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Approach to elucidation of the base excision mechanism of DNA oxidative damage repair enzyme, hOGG1, based on its intermediate structures

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1 Introduction

Human 8-Oxo Guanine Glycosylase 1 (hOGG1) is an enzyme that repairs 8-oxo-7,8-hydroxyguanine (8oxoG), a major DNA damage. hOGG1 is a bifunctional glycosylase that catalyzes two reactions: a glycosylase reaction that cleaves the *N*-glycosidic bonds of the damaged base and a β-lyase reaction that cleaves the 3' term of the DNA backbone at the debase site. In the β-lyase reaction, Lys249 has been shown to play a central role [1]. On the other hand, for the glycosylase reaction, although the catalytic residues have been identified [2], the catalytic mechanism involving these residues is still unclear. In this study, we aimed to elucidate the catalytic mechanism of the glycosylase reaction by directly observing the glycosylase reaction intermediates by X-ray crystallography.

2 Experiment

The hOGG1 gene was expressed using the *E. coli* expression system, and purified hOGG1 sample was obtained using chromatographic methods. Crystallization was performed with vapor diffusion method to prepare the ligand-free hOGG1(WT) crystals. A mutant of hOGG1 (hoGG1[K249X]) was prepare to trap the reaction intermediate. In addition, hOGG1(K249X)-8oxoG DNA complex crystals by co-crystallization using the hOGG1(K249X) mutant, which has high activity in the acidic region and low activity in the neutral to basic regions. The obtained crystals (Fig. 1) were subjected to X-ray diffraction experiments using the synchrotron radiation facility, PF, and the structures were obtained by molecular replacement method.

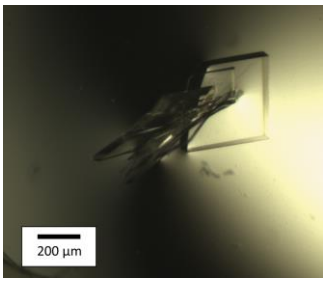

Fig. 1 Crystals of hOGG1(K249X)-8oxoG DNA complex obtained at a basic condition.

3 Results and Discussion

Previously, structural analysis of the hOOG1 mutant in complex with 8oxoG DNA from the crystals obtained at pH 7.0 showed the progression of the glycosylase reaction during the crystallization process. This has inhibited to analyse the structure. To obtain the static structure, at first, we crystallized the hOGG1(K249X)-8oxoG DNA at basic pH range. From the crystals obtained at the basic condition, we succeeded in obtaining diffraction data and the complete structure of the hOGG1(K249X)-8oxoG DNA at 1.91 Å resolution, which is the structure of the 8oxoG DNA complex before glycosylase reaction. Then the crystals were soaked in acidic solution to proceed the reaction, and the diffraction data was successfully obtained. The determined structure was a glycosylase reaction intermediate structure at 1.63 Å resolution. As a result of structural analysis, ring-opening of the 8oxoG deoxyribose sugar moiety was observed before the *N*-glycosidic bond cleavage. In addition, a water molecule that was present around Ser147 before the reaction moved approximately 2.5 Å toward the space between 8oxoG and the 249th residue during the reaction. These results suggest that the ring opening of the 8oxoG deoxyribose sugar moiety takes place before the *N*-glycosidic bond cleavage, and that a water molecule is activated by the catalytic residues and are involved in the glycosylase reaction.

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