Supplementary Materials

for

"Kinetics of Deamination and Cu(II)/H_2O_2/Ascorbate-induced Formation of 5-Methylcytosine Glycol at CpG sites in Duplex DNA"

by

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Table S1. Quantitative comparison of the yields (in lesions/10^6 nucleosides) for different types of lesions induced by different doses of Fenton reagents under Conditions A-E shown in Table 1)

Lesions[1]	Control	A	B	C	D	E
16mer self-complementary ODN 5'-XGXGXGXGXGXGXG-3' (X = mC)[2]						
5-HmdC	$(1.31 \pm 0.81) \times 10^2$	$(5.44 \pm 3.38) \times 10^2$	$(1.08 \pm 0.37) \times 10^3$	$(2.75 \pm 0.53) \times 10^3$	$(5.54 \pm 0.94) \times 10^3$	$(1.11\pm 0.03) \times 10^4$
5-FodC	95.9 ± 0.8	$(1.67 \pm 0.80) \times 10^3$	$(2.62 \pm 1.07) \times 10^3$	$(4.90 \pm 1.46) \times 10^3$	$(9.12 \pm 1.83) \times 10^3$	$(1.71 \pm 0.20) \times 10^4$
G[8-5m]mC	14.2 ± 3.8	21.3 ± 3.1	34.5 ± 1.1	62.4 ± 9.9	99.0 ± 3.4	$(1.89 \pm 0.16) \times 10^2$
mC[5m-8]G	1.31 ± 1.21	2.61 ± 2.69	2.75 ± 2.36	3.02 ± 2.29	5.35 ± 3.51	12.7 ± 3.7
16mer self-complementary ODN 5'-AXGTAXGTAXGTAXGT-3' (X = mC)						
5-HmdC	35.4 ± 6.7	$(1.41 \pm 0.39) \times 10^2$	$(4.67 \pm 1.48) \times 10^2$	$(1.32 \pm 0.52) \times 10^3$	$(2.36 \pm 0.93) \times 10^3$	$(5.97 \pm 1.01) \times 10^3$
Tg-dG	59.1 ± 17.4	$(1.81 \pm 0.07) \times 10^2$	$(4.01 \pm 0.48) \times 10^2$	$(8.90 \pm 1.18) \times 10^2$	$(3.48 \pm 0.47) \times 10^3$	$(5.52 \pm 0.49) \times 10^3$
8-oxodG	$(6.35 \pm 0.32) \times 10^2$	$(1.59 \pm 0.84) \times 10^3$	$(4.67 \pm 1.45) \times 10^3$	$(7.73 \pm 0.68) \times 10^3$	$(1.29 \pm 0.16) \times 10^4$	$(3.03 \pm 0.31) \times 10^4$
Tg-(8-oxodG)	5.01 ± 0.91	7.95 ± 3.68	11.1 ± 4.0	28.8 ± 9.6	$(1.56 \pm 0.22) \times 10^2$	$(5.64 \pm 0.11) \times 10^2$

[1] 5-HmdC: 5-(hydroxymethyl)-2'-deoxycytidine; 5-FodC, 5-formyl-2'-deoxycytidine; G[8-5m]mC and mC[5m-8]G, intrastrand cross-link lesions where the C8 of guanine is covalently bonded with the methyl carbon of its neighboring 3' and 5' 5-methylcytosine, respectively.

2 Data were from Ref. (11).

Retention Time	Area
8.283	539
14.333	998
18.533	1149
21.816	342
22.483	410
27.316	229
32.583	728

