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Covalent binding of uracil DNA glycosylase UdgX to abasic DNA upon uracil excision

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Supplementary Table 1. Data collection and refinement statistics (molecular replacement)

	Apo-UdgX	UdgX-5mer ssDNA	UdgX-UMP	UdgX-uracil	UdgX-H109S mutant	UdgX-E52Q mutant
Data collection						
Space group	P2	P222	P2	P2	P2	P2
Cell dimensions <i>a</i> , <i>b</i> , <i>c</i> (Å)	36.32, 51.64, 54.36	36.83, 64.45, 82.90	36.44, 51.21, 54.53	36.48, 51.77, 55.11	36.22, 51.39, 55.44	36.40, 51.60, 54.44
α , β , γ (°)	90.00, 105.31, 90.00	90.00, 90.00, 90.00	90.00, 104.98, 90.00	90.00, 104.98, 90.00	90.00, 105.21, 90.00	90.00, 105.35, 90.00
Resolution (Å)	50.00-1.314 (1.36-1.34) *	50.00-2.28 (2.32- 2.28)	50.00-1.33 (1.35- 1.33)	50.00-1.34 (1.36- 1.34)	50.00-1.63 (1.66- 1.63)	50.00-1.34 (1.36- 1.34)
<i>R</i> _{merge}	0.077 (0.187)	0.098 (0.938)	0.057 (0.153)	0.054 (0.238)	0.063 (0.129)	0.061 (0.383)
<i>I</i> / σ <i>I</i>	56.32 (18.20)	31.21 (1.7)	47.76 (5.00)	54.35 (10.33)	56.00 (28.67)	33.05 (4.64)
Completeness (%)	96.2 (86.1)	99.0 (86.1)	96.9 (68.3)	97.7 (98.4)	97.4 (92.0)	96.7 (98.2)
Redundancy	7.5 (6.6)	11.9 (8.2)	6.8 (5.5)	6.9 (5.9)	6.2 (5.9)	3.3 (3.0)
Refinement						
Resolution (Å)	1.34	2.28	1.33	1.34	1.63	1.34
No. reflections	42199	8569	43193	43379	23412	14956
<i>R</i> _{work} / <i>R</i> _{free}	13.3/16.0	19.4/26.5	17.2/18.6	13.84/16.57	15.99/19.71	17.34/19.33
No. atoms						
Protein	1539	1501	1531	1532	1538	1563
Ligand/ion	8	21	28	16	8	8
Water	190	15	244	299	216	250
<i>B</i> -factors						
Protein	15.05	54.17	20.60	20.15	24.62	20.71
Ligand/ion	14.06	52.79	19.04	17.23	23.48	18.95
Water	8.1	54.80	20.11	13.02	16.48	12.3
R.m.s. deviations						
Bond lengths (Å)	23.41	51.59	30.41	35.51	33.09	31.97
Bond angles (°)	0.007	0.019	0.008	0.007	0.007	0.007
	0.92	1.9	1.11	0.89	0.94	0.93

Each dataset was collected from a single crystal. *Values in parentheses are for highest-resolution shell.

Supplementary Figure 1

Multiple sequence alignment of *MsmUdgX* proteins with other UdgXs and Family 4 UDGs from other bacteria and the secondary structure of *MsmUdgX* protein. The signature motif UdgX is highlighted with a red box. Conserved motifs A and B are underlined in blue. Residues involved in the catalytic dyad are indicated with red diamonds. Black, dark gray, and light gray shading indicates residues that are conserved in 6/6, 5/6, and 4/6 of proteins, respectively. Accession numbers for these sequences are WP_011726794.1 (UdgX from *M. smegmatis*), WP_017983848.1 (UdgX from *A. methanolic*), WP_006237039.1 (UdgX from *S. xinjiangensis*), WP_011228142.1 (family4 UDG from *T. thermophilus*), WP_004081422.1 (family 4 UDG from *T. maritama*), and WP_013158605.1 (family 4 UDG from *M. silvanus*).

