SUPPORTING INFORMATION

Mutagenic Bypass of an Oxidized Abasic Lesion-Induced DNA Interstrand Cross-link Analogue by Human Translesion Synthesis DNA Polymerases

Wenyan Xu[1], Adam Ouellette[1], Souradyuti Ghosh[3], Tylor C. O' Neill[1], Marc M. Greenberg[3] and Linlin Zhao[1,2] *

[1]*Department of Chemistry and Biochemistry and* [2]*Science of Advanced Materials Program, Central Michigan University, Mount Pleasant, Michigan 48859, USA*

[3]*Department of Chemistry, Johns Hopkins University, Baltimore, Maryland 21218, United States*

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Table S1. Steady-state kinetic parameters for single-base incorporation at the (-1) position (illustrated in Figure 1B) with a regular (Reg) or a DOB-ICL-harboring duplex (ICL). Steady-state kinetic assays of single-nucleotide incorporation were performed at 37 °C with 60 nM 15/30-mer duplex, varying dNTP concentrations, 0.5-20 nM polymerase, 4 % (v/v) glycerol, 5 mM DTT, 50 mM NaCl, 5 mM $MgCl_2$, and 100 μg mL^{-1} bovine serum albumin (BSA) in 50 mM Tris-HCl (pH 7.4). Assays with pol ν contained 10% glycerol and 90 mM KCl. The correct nucleotide is shown in italic.

TLS pols	Template	Nucleotide	k_{cat} (min^{-1})	$K_{m,dNTP}$ (μM)	$k_{cat}/K_{m,dNTP}$ ($min^{-1}\mu M^{-1}$)	f[a]	Decrease in efficiency relative to a regular duplex[b]
pol η	Reg	*dTTP*	5.5 ± 0.5	0.92 ± 0.23	6.0 ± 1.6		
		dATP	1.4 ±0.2	28 ± 13	$(5.0 \pm 2.4) \times 10^{-2}$	8.3×10^{-3}	
		dCTP	0.27 ± 0.02	150 ± 60	$(1.8 \pm 0.7) \times 10^{-3}$	3.0×10^{-4}	
		dGTP	0.33 ± 0.03	160 ± 30	$(2.1 \pm 0.4) \times 10^{-3}$	3.5×10^{-4}	
	ICL	*dTTP*	4.4 ± 0.1	8.4 ± 0.7	$(5.2 \pm 0.5) \times 10^{-1}$		10-fold
		dATP	0.98 ± 0.06	88 ± 15	$(1.1 \pm 0.2) \times 10^{-2}$	2.1×10^{-2}	
		dCTP	0.32 ± 0.02	130 ± 30	$(2.5 \pm 0.6) \times 10^{-3}$	4.8×10^{-3}	
		dGTP	0.85 ± 0.12	230 ± 80	$(3.7 \pm 1.4) \times 10^{-3}$	7.1×10^{-3}	
pol κ	Reg	*dTTP*	14 ± 4	0.25 ± 0.01	56 ± 16		
		dATP	0.68 ± 0.03	200 ± 30	$(3.4 \pm 0.5) \times 10^{-3}$	6.0×10^{-5}	
		dCTP	6.1 ± 0.3	630 ± 60	$(9.7 \pm 1.0) \times 10^{-3}$	1.7×10^{-4}	
		dGTP	3.5 ± 0.2	260 ± 50	$(1.2 \pm 0.3) \times 10^{-2}$	2.3×10^{-4}	
	ICL	*dTTP*	6.9 ± 0.6	6.3 ± 1.8	1.1 ± 0.3		50-fold
		dATP	0.049 ± 0.003	360 ± 50	$(1.4 \pm 0.2) \times 10^{-4}$	1.3×10^{-4}	
		dCTP	0.095 ± 0.003	320 ± 30	$(3.0 \pm 0.3) \times 10^{-4}$	2.7×10^{-4}	
		dGTP	0.022 ± 0.001	290 ± 60	$(7.6 \pm 1.6) \times 10^{-5}$	6.9×10^{-5}	
pol ι	Reg	*dTTP*	8.9 ± 0.8	6.1 ± 0.8	1.5 ± 0.3		