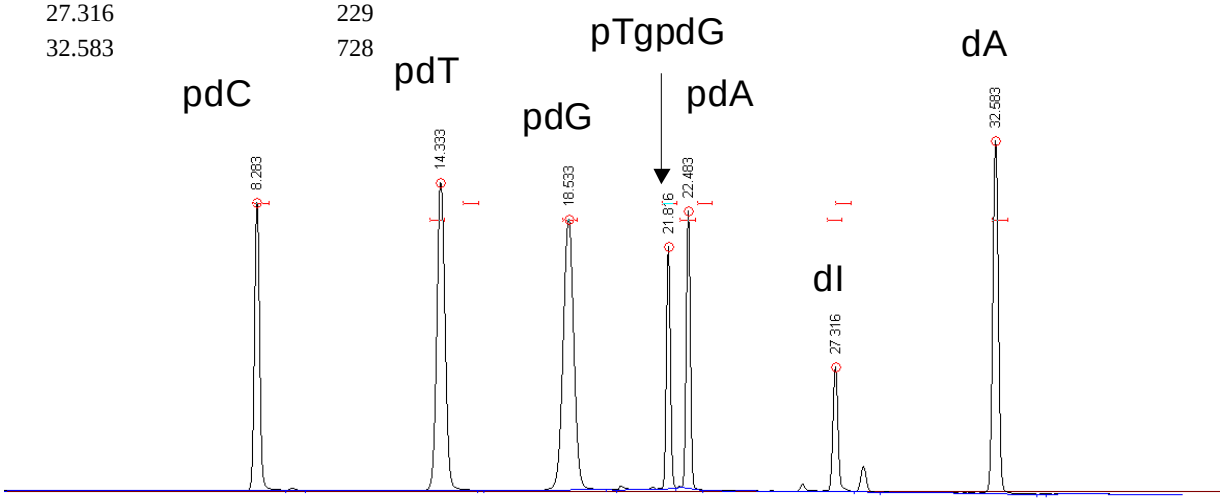


Figure S1. HPLC trace for monitoring the separation of the nuclease P1 digestion mixture of d(ATG GCTG TgGC TAT). The products released from the digestion were confirmed by ESI-MS and MS/MS analyses. The percentage of Tg released as pTgpdG is calculated by the relative peak area of the pTgpdG with respect to pdG and with the consideration that there are three other guanine residues that are released as pdG (342/1149*3 = 89%).