Supplementary Figure 2

(a) Side view of *MsmUdgX* structure emphasizing $\alpha/\beta/\alpha$ sandwich folding. The α -helices and β -strands are colored in blue and yellow, respectively. (b) B-factor putty presentation of the *MsmUdgX* apo-form structure. The wide yellow ribbon represents a higher b-factor compared to the thin blue ribbon with lower b-factor. (c) Electrostatic surface potential (scale of -10 to $+10$ kTe -1) of the apo form of *MsmUdgX*. Acidic and basic regions are colored in red and blue, respectively.

Supplementary Figure 3

Overlap of conserved residues in active site illustrates high similarity in the uracil-binding site of *Tth*UDG (white) and *Msm*UdgX (green) and highlights substitution of water-activating residue N89 in *Tth*UDG by H109 in *Msm*UdgX (green).

Supplementary Figure 4

Deoxyuridine residue with flipped geometry in the active site of *MsmUdgX* (presented with yellow sticks and spheres). Conserved residues in the active site, E52, Q53, P54, F65, N91, H178, and R184, are shown with wheat-colored sticks.

Human UDG (family 1)

MsmUdgX

Supplementary Figure 5

Interaction with the phosphate (orange spheres) which is 5' to deoxyuridine in family 1 human UDG (left) and *MsmUdgX* (right).

Supplementary Figure 6

Interaction between backbone phosphate of 5mer ssDNA and positive surface of *MsmUdgX*. Backbone phosphates (orange spheres) observed in the *MsmUdgX*-5mer DNA complex interact with the positively charged surface of *MsmUdgX*. Electrostatic surface potential is calculated at scale of -10 to $+10$ kTe^{-1} and colored red and blue for acidic and basic regions, respectively.

Supplementary Figure 7

Hydrogen bonding interactions (dashed line) of *MsmUdgX* with the phosphate, 3' to deoxyuridine (labeled P(+1)) and the next phosphate (labeled P(+2)).

Supplementary Figure 8

The $F_O - F_C$ electron density map around the ligand in the structure of *MsmUdgX* and uracil containing 5mer ssDNA. The density map is contoured at the 2.0σ level.

Supplementary Figure 9

Spectrum of MS/MS analysis with the 2,325.86 Da ion ($m/z = 776.23$); the 993.58 Da ion ($m/z=497.81$) abundance corresponds to the molecular weight of RIHKTPSR (993.583 Da). Representative result from three independent replicates is shown.

Supplementary Figure 10

Structure of *MsmUdgX*-dUMP complex. 2Fo-Fc map contoured at 1.0 σ , presented with blue mesh, illustrates strong clear density for dUMP in the active site of *MsmUdgX*.

a

b

c

Supplementary Figure 11

Determination of the status of the covalent bond between *MsmUdgX* and 5mer ssDNA (TTUTT) in crystals. (a) Electrophoresis of the *MsmUdgX* protein (lane 1), the mixture of *MsmUdgX* and 5mer ssDNA reacted for 20 min (lane 2), and the protein crystals (lane 3) obtained 3 weeks after crystallization setup with SaltRxI #18 solution (2.0 M ammonium citrate tribasic, 0.1 M Bis-Tris propane pH 7.0). **MsmUdgX* and 5mer ssDNA complex were freshly incubated before the electrophoresis. (b) The *MsmUdgX*-5mer ssDNA complex was incubated in the presence of 2.0 M ammonium citrate tribasic pH 7.0 or 0.1 M Bis-Tris propane pH 7.0 for 3 weeks in 18°C. None of the crystallization chemicals resulted in the cleavage of the bond between protein and DNA. (c) Analysis of pH effect on covalent bond cleavage. *MsmUdgX* was reacted with 5mer ssDNA to allow complex formation and dialyzed against the buffers with various pH ranging pH 6.0 to pH 9.0 before the SDS-PAGE. At none of the pH, the complex was seen to be dissociated. Each shown panels are representative results from three independent replicates.

