MS5-P27 Purine nucleoside phosphorylase from bacterium *Helicobacter pylori* strain 26695: cloning, expression, purification, characterisation and crystallisation

Karolina Gucunski[1], Ivana Leščić Ašler[1], Marija Luić[1]

1. Laboratory for Chemical and Biological Crystallography, Division of Physical Chemistry

email: karolina.gucunski@gmail.com

Purine nucleoside phosphorylase (PNP) is the key enzyme in the purine salvage pathway. It catalyses the reversible phosphorolytic cleavage of the glycosidic bond of ribo- and deoxyribonucleosides, in the presence of inorganic orthophosphate as a second substrate to generate the purine base and ribose(deoxyribose)-1-phosphate.

Helicobacter pylori is a Gram-negative, microaerophilic bacterium, human pathogen involved in development of many diseases as gastric ulcers and stomach cancer, and therefore known for its ability to colonize human stomach. Study of the *H. pylori*, due to the ever growing infection rate and increase of *H. pylori* antibiotic resistance, is centred on understanding pathogenesis and finding a way to attack and eradicate *H. pylori*.

H. pylori PNP represents potential drug target as this bacterium cannot synthesize purine rings through *de novo* pathway and has to rely on purine production through purine salvage pathway. It belongs to the class of bacterial high-molecular-mass homohexamers with specificity for both 6-oxo- and/or 6-aminopurines.

Purine nucleoside phosphorylase gene *deoD* was isolated from genomic DNA of *Helicobacter pylori* (strain 26695) and amplified using Phusion High-Fidelity PCR kit with the set of specific DNA primers for both 5' and 3' ends of the gene. Resulting plasmid pET21b-HP26695*deoD*, with ampicillin resistance and without purification tag, was transformed into *E. coli* strain BL21-CodonPlus(DE3)RIL. Induction conditions for PNP expression in *E. coli* were optimised and evaluated by SDS-PAGE electrophoresis of bacterial cultrate filtrate.

Purification of overexpressed PNP from the bacterial culture filtrate was performed by anion exchange chromatography on Q-Sepharose FF column. Next step, which gave single protein band on SDS-PAGE was affinity chromatography, performed on Sepharose-FormycinA column.

Biochemical characterisation involves kinetic studies, as well as temperature and pH effects on stability and activity of PNP. Crystallisation experiments with purified purine nucleoside phosphorylase from *H. pylori* are under way.

Keywords: Helicobacter pylori, purine nucleoside phosphorylase, biochemical characterisation, enzyme kinetics, crystallisation

MS5-P28 A sequence-specific DNA glycosylase mediates restriction-modification in *Pyrococcus abyssi*

Masaru Tanokura[1], Ken-ichi Miyazono[1], Yoshikazu Furuta[2,3], Miki Watanabe-Matsui[3], Takuya Miyakawa[4], Tomoko Ito[4], Ichizo Kobayashi[1,2,3]

1. Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo
2. Department of Medical Genome Sciences, Graduate School of Frontier Science, The University of Tokyo
3. Institute of Medical Science, The University of Tokyo
4. Graduate Program in Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo

email: amtanok@mail.ecc.u-tokyo.ac.jp

Restriction-modification systems consist of genes that encode a restriction enzyme and a cognate modification methyltransferase. It was believed that restriction enzymes are sequence-specific endonucleases that cleave double-stranded DNAs at specific sites by catalyzing the hydrolysis of phosphodiester bonds. R.PabI is a type II restriction enzyme from a hyperthermophilic archaea *Pyrococcus abyssi* that recognizes 5'-GTAC-3' sequence and cleaves double-stranded DNAs without the addition of a divalent cation, although most restriction enzymes require divalent cations for their activity. The structural and mutational analyses of R.PabI in our previous work showed that R.PabI forms a homodimer and has a novel DNA-binding fold called a "half-pipe," which consists of a highly curved anti-parallel b-sheet. Because the structure of R.PabI shares no structural similarity to any other protein with a known function, the structural basis for the sequence-recognition and DNA-cleavage mechanisms of R.PabI remained unclear. In this study, we report the crystal structure of R.PabI in complex with a double-stranded DNA containing the R.PabI recognition site. The structure of the R.PabI-DNA complex shows that R.PabI unwinds a double-stranded DNA at the 5'-GTAC-3' site and flips the guanine and adenine bases out of the DNA helix to recognize the sequence (Figure). The electron-density map of the R.PabI-DNA complex shows that R.PabI releases adenine bases from the R.PabI recognition site. This suggests that R.PabI catalyzed the cleavage of the *N*-glycosidic bond of adenine nucleotide in the same way as DNA glycosylases. Biochemical assays using HPLC and MALDI-TOF MS spectrometry also support the observation that R.PabI catalyzes the hydrolysis of the *N*-glycosidic bond of adenine nucleotide. These results show that R.PabI is not an endonuclease but a sequence-specific adenine DNA glycosylase. R.PabI is the first example of a restriction enzyme that shows DNA glycosylase activity. Mutational analyses reveal the active site of the adenine DNA glycosylase activity of R.PabI. The two opposing apurinic/apyrimidinic (AP) sites generated by R.PabI are cleaved by heat promoted b elimination and/or by endogenous AP endonucleases of