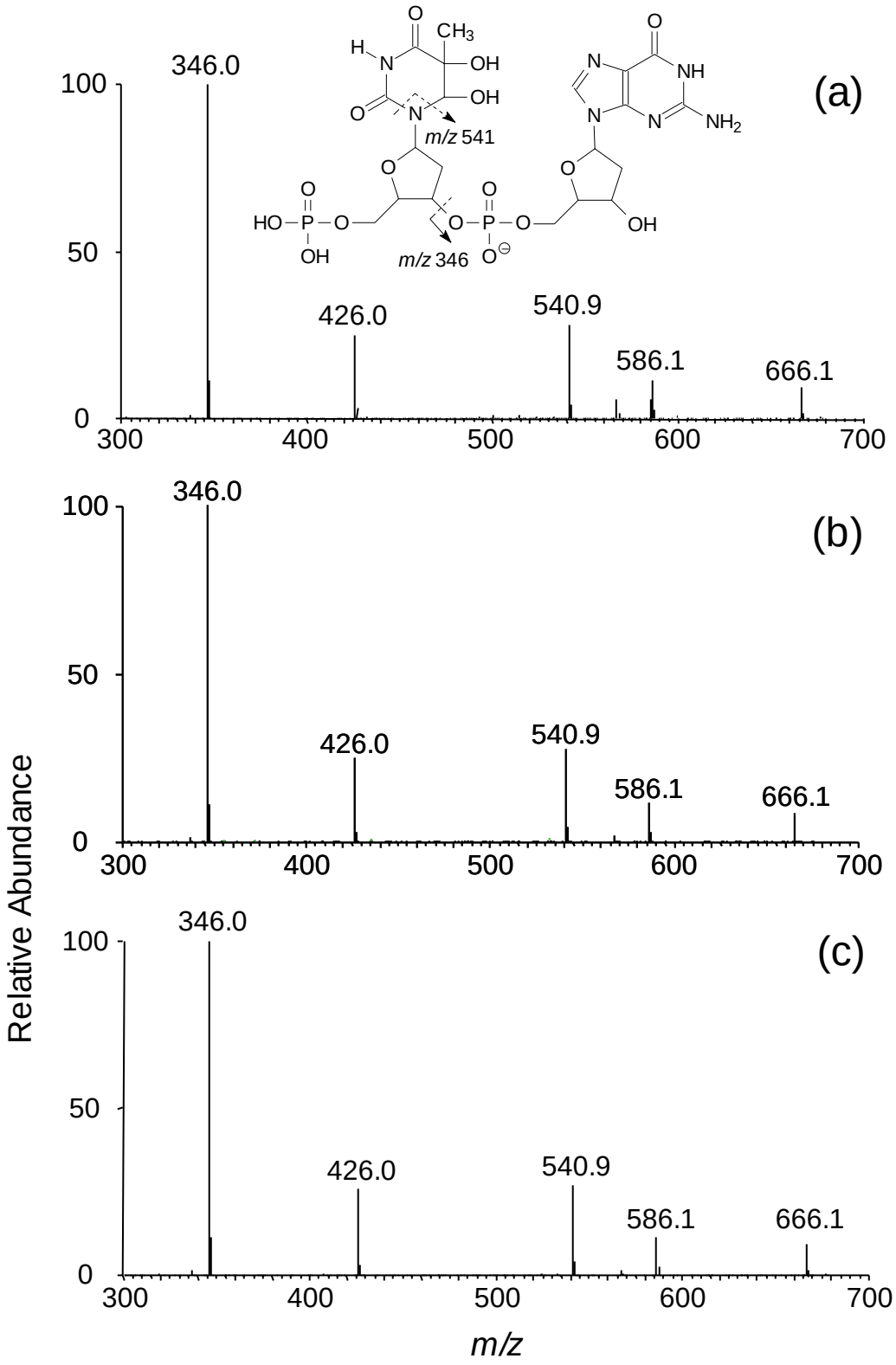


Figure S2. MS/MS of the $[M - H]^-$ ions of dinucleotide pTgpdG generated from the nuclease P1 digestion of: (a) The Fenton-reaction mixture of mC-containing 27-mer duplex DNA (the 19.4-min fraction in Figure 1c); (b) d(ATGGCTgGGCTAT), which contained the authentic (*5R,6S*) diastereomer of Tg (the 19.6-min fraction in Figure 1e); (c) OsO_4 reaction mixture of d(ATGC) (The 19.6-min fraction shown in Figure 1f).

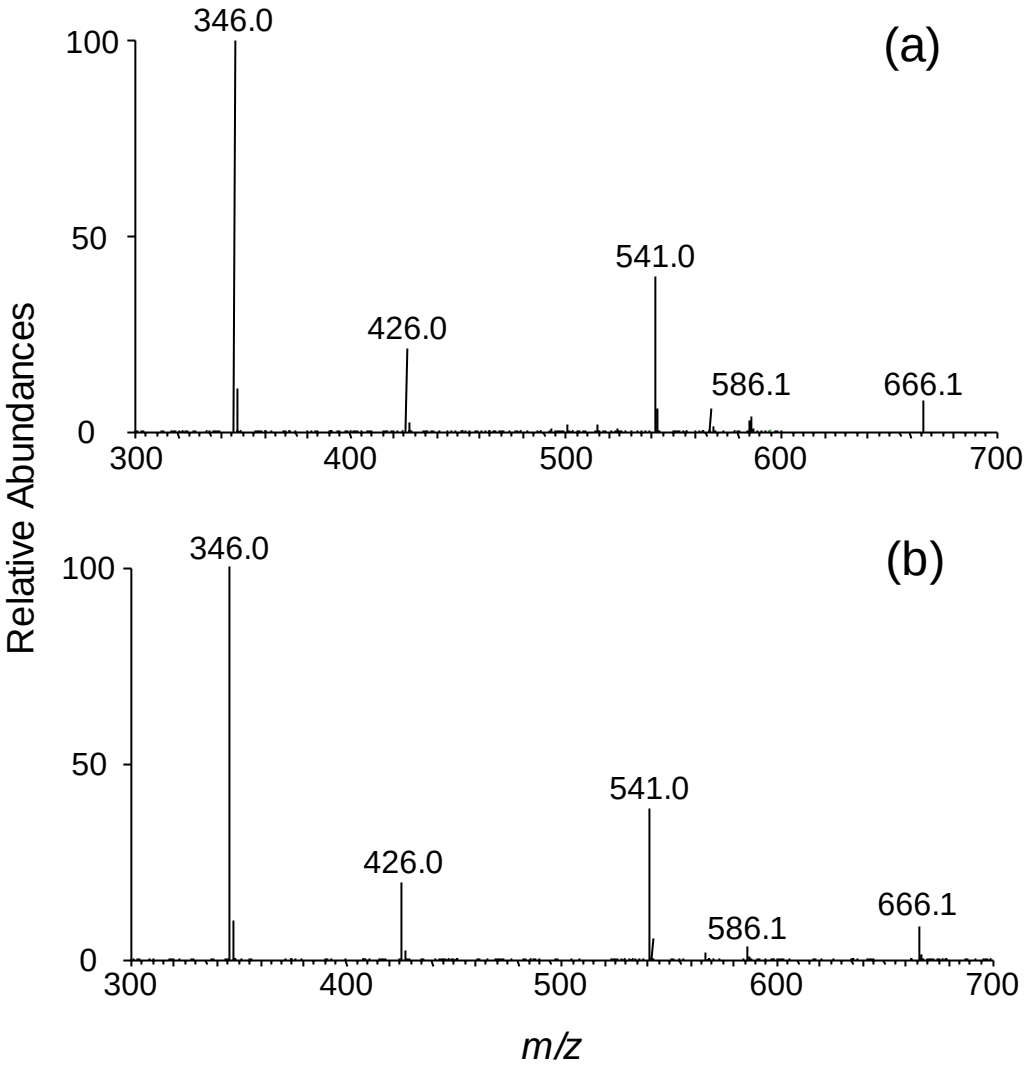


Figure S3. MS/MS of the $[M - H]^-$ ions of dinucleotide pTgpdG generated from the nuclease P1 digestion of: (a) The Fenton-reaction mixture of mC-containing 27-mer duplex DNA (the 20.1-min fraction in Figure 1d); (b) OsO_4 reaction mixture of d(ATGC) (The 20.3-min fraction shown in Figure 1f).