Supplementary Figure 12

Structure of *MsmUdgX*-uracil complex. Clear electron density for uracil, with the 2Fo-Fc map contoured at 1.0 σ , is observed in the active site of *MsmUdgX*. Crystal structure obtained from solution of *MsmUdgX*-5mer ssDNA complex; its formation was determined via SDS-PAGE electrophoresis.

Supplementary Figure 13

Time course of activity analyses of *MsmUdgX* (WT), and its E52Q and E52A mutants. Assays were performed with the 0.1 pmol of SSU9 DNA oligomer with 125 ng (5.14 pmol) of proteins at 37 °C for 0 min to 20 min. Lanes 3 to 18 in *MsmUdgX* (WT) correspond to reaction times of 0, 5, 10, 20, 40, 60, 120, 180, 240, 300, 420, 540, 660, 840, 1080, and 1200 seconds, respectively. In the E52Q, and E52A mutants, the lanes 4 to 17 correspond to reaction times of 0, 20, 40, 60, 120, 180, 240, 300, 420, 540, 660, 840, 1020, and 1200 seconds, respectively. Reactions were resolved on 8 M urea PAGE (15%) and visualized by phosphoimaging. Plots of the complex accumulation against time for each experiment are presented in the right panels. Values for 20 s time points are indicated in all the plots. Each shown panels are representative results from three independent replicates. Data in graphical graphs on right side represent the mean of three independent experiments $\pm$ s.d.

Supplementary Figure 14

Time course of activity analyses of *MsmUdgX* (WT) and its mutants. The activity assays were carried out with SSU9 DNA substrate at 37 °C for 15 min. The reaction times for lanes 3 to 13 in *MsmUdgX* correspond to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 15 min, respectively. The reaction times for lane 3 to 12 in H109S corresponds to 1, 2, 3, 4, 5, 6, 7, 8, 9 and 15 min, respectively. The reaction times for lane 4 to 13 in H178A, H178S, H109S/H178S and R184A correspond to 1, 2, 3, 4, 5, 6, 7, 8, 9 and 15 min, respectively. The reactions were resolved on 8M urea PAGE (15%) and analyzed by phosphoimaging. Each shown panels are representative results from three independent replicates.

Supplementary Figure 15

(a) Schematic diagram of the experimental conditions used to determine turnover activity. (b) Turnover activity assays for the wild type *MsmUdgX*, and H109S, H109A, H109G, and H109Q mutants. Under condition (A), the protein (0.5 pmol) and 5'-³²P-radiolabeled (hot) substrate (0.1 pmol) were incubated for 20 min. Under condition (B), the protein (0.5 pmol) was reacted for 5 min with excess of unlabeled (cold) substrate (1 pmol) before addition of 5'-³²P-radiolabeled (hot) substrate (0.1 pmol) and further reaction for 15 min. Under condition (C), the protein (0.5 pmol), 5'-³²P-radiolabeled (hot) substrate (0.1 pmol), and unlabeled (cold) substrate (1 pmol) were reacted together for 20 min. The substrate was SSU9 DNA oligomer. The reacted samples were resolved on 8 M urea PAGE (15%). Red squares indicate the areas where radiolabeled product/complex should appear in case of reaction with a turnover. No radiolabeled complex was seen under condition (B) for *MsmUdgX* (WT) suggesting no turnover. However, radiolabeled product was seen under condition (B) for the mutant *MsmUdgX* proteins, suggesting turnover. Representative result from three independent replicates are shown.

Supplementary Figure 16

Isothermal titration calorimetry (ITC) profiles for *MsmUdgX* (a) and H109S mutant (b) to uracil base. The upper panels show the raw data and the bottom panels show integrated heats of injection and the best fit with MicroCal Origin (ver 7.0) software. Representative titration images for three independent replicates are shown.

Supplementary Figure 17

Water molecules in the vicinity of general bases in the active site of *Tth*UDG (left) and *Msm*UdgX H109S (right). Hypothetical position of uracil DNA based on *Msm*UdgX-5mer ssDNA complex, presented as transparent yellow sticks.

Figure 5a

Figure 5b

Supplementary Figure 18
Uncropped gel of Fig. 5a and b.