		dATP	0.23 ± 0.01	160 ± 30	$(1.4 \pm 0.3) \times 10^{-3}$	9.3×10^{-4}	
		dCTP	0.16 ± 0.01	590 ± 140	$(2.5 \pm 0.7) \times 10^{-4}$	1.6×10^{-4}	
		dGTP	0.049 ± 0.003	130 ± 30	$(3.7 \pm 0.9) \times 10^{-3}$	2.5×10^{-3}	
	ICL	*dTTP*	0.28 ± 0.01	14 ± 1	$(2.0 \pm 0.2) \times 10^{-2}$		75-fold
		dATP	0.051 ± 0.001	120 ± 7	$(4.3 \pm 0.3) \times 10^{-4}$	2.2×10^{-3}	
		dCTP	0.054 ± 0.003	1900 ± 180	$(2.8 \pm 0.3) \times 10^{-5}$	1.4×10^{-4}	
		dGTP	0.0070 ± 0.0005	230 ± 50	$(3.0 \pm 0.7) \times 10^{-5}$	1.5×10^{-4}	
pol ν	Reg	dTTP	0.085 ± 0.008	3.2 ± 0.6	$(2.7 \pm 0.6) \times 10^{-2}$		
		dATP	0.054 ± 0.002	240 ± 30	$(2.3 \pm 0.3) \times 10^{-4}$	8.5×10^{-3}	
		dCTP	0.092 ± 0.008	190 ± 40	$(4.8 \pm 1.1) \times 10^{-4}$	1.8×10^{-2}	
		dGTP	0.010 ± 0.001	440 ± 110	$(2.3 \pm 0.6) \times 10^{-5}$	8.5×10^{-4}	
	ICL	dTTP	0.060 ± 0.002	100 ± 10	$(6.0 \pm 0.7) \times 10^{-4}$		44-fold
		dATP	0.0072 ± 0.0002	74 ± 12	$(9.7 \pm 1.6) \times 10^{-5}$	0.16	
		dCTP	0.013 ± 0.001	260 ± 30	$(5.0 \pm 0.7) \times 10^{-5}$	0.083	
		dGTP	0.0015 ± 0.0001	73 ± 25	$(2.1 \pm 0.7) \times 10^{-5}$	0.035	
REV1	Reg	*dTTP*	0.062 ± 0.005	40 ± 10	$(1.6 \pm 0.4) \times 10^{-3}$		
		dATP	0.010 ± 0.001	110 ± 30	$(9.1 \pm 2.6) \times 10^{-5}$	5.7×10^{-2}	
		dCTP	1.0 ± 0.1	2.0 ± 0.4	0.50 ± 0.11	310	
		dGTP	0.057 ± 0.002	140 ± 20	$(4.1 \pm 0.6) \times 10^{-4}$	0.26	
	ICL	*dTTP*	0.0073 ± 0.0004	42 ± 8	$(1.7 \pm 0.3) \times 10^{-4}$		9-fold
		dATP	0.0026 ± 0.0001	0.73 ± 0.34	$(3.6 \pm 1.7) \times 10^{-3}$	21	
		dCTP	0.080 ± 0.003	3.9 ± 0.5	$(2.1 \pm 0.3) \times 10^{-2}$	120	
		dGTP	0.0021 ± 0.0001	29 ± 9	$(7.2 \pm 2.3) \times 10^{-5}$	0.42	

[a] Misincorporation frequency, $f = (k_{cat}/K_{m,dNTP})_{incorrect}/(k_{cat}/K_{m,dNTP})_{correct}$.

[b] Decrease in catalytic efficiency relative to an undamaged duplex is calculated from $(k_{cat}/K_{m,dNTP})_{undamaged,\ dTTP}/(k_{cat}/K_{m,dNTP})_{ICL,\ dTTP}$.

Figure S1. LC-MS/MS fragmentation of pol η-catalyzed primer extension with an ICL-containing duplex. A (product ions of m/z 616.7, identical species as m/z 1646.5 but with -6 charge) and B (product ions of m/z 656.0, identical species as m/z 1751.0 but with -6 charge) with product ions that matched theoretical fragmentation patterns labeled. Reactions contained 1 μM pol η, 2 μM primer-template complex; 2% (v/v) glycerol, four dNTPs (100 μM each), 5 mM DTT, 50 mM NaCl, 5 mM $MgCl_2$, and 50 μg mL^{-1} BSA in a total volume of 50 μL.