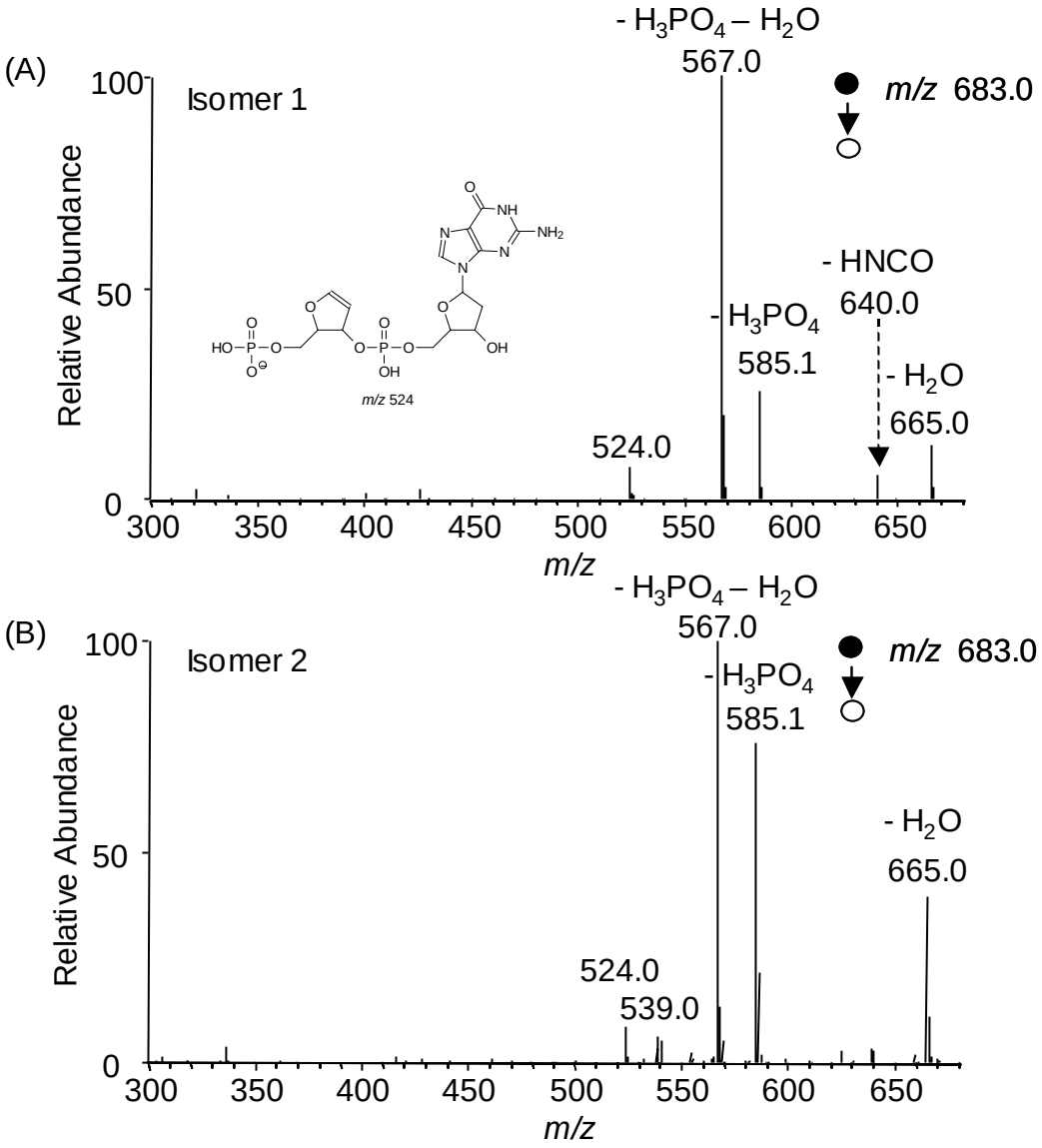


Figure S4. MS/MS of the $[M - H]^-$ ions of dinucleotide p(mdCg)pdG generated from the nuclease P1 digestion of the Fenton-reaction mixture of mC-containing 27-mer duplex DNA: (a) The 19.5-min fraction in Figure 1b); (b) The 20.3-min fraction in Figure 1b. The proposed structure of the fragment ion of *m/z* 524 is depicted in the inset of panel (a).

