In aerobic organisms, cellular DNA is easily damaged by activated oxygen species resulting from aerobic energy metabolism or oxidative stress. Highly reactive oxygen accelerates the spontaneous mutation rate and therefore has been implicated as a causative agent for aging or the pathogenesis of disease, including cancer. One of the most common products of oxidative DNA damage is the 8-oxoguanine (GO) lesion. GO can pair with cytosine (C) as well as adenine (A), causing conversion from guanine (G) to thymine (T). To prevent mutation, the MutM protein removes GO bases from GO:C pairs in DNA. The \textit{mutM} (\textit{fpg}) gene encoding the MutM protein is highly conserved across a wide range of aerobic bacteria. These enzymes (\textit{Mr}= 30 \text{ kDa}) possess the invariant N-terminal sequence Pro-Glu-Leu-Pro-Glu-Val-, two strictly conserved lysine residues (Lys52 and Lys147), and a zinc finger motif (-Cys-X2-Cys-X16-Cys-X2-Cys-) at the C-terminus.

We determined the structure of the MutM enzyme derived from an extremely thermophilic bacterium, \textit{Thermus thermophilus} HB8 at 1.9 Å resolution using MAD phasing of the intrinsic Zn\textsuperscript{2+} ion of the zinc finger at beamline BL45XU [1-4]. The crystal structure of MutM comprises two distinct domains and a new fold connected by a flexible hinge (Fig. 1). Two molecules are detected in an asymmetric unit within the crystal. The overall conformations of the two independent molecules

**Fig. 1.** Overall structure of MutM. The MutM molecule consists of an N-terminal domain (blue), a C-terminal domain (red, orange and green) and two long loops (yellow). The N-terminal domain consists of a two-layered \( \beta \)-sandwich with two alpha helices. The C-terminal domain consists of four \( \alpha \)-helix bundles (red and orange) and a \( \beta \)-hairpin loop of the zinc finger motif (green).
indicate the existence of significant hinge movement, whereas the individual domains of the two molecules in the asymmetric unit are substantially similar (Fig. 2B). A large, electrostatically positive cleft lined by highly conserved residues exists between the domains.

Based on previous biochemical experiments and the three-dimensional structure, we constructed a structural model of the dsDNA-MutM complex (Figs. 2A and 3) and propose a new reaction mechanism for MutM (Fig. 4). The locations of the putative catalytic residues and the two DNA-binding

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**Fig. 2.** (A) The model of the MutM - DNA complex between the flipped-out DNA and MutM in the closed form was obtained by molecular dynamic calculation. The kinked DNA is drawn as a CPK model with backbones colored in steel blue and with bases in steel gray. C and GO bases and their sugar residues before and after flipping out are shown by ball-and-stick models. All the four conserved regions (I-IV) are in the large cleft of the MutM molecule. The N-terminal domain has access to the major groove of DNA and the zinc finger motif of the C-terminal domain to the minor groove. The H2TH motif of the C-terminal domain is situated near the active site, and may interact with the damaged base of the DNA backbone. (B) There are two conformers in an asymmetric unit. The conformations of the two long loops in the inter-domain cleft differ between the two conformers. These two long loops would work as a hinge in domain movement.

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**Fig. 3.** Architecture of the active site of MutM. (A) The nucleophile Pro1 of MutM is surrounded by the invariant charged residues Glu2, Glu5 and Lys52, accompanied by several bound water molecules. (B) Model of docking of the flipped-out GO nucleotide to MutM active site bases on the dsDNA complex. (C) The putative reaction intermediate adduct with Pro1 after β-elimination, which is well defined due to its conjugated double bond and can fit to the active site; the hydroxyl group at C4’ of the opened deoxyribose reaches to the carboxylic acid of Glu2, which is a good candidate for proton acceptor.
motifs (the zinc finger and the helix-two turns-helix motifs) suggest that the oxidized base is flipped-out from double-stranded DNA and excised by a catalytic mechanism in a manner similar to that of bifunctional base-excision-repair enzymes. This model detailing the formation of a dsDNA-MutM complex accounts for the multiple enzymatic activities assigned to MutM. The DNA glycosylase excises various damaged bases from DNA by forming a covalent Schiff base intermediate, which is formed at the damaged site by the nucleophilic attack of the Pro1 secondary amino group at the N-terminus of the deoxyribose C1’ to produce an aldehydic abasic site. The AP lyase cleaves the 3’-phosphodiester bond at AP sites through β-elimination. In addition, an alternative AP lyase cleaves the 5’-phosphodiester bond through δ-elimination. These reaction mechanisms also explain the different N-glycosylase/AP-lyase activities among MutM, *E. coli* endonuclease III and T4 endonuclease V. To confirm the detailed mechanism of the MutM N-glycosylase/AP-lyase reactions, mutational analyses of the active-site residues are currently undertaken.

Fig. 4. Schematic representation of the reaction mechanism of MutM N-glycosylase/AP-lyase. We propose that the invariant amino acid residues (Glu2, Glu5, and Lys52) are in the vicinity of the primary catalytic residue Pro1. In this highly electrostatically positive environment, Lys52 may act as a proton donor for depuration of the damaged base (Fig. 3B). After the C2’ of deoxyribose forms a Schiff base with Pro1, Glu5 can withdraw the proton of C2’ via a bound water molecule, leading to β-elimination. The resulting adduct intermediate (Fig. 3C) would deprotonate at C4’ of the opened deoxyribose, leading to δ-elimination. Finally, the recapture of the proton by Lys52 would release the other product, 4-oxo-2-pentenal, to form the gapped dsDNA product. The residues that will contribute to each reaction step were deduced from the crystal structure and are shown in red.

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