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Crystallization and preliminary X-ray analysis of *Escherichia coli* MutT in binary and ternary complex forms

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During replication, *Escherichia coli* MutT prevents the misincorporation of mutagenic 8-oxoguanine into nascent DNA strands opposite adenine by hydrolyzing 8-oxo-dGTP in nucleotide pools to 8-oxo-dGMP. *E. coli* MutT is the most widely investigated member of the Nudix hydrolase family, which is large and found in all organisms. By co-crystallization of MutT with 8-oxo-dGMP, a reaction product, crystals of the binary complex were obtained using ammonium sulfate as a precipitant. The crystals belong to space group $P2_12_12_1$, with unit-cell parameters $a = 37.9$, $b = 56.0$, $c = 59.4$ Å. Assuming the presence of one protein–nucleotide complex in the asymmetric unit, the Matthews coefficient V_M is 2.1 Å^3 Da^{-1}. Crystals of the ternary complex were prepared by soaking crystals of the binary complex in 1 m*M* $MnCl_2$ solution. They diffracted to 1.96 and 2.56 Å resolution, respectively.

1. Introduction

The integrity of DNA is constantly challenged by a number of endogenous and environmental DNA-damaging agents (Lindahl, 1993). Hydroxyl radicals easily react with the C8 position of guanine to generate highly mutagenic 8-oxoguanines that can mispair with adenine during replication and transcription. 8-Oxoguanines occur in the chromosomal DNA, RNA and free nucleotides such as dGTP and GTP. *Escherichia coli* MutT protein (129 amino acids, M_r = 14 900) hydrolyzes 8-oxo-dGTP and 8-oxo-GTP to their corresponding nucleoside monophosphates and inorganic pyrophosphate in the presence of Mg^{2+} (Maki & Sekiguchi, 1992; Taddei *et al.*, 1997). These events effectively prevent the misincorporation of 8-oxoguanine opposite adenine. Therefore, *mutT*-defective mutants cause a 100- to 10 000-fold increase in the occurrence of the A:T to C:G transversion. On the other hand, 8-oxoguanines paired with cytosines in the chromosomal DNA are removed by MutM, while MutY removes adenines paired with 8-oxoguanine in *E. coli* (Tajiri *et al.*, 1995). Similar enzymes are found in humans and many other species. In humans, MTH1, OGG1 and MYH are functional homologues of MutT, MutM and MutY, respectively (Furuichi *et al.*, 1994; Nakabeppu, 2001). Thus, all forms of living cells are protected from nucleic acid base damage caused by oxygen attack.

It is interesting to note the structural basis for the specificity of these enzymes in recognizing 8-oxoguanines. Solution structures of *E. coli* MutT have been determined (Abeygunawardana *et al.*, 1995; Lin *et al.*, 1997) and several recognition models of 8-oxo-dGMP have been proposed using NMR studies and mutational analyses (Massiah *et al.*, 2003; Saraswat *et al.*, 2004). It is uncertain whether there is an accurate model amongst those that have been proposed. Structures of hOGG1, MutM and MutY complexed with 8-oxoguanine-containing DNA have been determined by X-ray crystalloraphic analyses, (Bruner *et al.*, 2000; Fromme & Verdine, 2003; Fromme *et al.*, 2004). Unexpectedly, the O atoms at position C8 of the 8-oxoguanine moiety did not interact with any residues of enzymes in the crystal structures of hOGG1 and MutM. The discrimination of 8-oxoguanine from guanine is a result of protonation at N7 accompanied by oxidation of C8 in these enzymes. Whether this is a universal recognition mechanism for 8-oxoguanine in other enzymes is a question that needs to be addressed. The apparent K_m of *E. coli* MutT for 8-oxo-dGTP is 2300-fold lower than that for dGTP (Maki & Sekiguchi, 1992). On the other hand, the substrate-specificity of hOGG1, MutM and MutY is not very high. For example, the kinetic preference of MutY for A:8-oxoG is only sixfold greater than that for A:G (Porello *et al.*, 1998) and MutM possesses the ability to recognize several bases. The implications of this higher specificity of MutT for 8-oxo-dGTP remain unknown.

In addition, *E. coli* MutT is the most investigated protein in the Nudix (nucleoside diphosphate linked to some other moiety *X*) hydrolase superfamily. Nudix superfamily members contain the conserved MutT signature, $GX_5EX_7REUXEEXGU$, where *U* is a hydrophobic residue and *X* is any amino acid

(Bessman *et al.*, 1996). Several studies on *E. coli* MutT have shown that this common region plays an important role in the hydrolysis of the nucleoside diphosphate linked to some moiety. However, there are some results that are unexplainable; for example, by NMR studies it was interpreted that Glu53, Glu56, Glu57 and Glu98 were coordinated to the metal ion (Harris *et al.*, 2000); however, their contribution to the hydrolysis differed considerably. Besides, there is little information about the crystal structures of Nudix enzymes with active metals.

The crystallization of *E. coli* MutT has been described previously (Bessman *et al.*, 1991); however, no X-ray structure has been reported so far. In this study, we have obtained crystals of *E. coli* MutT in a complex with 8-oxo-dGMP. We have also presented preliminary X-ray diffraction studies of the binary and ternary complexes with 8-oxo-dGMP (MutT–8oxodG) as well as 8-oxo-dGMP and Mn^{2+} (MutT–8oxodG–Mn^{2+}), respectively.

2. Crystallization

Details of the protein expression and purification will be published elsewhere. The purified protein solution was concentrated to about 12 mg ml^{-1}. 8-Oxo-dGMP was synthesized by the oxidation of dGMP with hydrogen peroxide (Bialkowski & Kasprzak, 1998).