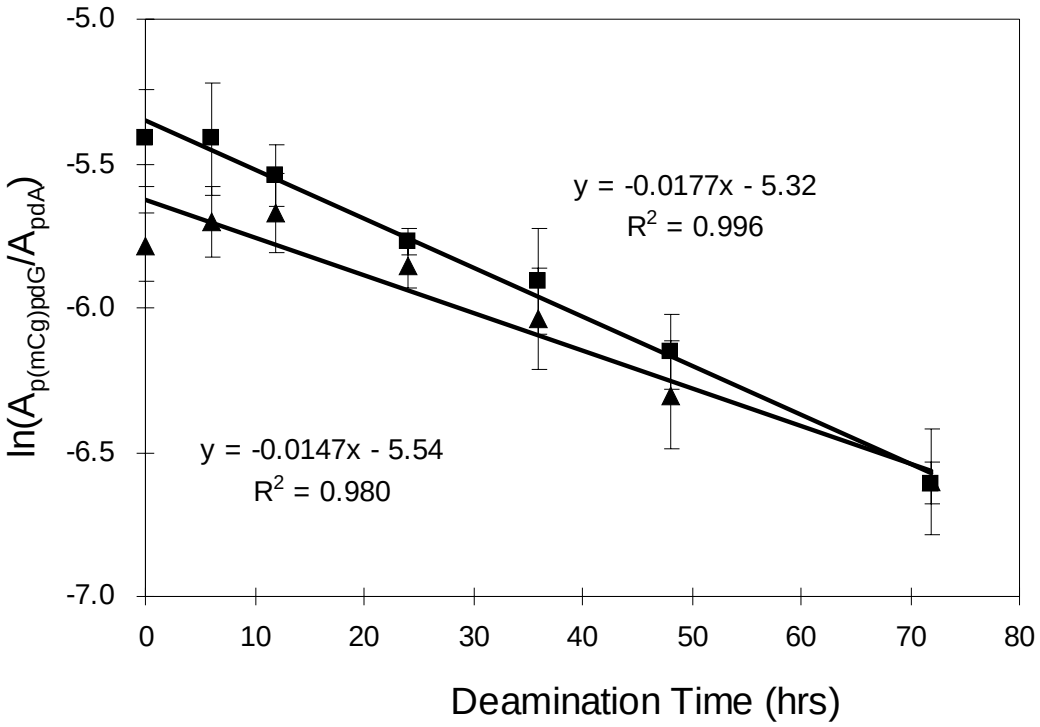


Figure S5. Determination of the first-order rate constants for the deamination of 5-methylcytosine glycol in a 27-mer duplex DNA. Plotted are the ratios of peak area found in the SIC for the *m/z* 683→524, 567 transitions [for p(mdCg)pdG] over that found in the SIC for monitoring the fragmentation of the ion of *m/z* 330 (for pdA) versus the deamination time. The solid square and triangle represent the results based on the formation of the major (the 19.5-min peak in Figure 1b) and minor (the 20.3-min peak in Figure 1d) components of p(mdCg)pdG, respectively. The 27-mer duplex sequence and deamination reaction condition were described in Materials and Methods.

