crystal Screen, Crystal Screen II and Grid (ammonium sulfate) kits (Hampton Research) was performed using the hanging-drop vapor diffusion method at 20°C. Crystals of hPRS (and mutant) in complex with ligands were mounted on a cryo-loop and flash-frozen in liquid nitrogen. Data collection was carried out using the ADSC CCD detector of BL6A at PF. Data processing and scaling were performed using the HKL2000 suite.

Crystals of the hPRS and mutant both belong to space group *R3*. The refinement for hPRS was converged to final R-factor of 21.2%, with Rfree value of 24.6%. The final R factor and Rfree factor for hPRS mutant were reduced to 20.6% and 25.7%, respectively.

Comparison of the crystal structures between the wild-type hPRS, the mutant form and the homologs from prokaryotic organism has revealed that a second regulatory site probably exists in hPRS.

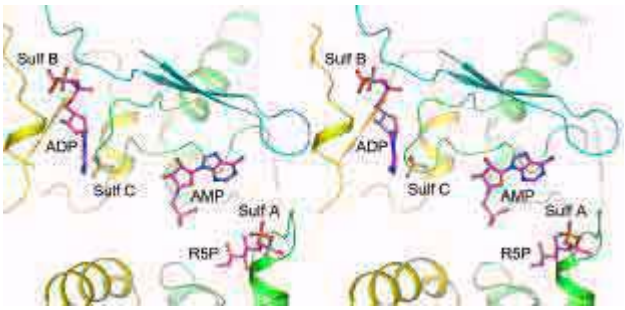

Fig. 1. Structure of the catalytic and regulatory sites of hPRS

References

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