Structures of Escherichia coli and Mouse 8-Oxo-dGTPases that Hydrolyze the Mutagenic 8-Oxo-dGTP to 8-Oxo-dGMP

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8-Oxoguanine is produced in nucleotide pools, as well as in DNA of cells by active oxygen species normally formed during cellular metabolic processes. 8-Oxo-dGTP in the nucleotide pools is a potent mutagenic substrate for DNA synthesis and can pair with cytosine and adenine. 8-oxoG:A mispairing causes transversion mutation. 8-Oxo-dGTPase hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP with magnesium ions and is responsible for preventing misincorporation of 8-oxoguanine into DNA. Our final goal is to understand the structural basis for the recognition and hydrolysis of 8-oxo-dGTP by 8-oxo-dGTPase from the three-dimensional structures of 8-oxo-dGTPase and 8-oxo-dGTPase complexed with 8-oxoguanine analogues and magnesium ion.

Crystals of Escherichia coli 8-oxo-dGTPase (MutT) were obtained and X-ray diffraction data (2.2 Å resolution) of the native crystals were collected at BL6B of Photon Factory (PF). Consequently we planed to collect derivative data for the multiple isomorphous replacement method. However, it was so difficult to get diffraction quality crystals of MutT reproduciblly. Therefore, we tried again to search the new crystallization condition using the dynamic light scattering method and found that the solution of MutT-Mn^{2+} complex is monomodal and suitable for the crystallization. The crystals of MutT-Mn^{2+} complex were grown with the similar conditions to those for MutT crystals. The native data of the complex were measured at BL6B of PF at 293K, but the quality of the data set collected was low (2.8 Å, R_{merge} = 0.10, Completeness 80%) because of the radiation damage. Then we decided that the data collection was going to be carried out at low temperature (100K) and screened several cryoprotectants. The mixture of 20% glycerol and 1.6 M tartrate was found as the suitable cryoprotectant from a preliminary data collection using R-axis IV at 100K. In our beam time period of the 3rd cycle we tried to collect the native and derivative (soaked Pt compounds) data of MutT-Mn^{2+} crystals at 100K. Several crystals checked for the data collection had extremely large mosaicities due to the failure in flash cooling in a stream of 100K nitrogen gas or the low quality crystals. Some crystals were twin and the resolution of small single crystals was too low (~ 5 Å).

We investigated which kinds of crystals could produce the high quality data at BL41XU of SPring-8 at 100K using the crystals of mutant DNA repair enzymes and mutant human lysozymes. From the crystal of the mutant human lysozyme replaced Val74 by Ser, the usual data set was obtained (2.0 Å, R_{merge} = 0.056, Completeness 89%). The refinement of the V74S crystal structure gave the conventional R value of 0.188.

We are trying to make the high quality crystals of MutT-Mn^{2+} suitable for the measurement of diffraction data at SPring-8.