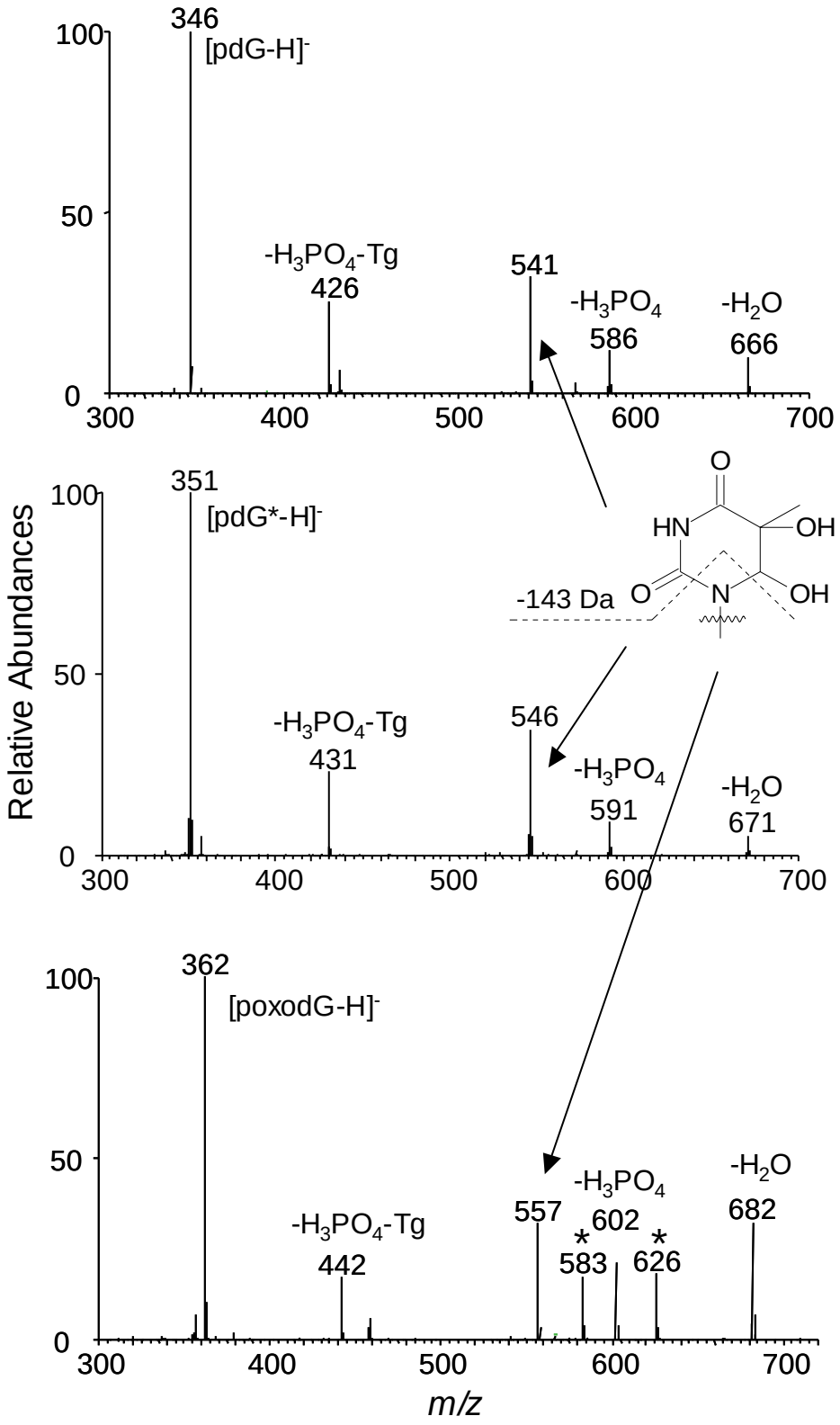


Figure S6. Product-ion spectra of the $[M - H]^-$ ions of the unlabeled pTgpdG (a), labeled pTgpdG* (b), and unlabeled pTgp(8-oxodG). "dG*" represents uniformly ^{15}N-labeled 2'-deoxyguanosine. Ions labeled with "*" are due to the fragmentation of the other co-eluting species.

