Structural study on human oxidative hydrolase using high-quality crystals

Teruya Nakamura and Yuriko Yamagata

Grad. Sch. Pharmaceut. Sci., Kumamoto Univ., Japan


**Mispair caused by 8-oxoG**

8-oxo-dGTP

![Chemical structure of 8-oxo-dGTP]

8-oxoG

Cytosine

![Chemical structure of 8-oxoG and Cytosine mispair]

8-oxoG

Adenine

![Chemical structure of 8-oxoG and Adenine mispair]
MutT family prevents transversion mutations caused by 8-oxo-dGTP

8-oxo-dGTP

E. coli MutT
Human MTH1

8-oxo-dGMP

DNA
Replication
8oxoG
Replication
8oxoG

A → C:G

transversion mutation

Mo J. Y. et al. PNAS (1992)
# Substrate specificity of MutT and MTH1

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-oxo-dGTP</td>
<td>0.081</td>
</tr>
<tr>
<td>dGTP</td>
<td>1,100</td>
</tr>
<tr>
<td>2-oxo-dATP</td>
<td>8.3</td>
</tr>
<tr>
<td>8-oxo-dGTP</td>
<td>15.2</td>
</tr>
<tr>
<td>dGTP</td>
<td>258</td>
</tr>
<tr>
<td>dATP</td>
<td>-</td>
</tr>
</tbody>
</table>

**MutT**

**MTH1**

*Notes*
- 2-oxo-dATP: *Fujikawa K., et al., JBC (1999)*
- 8-oxo-dGTP: *Nakamura T., et al., JBC (2010)*
Recognition of oxidized nucleotides by MTH1

Overall structure of MTH1-8-oxo-dGTP complex

8-oxo-dGTP complex 2.0 Å resolution

2-oxo-dATP complex 2.2 Å resolution

Asp119
Asp120
Gly34
Thr8
Asn33
2-oxo-dATP

Overall structure of MTH1-8-oxo-dGTP complex
MTH1 recognizes 8-oxoG and 2-oxoA by switching the protonation site at the neighboring Asp residues
MTH1 inhibition eradicates cancer by preventing sanitation of the dNTP pool

Stereospecific targeting of MTH1 by (S)-crizotinib as an anticancer strategy

Nature 2014 April
Proposed protonation sites in the reported MTH1-ligand/inhibitor complexes

Determination of H-atom positions by neutron crystallography by ultra-high resolution X-ray crystallography

(S)-crizotinib
IC$_{50}$ = 72 nM

(S)-4
IC$_{50}$ = 6 nM

(TH287
IC$_{50}$ = 0.8 nM

8-oxoG
IC$_{50}$ = 72 nM

2-oxoA
**Wildtype MTH1**

- **Crystals**
  Medium resolution (~2.0 Å resolution) at an acidic pH (<4.5)

- **Purity of crystallization sample**
  Heterogeneous N-termini of Met1 and Gly2 due to MAP activity
  - Met1 form: N term — MGASRLYTLV · · · · · · REVDTV — C term
  - Gly2 form: N term — GASRLYTLV · · · · · · REVDTV — C term

---

**MTH1(G2K) mutant for high-quality crystals**

- **Purity of crystallization sample**
  Homogeneity by inhibition of MAP activity
  - Met1 form: N term — MKASRLYTLV · · · · · · REVDTV — C term
Crystals of MTH1(G2K)

Crystal 1
Reservoir
1.0 M Sodium citrate
0.1 M Imidazole pH8.0
0.3 x 0.2 x 0.2 mm

Crystal 2
Reservoir
2.0 M (NH₄)₂SO₄
10% Glycerol
0.1 M Mg₂SO₄
0.1 M Imidazole pH6.5
0.1 x 0.1 x 0.1 mm

Crystal 3
Reservoir
1.0M Sodium citrate
0.1M Tris-HCl pH7.0
0.2M NaCl
0.2 x 0.4 x 0.1 mm

1.1 Å resolution!

