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Trapping Reaction Intermediates of the DNA Oxidative Damage Repair Enzyme, hOGG1 and Their Structures

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1 Introduction

Oxidatively damaged bases in DNA are repaired by the base excision repair (BER) pathway. Human 8-oxoguanine glycosylase (hOGG1) is an enzyme that removes and repairs 8-oxoguanine (8oxoG), an oxidatively damaged base of DNA. hOGG1 catalyses two reactions in BER after recognizing the damaged base: a glycosylase reaction that cleaves 8oxoG and a β-lyase reaction that cleaves the 3' end of the abasic site. For these two reactions, Lys249 and Asp268 have been identified as catalytic residues [1]. Lys249 has been shown to play a central role in the mechanism β-lyase reaction [2], however the mechanism of glycosylase reaction remains unclear. Therefore, we aimed to elucidate the catalytic mechanism of the glycosylase reaction by directly observing the reaction intermediates by X-ray crystallography.

2 Experiment

The hOGG1 gene was expressed using the *E. coli* expression system, and highly purified hOGG1 (K249X) sample was obtained using three types of chromatography. Crystallizations were performed by vapor diffusion method. Crystals of the complex with hOGG1 (K249X), which has glycosylase activity only under acidic conditions (～pH 4.0), and model DNA containing 8oxoG (8oxoG DNA, 16mer) were obtained under basic conditions (pH 8.0). The obtained crystals were soaked in acidic solution at pH 4.0 for various temperatures/periods to proceed the reaction and flash frozen under liquid nitrogen temperature. X-ray diffraction experiments using synchrotron radiation facilities and structural analyses of the crystals were performed.

3 Results and Discussion

The hOGG1 (K249X)-8oxoG DNA complex was crystallized at pH 8.0, and X-ray diffraction data of the crystals in which the state was before glycosylase reaction were successfully obtained at the highest resolution of 1.45 Å. In addition, the structure of the reaction intermediates were successfully obtained at the highest resolution of 1.54 Å from the crystals soaked in the pH4.0 solutions with the various temperatures/periods. The structural analyses suggested that in the crystal of hOGG1 (K249X)-8oxoG DNA complex, a water molecule in the vicinity of Ser147 is activated and is involved in the reaction (Fig. 1). In the crystals after the reaction, the ribose was found to open the ring near the O4' atom but not to dissociate the 8oxoG base, indicating that the mechanism of hOGG1 (K249X) glycosylase reaction is related to that proposed by Ochsenfeld's group in which Asp268 residue was suggested to act as a proton donor [3].

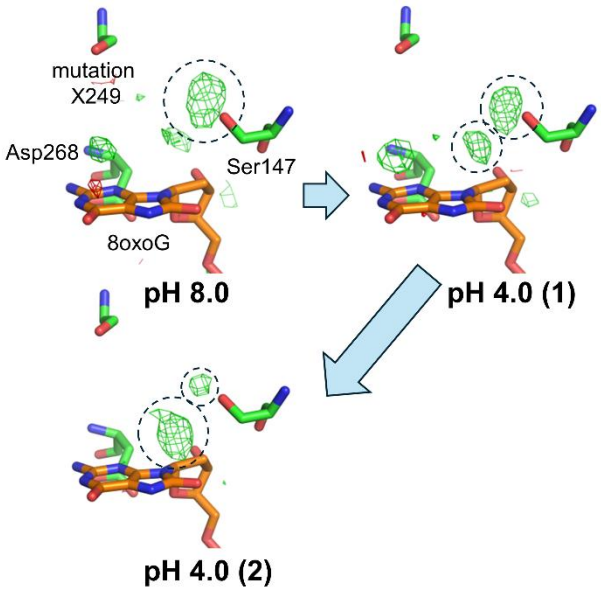

Fig. 1: Activated water molecule in hOGG1(K249X)

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