The MutT Crystal Retains the Ability to Hydrolyze 8-oxo-dGTP

Yuriko Yamagata, Teruya Nakamura, *Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan*. E-mail: yamagata@gpo.kumamoto-u.ac.jp

Escherichia coli MutT specifically hydrolyzes a potently mutagenic and DNA replicative substrate, 8-oxo-dGTP to 8-oxo-dGMT and pyrophosphate in the presence of Mg^{2+} (Mn^{2+}) so as to prevent misincorporation of 8-oxoguanine (8-oxoG) opposite adenine and A:T to C:G transversion by the resulting A:8-oxoG mispair. Recently we have determined the crystal structures of MutT in the presence and in the absent of the reaction product 8-oxo-dGMP. The structures reveal that MutT specifically recognizes 8-oxo-dGMP through a wealth of hydrogen bonds to the protein and waters in the binding pocket with the large ligand-induced conformational change. The catalytic mechanism of MutT still remains unclear.

In this paper we report the crystal structure of MutT in complex with its substrate, 8-oxo-dGTP at 1.8 Å resolution. The structure confirms the substrate induced conformational change and sodium ions bound to the triphosphate moiety and residues in the MutT (Nudix) motif. In order to elucidate the reaction mechanism of MutT, the MutT-8-oxo-dGTP crystals were soaked in $MnCl_2$ under a variety of conditions and freeze-trapped. The crystal soaked in 2mM $MnCl_2$ for 2 days showed that a manganese ion occupied one sodium ion site in the two ones. When the crystals were soaked in 20mM $MnCl_2$ for 4 hours, the substrate was perfectly hydrolyzed. The trial of the catching the intermediate state of catalysis is in progress. These results would provide insights into the hydrolysis mechanism.

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