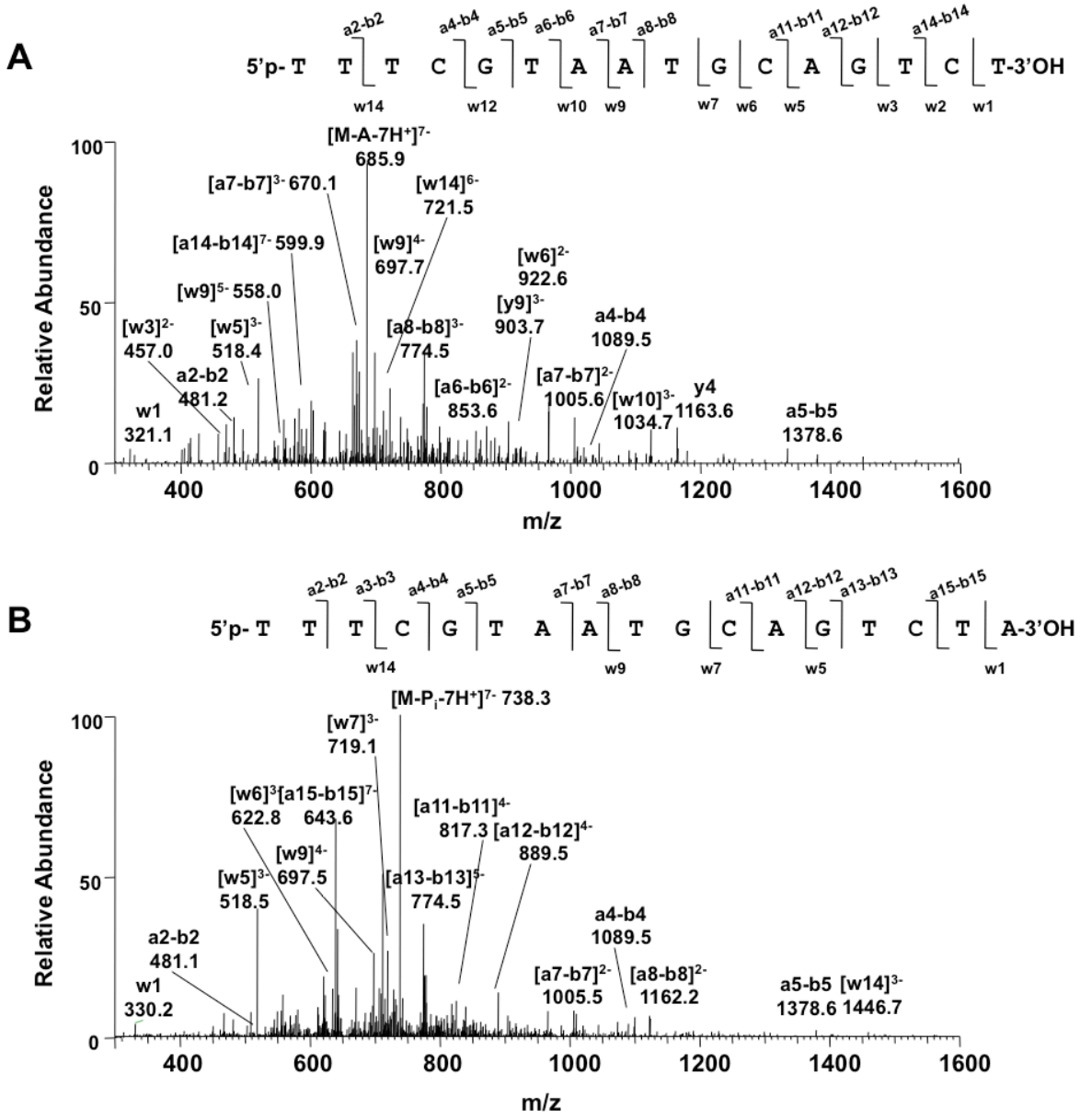

Figure S2. Extracted ion chromatograms (A) and LC-MS/MS fragmentation of authentic standards of full-length extension product (*m/z* 1646.5, $[M\text{-}3H^{+}]^{3-}$) and a product with an extra dA added at the 3'-end (*m/z* 1751.0, $[M\text{-}3H^{+}]^{3-}$). MS/MS fragmentation patterns are shown in B (product ions of m/z 616.7, identical species as m/z 1646.5 but with -6 charge) and C (product ions of m/z 656.0, identical species as m/z 1751.0 but with -6 charge)