Best crystals of wildtype
Diffracted to 2.0 Å at pH 4.0
Recognition of oxidized nucleotides at neutral pH

8-oxo-dGTP
1.21 Å resolution

2-oxo-dATP
1.20 Å resolution
Structures of hydrolase motif at neutral pH

8-oxo-dGMP

Hydrolysis in crystal = Active form
Recognition of oxidized nucleotides at neutral pH

8-oxo-dGTP

1.21 Å resolution

Asp119

Asp120

Asn33

Asn33

Asp120

Asn/Ala

8-oxo-dGTP

2-oxo-dATP

1.20 Å resolution

Asp119

Asp120

Asn33

Asn33

Asp120

Asn/Ala
**Enzymatic activities of hMTH1 mutants**

### 8-oxo-dGTP activity

- **G2K**
- **G2K/D120N**
- **G2K/D120A**

![Graph showing 8-oxo-dGTP activity with different enzyme concentrations and activities.]

### 2-oxo-dATP activity

- **G2K**
- **G2K/D120N**
- **G2K/D120A**

![Graph showing 2-oxo-dATP activity with different enzyme concentrations and activities.]

**Key Structures:**
- **Asp120 Accepter**
- **Asp120 Donor**
- **8-oxoG**
- **2-oxoA**
Asn120

D120N mutant

8-oxo-dGTP

\[ K_m: \text{5-fold } \uparrow \]
\[ k_{cat}: \text{5-fold } \downarrow \]

D120N vs WT

D120A mutant

8-oxo-dGTP

\[ K_m: \text{5-fold } \uparrow \]
\[ k_{cat}: \text{2-fold } \downarrow \]

D120A vs WT

Structures of D120 mutants in complex with 8-oxo-dGTP

D120N vs WT

D120A vs WT
Asn120

D120N mutant

2-oxo-dATP
Similar to WT

D120A mutant

2-oxo-dATP

$K_m$: 6-fold $\uparrow$

$k_{cat}$: Similar to WT
MTH1 recognizes 8-oxoG and 2-oxoA by switching the protonation site at the neighboring Asp residues
Further improvement of crystal quality

Ultra-high resolution X-ray crystallography (Neutron crystallography)

- Mutation of Cys residues (Cys -> Ser or Ala)
- Preparation of big crystals using macro-seeding technique

![Crystal Image](image)

Resolution (Å) 0.98
Completeness (%) 98.4 (79.0)
Redundancy 8.1 (4.4)
$I/\sigma(I)$ 58.4 (7.6)
$R_{merge}$ 6.9 (29.8)

Collected at PF NW12A
Further improvement of crystal quality

Ultra-high resolution X-ray crystallography (Neutron crystallography)

- Mutation of Cys residues (Cys -> Ser or Ala)
- Preparation of big crystals using macro-seeding technique
Further improvement of crystal quality

Ultra-high resolution X-ray crystallography

- High quality protein crystal growth experiment using “Kibo”
  Collaborated with JAXA

Resolution (Å) 0.97
Best record!
Completeness (%) 98.6 (99.1)
Redundancy 6.6 (4.5)
$I/\sigma(I)$ 46.4 (2.8)
$R_{merge}$ 7.1 (66.7)

Collected at SPring-8 BL44XU
Acknowledgement

Kumamoto University
    Keisuke Hirata, Shaimaa Waz, Dr. Mami Chirifu, Dr. Shinji Ikemizu

Maruwa Foods and Biosciences, Inc
    Dr. Koji Inaka

JAXA
    JAXA-GCF project members

Confocal Science, Inc
    Dr. Hiroaki Tanaka

JAEA
    Dr. Takeshi Hiromoto, Dr. Takao Arimori, Dr. Motoyasu Adachi,
    Dr. Taro Tamada, Dr. Ryota Kuroki

Kyushu University
    Dr. Yusaku Nakabeppu