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# Recognition model of a uracil residue in DNA by AP endonuclease from *Methanothermobacter thermautotrophicus*

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## 1 Introduction

AP endonuclease is a DNA repair enzyme that cleaves DNA sugar-phosphate backbone at the 5'-side of Apyrimidinic/apurinic (AP) sites. *Methanothermobacter thermautotrophicus*, a thermophilic archaeon has an exonuclease III family AP endonuclease (*Mth*Exo). In addition to AP endonuclease activity, *Mth*Exo has DNA uridine endonuclease activity that cleaves the phosphoester bond at the 5'-side of 2'-deoxy-uridine residue in the double-stranded DNA [1]. This activity was not found in the other AP endonucleases. In order to understand the recognition mechanism of the uracil residue by *Mth*Exo, we analysed the structural differences between wild-type *Mth*Exo and its mutant W205S with higher activity of DNA uridine endonuclease than the activity of wild type.

## 2 Experiment

Wild-type *Mth*Exo and its mutant W205S were crystallized at 20ºC using the hanging drop vapor diffusion method. 1µl of *Mth*Exo was mixed with the same volume of reservoir solution (50 mM magnesium formate, 10% PEG 3,350 MME) and 0.3 µl of 100 µM uracil. X-ray diffraction data collections were performed at Photon Factory BL-5A or NW12A at 95 K with 22.5% PEG 400 as cryoprotectant. The structure was solved by molecular replacement method using MOLREP in the CCP4 with the structure (PDB: 3FZI) of *Mth*Exo [2] as a search model. The crystal structures were refined to 1.6 Å or 1.9 Å resolution using REFMAC5. The atomic coordinates and the structure factors have been deposited in the Protein Data Bank, with the accession codes 3W2X and 3W2Y.

## 3 Results and Discussion

There are no differences in the overall structures between the wild-type *Mth*Exo and the mutant W205S. Unfortunately, electron density for uracil was not observed in these structures. The pocket space near the active site in W205S is wider than that of wild type because of reduction of the steric hindrance of the aromatic ring of tryptophan (Fig. 1). The pocket is comprised of six amino acids including Asn114 and Asn153 and the mutants N114Q and N153Q lack DNA uridine endonuclease activity. The docking simulation of 2'-deoxyuridine 5'-monophosphate (dUMP) into the pocket shows that the uracil residue can fit in the pocket with the five hydrogen bonds formation between uracil and *Mth*Exo (Fig. 2). These findings suggest the recognition model that the pocket site of *Mth*Exo binds to the uracil residue in DNA.

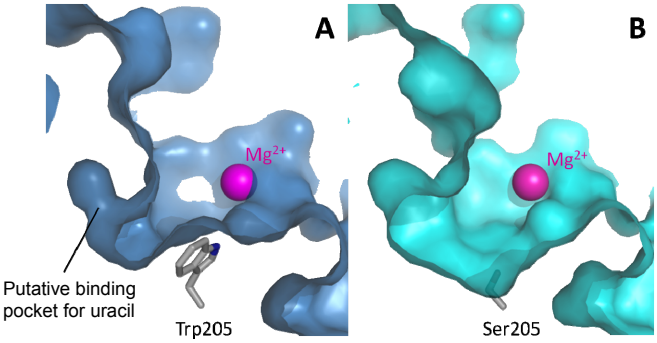


Fig. 1: The structural differences of the putative binding pocket for a uracil residue between *Mth*Exo (A) and the mutant W205S (B).

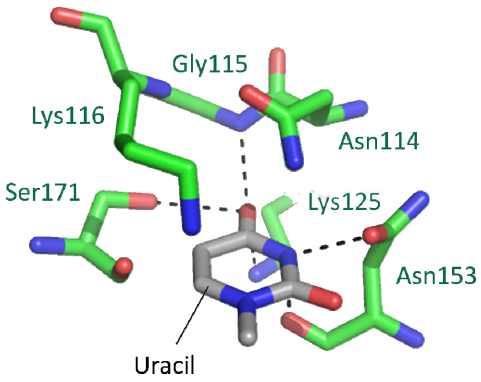


Fig. 2: The putative interactions of docking simulation between uracil and the amino acid residues in the binding pocket of *Mth*Exo.

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## References

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