DNA-Protein Crosslinks Induced by *Multiple-e^-* Oxidation of Guanosine

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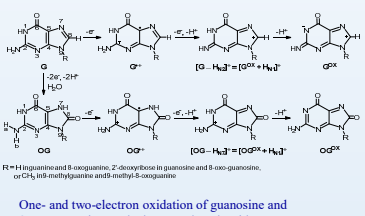
Abstract & Introduction

- DNA–protein crosslinks (DPCs) represent a significant yet poorly understood class of DNA lesions. Oxidation of guanosine (**G**) and its major lesion 8-oxo-guanoisne (**OG**) generates intermediates capable of covalently bonding with nucleophilic amino acid residues. Prior studies focused on reactivities of one-electron-oxidized guanosine radical cations (**G$^{\bullet+}$**) and two-electron-oxidized intermediate [**OG – H**]$^+$(2-amino-7,9-dihydro-purine-6,8-dione), whereas the roles of one-electron-oxidized 8-oxoguanosine radical cations (**OG$^{\bullet+}$**) and two-electron-oxidized non-radical guanine species [**G – H**]$^+$ remain unexplored.
- We investigated systems employing 9-methylguanine (**9MG**) and 9-methyl-8-oxoguanoisne (**9MOG**) as nucleoside analogues and **CD_3NH_2** as a mimic of the lysine ε-amine. Guided-ion-beam MS, complemented by electronic structure calculations, MD simulations, and kinetic modeling, revealed new DPC pathways and helped understand how DPCs arise during the progression of DNA oxidation.



One- and two-electron oxidation of guanosine and 8-oxoguanosine nucleobases and nucleosides.

Instrumentation & Experiment

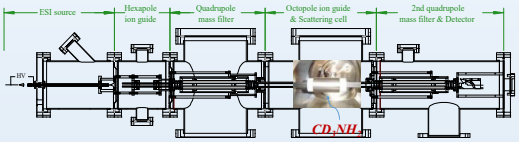
Formation of primary ions via post-ESI, in-source reactions

$[Cu^{II}(\text{nucleobase})_3]^{\bullet 2+} \rightarrow [Cu^{II}(\text{nucleobase})_2]^+$ + **nucleobase$^{\bullet+}$**

nucleobase$^{\bullet+} \rightarrow$ [nucleobase – H]$^\bullet$ + H$^+$

nucleobase$^{\bullet+}$ + [nucleobase – H]$^\bullet \rightarrow$ **[nucleobase – H]$^\bullet$** + nucleobase

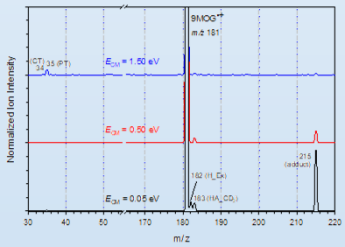
Ion-molecule reactions using ESI guided-ion beam MS



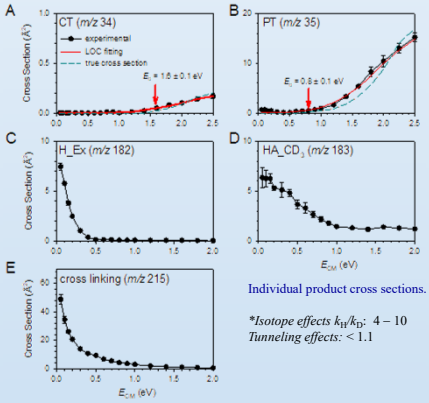
Crosslinking Products, Structures , Mechanisms, & Energetics

1. DPCs between OG$^{\bullet+}$ and CD_3NH_2

***m/z* 34**	$CD_3NH_2^{\bullet+}$ + 9MOG	charge transfer (CT)
***m/z* 35**	$CD_3NH_3^+$ + [9MOG – H]$^\bullet$	proton transfer (PT)
***m/z* 182**	d_1-9MOG$^{\bullet+}$ + CHD_2NH_2	H/D exchange (H_Ex)
	or [9MOG + H]$^+$ + $CD_2NH^\bullet$	amine-H abstraction (HA_NH$_2$)
***m/z* 183**	[9MOG + D]$^+$ + $^\bullet CD_2NH_2$	methyl-H abstraction (HA_CD$_3$)
***m/z* 215**	[CD_3NH_2-9MOG]$^{\bullet+}$ adduct	crosslinking

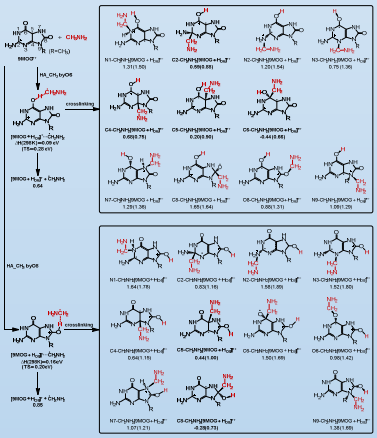


Product-ion MS at different E_{CM}. Primary ion intensities were normalized to a unit for comparation.