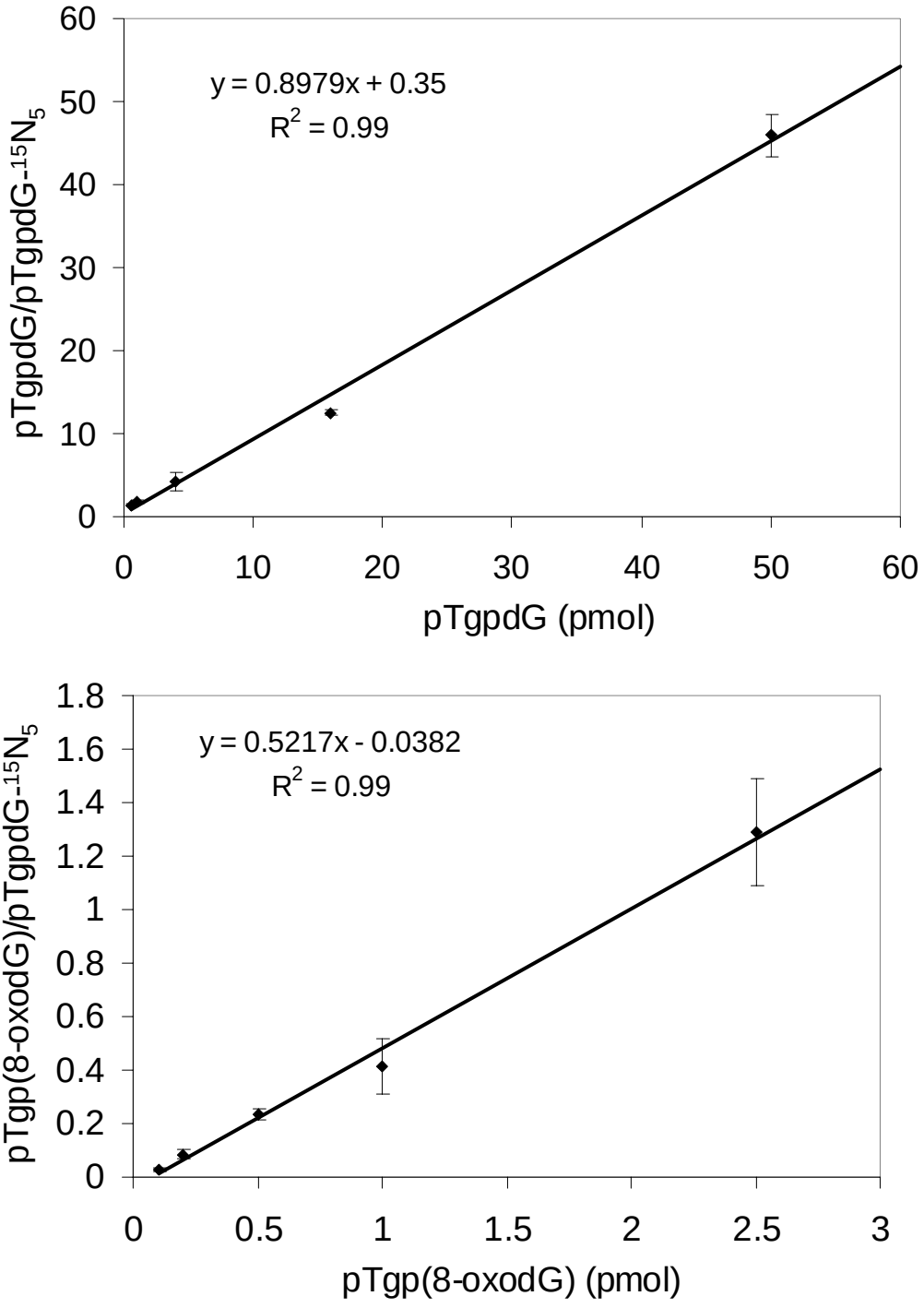


Figure S7. Calibration curves for the quantifications of pTgpdG and pTgp(8-oxodG). The authentic synthetic oligomer containing an isolated pTgpdG or pTgp(8-oxodG) site was used as standard. 4 µL of oligomer mixture containing ^{15}N-labeled pTgpdG from stock solution was added to each Fenton-reaction and calibration standard samples.

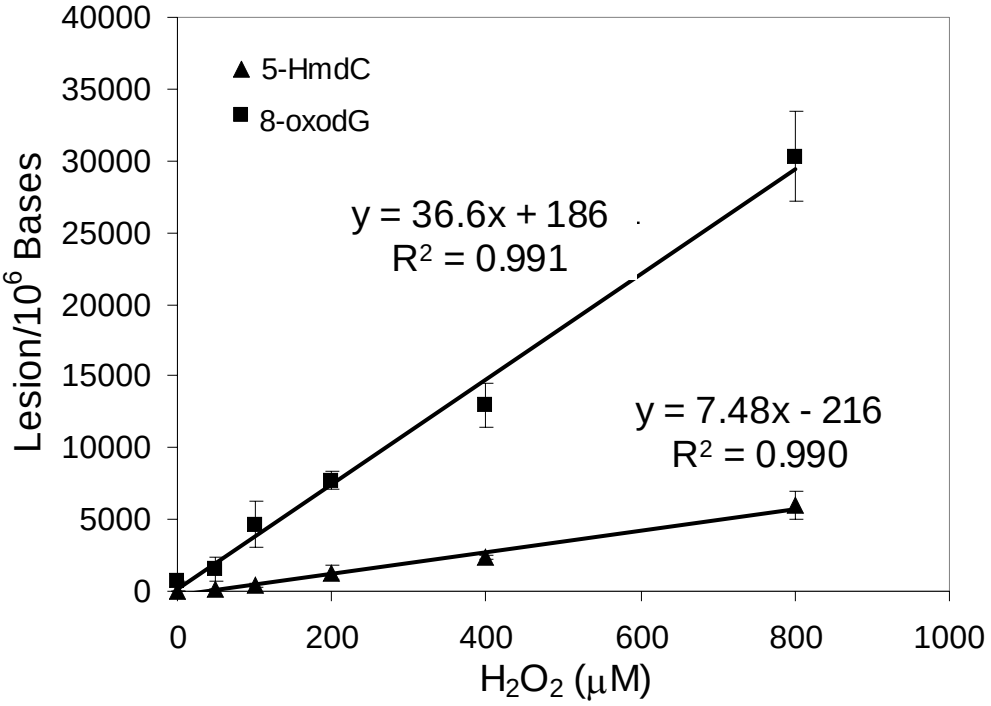
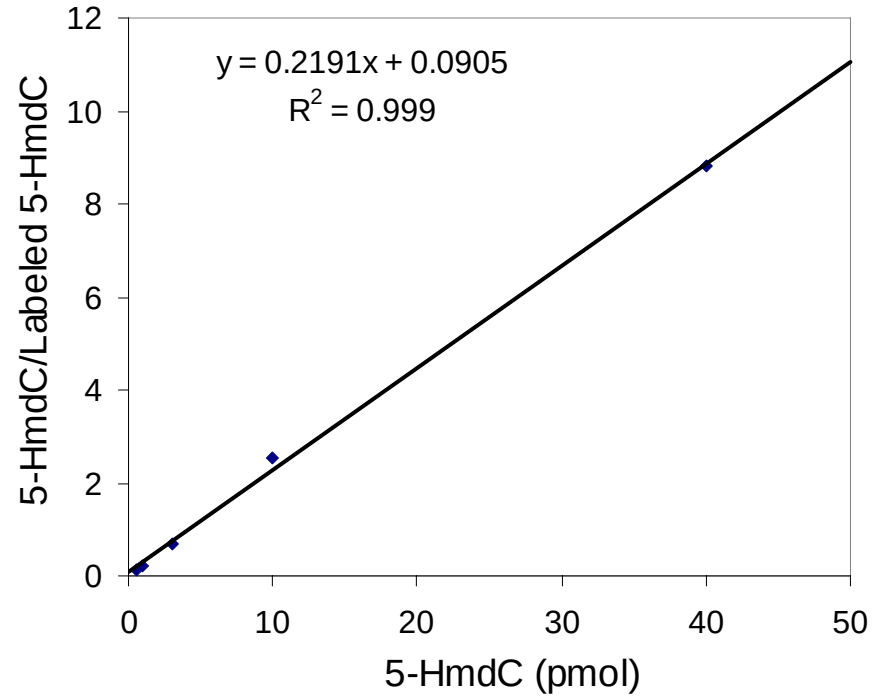