The initial screening of co-crystallization conditions of MutT and 8-oxo-dGMP was carried out by the hanging-drop vapour-diffusion method using Hampton Crystal Screen 2 and Lite kits. 1 µl protein solution and an equal quantity of 8-oxo-dGMP solution were mixed with 2 µl reservoir solution and equilibrated at 288 K. Initially, a large number of thin needle-shaped crystals appeared under several conditions. A systematic optimization of crystallization conditions resulted in thick and long needle-shaped crystals. The best crystals of MutT–8oxodG were obtained from a droplet containing 3 mg ml^{-1} protein, 2 m*M* 8-oxo-dGMP, 5 m*M* Tris–HCl pH 7.5, 0.25 m*M* EDTA, 1.25%(*v*/*v*) glycerol, 0.25 m*M* 2-mercaptoethanol, 0.1 *M* potassium sodium tartrate, 0.05 *M* sodium citrate pH 5.6 and 1.0 *M* ammonium sulfate equilibrated against a reservoir containing 0.2 *M* potassium sodium tartrate, 0.1 *M* sodium citrate pH 5.6 and 2.0 *M* ammonium sulfate after two weeks (Fig. 1). Crystals of MutT–8oxodG–Mn^{2+} were obtained by soaking MutT–8oxodG crystals in reservoir supplemented with 1 m*M* $MnCl_2$ for 2 d.

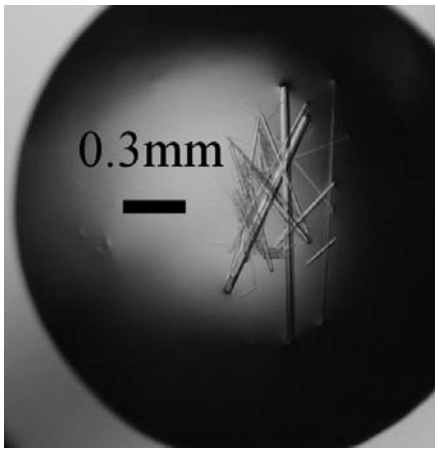

Figure 1
Crystals of MutT–8oxodG. A crystal with dimensions of 0.05 × 0.05 × 1.4 mm was used in data collection.

3. Data collection

Crystals were transferred to a cryoprotectant composed of the above-mentioned reservoir solution with 30% sucrose. Crystals were scooped up with a Cryo-loop (Hampton Research) and immediately flash-frozen in a nitrogen stream at 100 K. MutT–8oxodG and MutT–8oxodG–Mn^{2+} diffraction data were collected at 100 K on beamlines 44XU, 38B1, 41XU and 40B2 of SPring-8 (Harima). Data were processed, integrated and scaled using *HKL*2000 (Otwinowski & Minor, 1997). It was found that the crystals belonged to the space group $P2_12_12_1$, with unit-cell parameters a = 37.9, b = 56.0, c = 59.4 Å for MutT–8oxodG and a = 38.2, b = 56.0, c = 59.3 Å for MutT–8oxodG–Mn^{2+}. There is one complex in the asymmetric unit of each crystal with a volume per unit molecular weight of the protein of 2.1 Å^3 Da^{-1} and a calculated solvent content of 42% (Matthews, 1968). The statistics of the data used for structure determination are listed in Table 1.

Table 1
Data-collection statistics.

Values in parentheses correspond to the highest resolution shell.

Crystal	MutT–8oxodG	MutT–8oxodG–Mn^{2+}
Beamline	SPring-8 BL41XU	SPring-8 BL40B2
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å)	a = 37.9, b = 56.0, c = 59.4	a = 38.2, b = 56.0, c = 59.3
Resolution range (Å)	20–1.96 (2.08–1.96)	18.56–2.56 (2.72–2.56)
No. observed reflections	54195	26502
No. unique reflections	9344	4395
Completeness (%)	97.6 (93.2)	99.2 (95.7)
$R_{\rm merge}$† (%)	6.5 (18.1)	7.7 (15.2)
$\langle I/\sigma(I)\rangle$	29.6 (6.2)	45.9 (22.6)

† $R_{\rm merge} = 100 \times \sum |I_{hkl} - \langle I_{hkl}\rangle| / \sum I_{hkl}$, where $\langle I_{hkl}\rangle$ is the mean value of I_{hkl}.

4. Crystallographic analysis

In order to ascertain the structure of MutT–8oxodG, we carried out molecular-replacement calculations using the crystal structure of the apo form of MutT solved by the MAD method (Nakamura *et al.*, unpublished data) as a search model with the *AMoRe* program (Navaza, 1994). A single solution with a correlation coefficient of 0.43 and an R factor of 49% (8–4 Å) was obtained after the translation-function calculation. The next best solution had a correlation coefficient of 0.18 and an R factor of 57%. The first $2F_o - F_c$ map contoured at the 1σ level after positional refinement (R = 38.6% and $R_{\rm free}$ = 45.0%) using *CNS* (Brünger *et al.*, 1998) showed unambiguous density for 8-oxo-dGMP and conformationally changed loops of MutT. Molecular-replacement searches using NMR structures (PDB codes 1mut, 1tum, 1ppx, 1pun, 1puq and 1pus; Abeygunawardana *et al.*, 1995; Lin *et al.*, 1997; Massiah *et al.*, 2003) as the search model, however, did not yield a solution. The Mn^{2+}-ion sites in MutT–8oxodG–Mn^{2+} were determined by visual inspection (at the 8σ level) of the $F_o - F_c$ map calculated using the MutT–8oxodG structure.

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