Crystallographic analysis of the oxidative DNA repair enzyme from human.

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Introduction

By several aerobic systems in the nature and exogenous agents, reactive oxygen species generate and they also induce oxidative damage to DNA. In order to avoid the unusual DNA synthesis or unusual DNA replication derived from the DNA damages, cells have several DNA repair mechanisms. For example, oxidative pyrimidine lesions such as thymine glycols are primarily excised from DNA by endonuclease III in E.coli. But mammalian cells have a homologue of Endo III (NTH1). Recently, Ide et al. found the broad specificities to the oxidative bases in the mammalian DNA repair enzymes through the activity studies. In this study, by analyzing repair enzyme structure of atomic resolution, we would like to reconstitute the oxide base recognition mechanism.

Results and Discussion

At first, human NTH1 and human SMUG1 were selected to crystallize for X-ray structure analysis. Both recombinant enzymes were expressed in E.coli as GST fusion protein. After thrombin treatment, those repair enzymes were isolated, but unstable after the condense for crystallization. Thus, crystallizations were performed by coupling with purification, and protocols were optimized to avoid aggregation.

Crystal of SMUG1 was obtained by the micro batch method. The crystal size was 0.1 x 0.1 x 0.2mm. Although the crystal was very hard to obtain (1 crystal of 300 trials), the edge shape was quite clear. The cell dimensions were, a = 50.19 Å, b = 60.16 Å, c = 91.38 Å, and the space group belonged to orthorhombic P212121. The data collections were performed using Quantum 4R CCD detector using synchrotron radiation. The reflection images were processed using the programs HKL2000 and Mosflm. The crystal diffracted x-ray up to 2.0 Å resolution, and 17,125 independent reflections were collected and the R_{sym} was 0.111. Initial phase were determined by molecular replacement method by using program MolRep using the coordinate set of *Xenopus* SMUG1, which was recently determined by Wibley et al. Crystallographic refinement were performed using REFMAC and the R and R_{free} were converged to 0.173 and 0.229, respectively. The main chain structure of human and *Xenopus* SMUG1s are almost identical. In the case of *Xenopus* SMUG1, nucleic base such as hydroxymethyl uracil (hmU) and uracil were used as substrate. But in the case of human enzyme, hydroxyl uracil were used as substrate. Comparing the binding scheme of these three substrates, the recognition of the 5th site of base became clearly. And combing with the result of the site directed mutagenesis, SMUG1 was found to require hydroxyl group at 5th site or, in the case of uracil, this space is filled up with free water molecule in order to form hydrogen bond. In addition, only in the case of human enzyme, one side chain of glutamic acid were found to involved for the substrate binding.

In the case of NTH1, obtained crystals were quite small and gave low resolution as 4.5 Å. But recently, we could obtain larger crystal, of which resolution was 3.2 Å. Now, heavy atom search is in progress.

References


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