Figure S8. Cu(II)/H_2O_2/ascorbate-induced formation of single-base lesions in ODN d(AXGTAXGTAXGTAXGT) ("X" represents 5-methylcytosine). The values represent the means ± SD from three independent oxidation and quantification experiments.



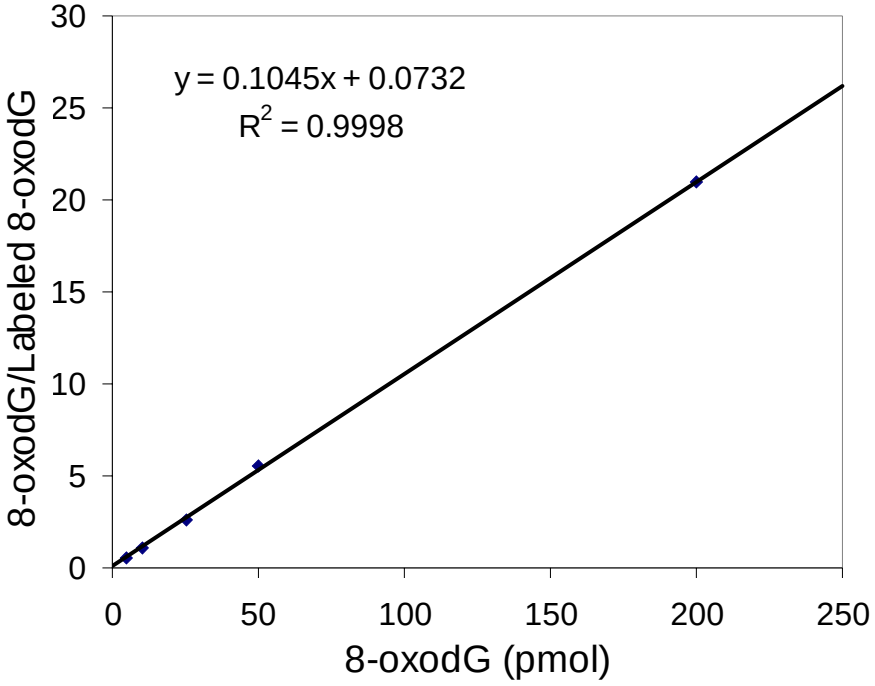


Figure S9. Calibration curves for the quantifications of 5-HmdC (top) and 8-oxodG (bottom). The amount of labeled 5-HmdC and 8-oxodG used for calibration were 5 and 10 pmol, respectively.