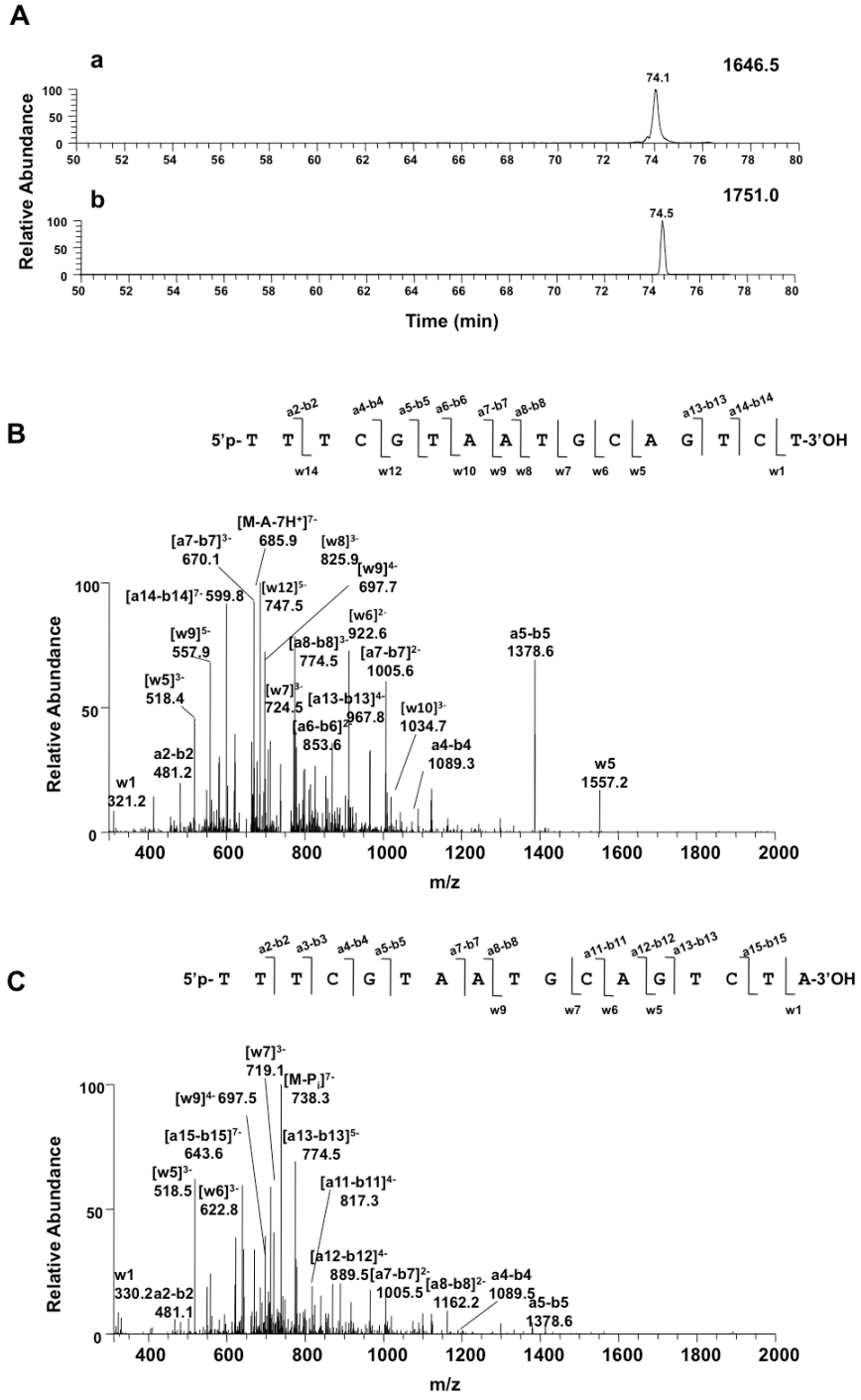

Figure S3. Extracted ion chromatograms and LC-MS/MS fragmentation of authentic standards of two partially extended products (*m/z* 1127.8, $[M\text{-}3H^+]^{3-}$ and *m/z* 1130.8, $[M\text{-}3H^+]^{3-}$). (A) Extracted ion chromatograms. (B) MS/MS fragmentation of *m/z* 1130.8, $[M\text{-}3H^+]^{3-}$. (C) MS/MS fragmentation of *m/z* 1127.8, $[M\text{-}3H^+]^{3-}$.