Individual product cross sections.

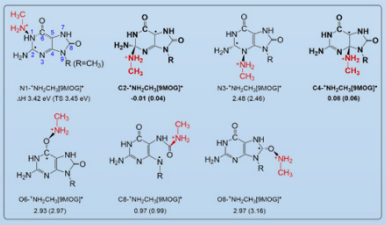
**Isotope effects* k_H/k_D: 4 – 10
Tunneling effects: < 1.1



Representative DPC pathways via **methyl-H abstraction mediated**

and

direct NH_2-addition



Structures in bold represents the most probable DPCs

2. DPCs between [G – H]$^+$ and CD_3NH_2

PT ***m/z* 35**
$CD_3NH_3^+$ + 9MG^{ox}

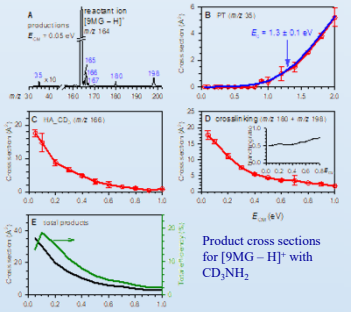
H_Ex ***m/z* 165**
d_1-[9MG – H]$^+$ + CHD_2NH_2

H_NH$_2$ ***m/z* 165**
9MG$^{\bullet+}$ + $CD_3NH^\bullet$

HA_CD$_3$ ***m/z* 166**
d_1-9MG$^{\bullet+}$ + $^\bullet CD_2NH_2$

H$^\ominus$A + PT ***m/z* 167**
d_1-[9MG + H]$^+$ + CD_2NH

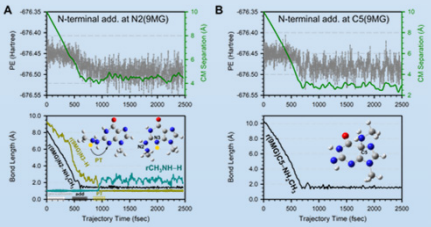
DPC ***m/z* 198**



Product cross sections for [9MG – H]$^+$ with CD_3NH_2

[9MG – H]$^+$ enhances DPCs via direct addition, abstraction of H or H$^\ominus$

1) N2-, N3-, -C5-, and C8-addition, exemplified by trajectory simulations



2) Methyl-H$^\ominus$ abstraction enhances DPCs within primary products

[9MG – H$_{N2}$]$^+$ + CH_3NH_2 →	[9MG – H$_{N2}$ + H$_{C5}$]···CH_2-NH_2	ΔH/TS = -2.39 / -0.47 eV
	[9MG – H$_{N2}$ + H$_{O6}$]···CH_2-NH_2	ΔH/TS = -2.30 / 0.32 eV
	[9MG – H$_{N2}$ + H$_{N7}$]···CH_2-NH_2	ΔH/TS = -2.30 / 0.18 eV
	9MG···$^+NH_2CH_2$	ΔH/TS = -3.96 / -0.40 eV
	[9MG – H$_{N2}$ + H$_{N3}$]···$^+NH_2CH_2$	ΔH/TS = -3.20 / -0.38 eV

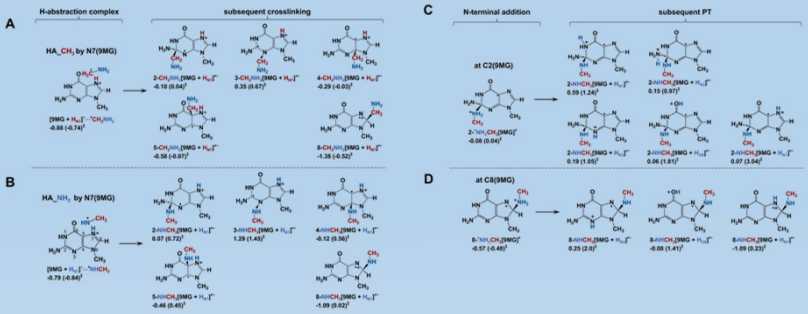
3) H abstraction and combination of radical product pairs

[9MG – H$_{N2}$]$^+$ + CH_3NH_2 →

[9MG – H$_{N2}$ + H$_{O6}$]$^\bullet$···$^\bullet CH_2NH_2$	ΔH/TS = -0.97 /-1.0 eV
9MG$^\bullet$···$^\bullet NHCH_3$	ΔH/TS = -1.24 / -0.51 eV
[9MG – H$_{N2}$ + H$_{N3}$]$^\bullet$···$^\bullet NHCH_3$	ΔH/TS = -0.27 / -0.20 eV
[9MG – H$_{N2}$ + H$_{O6}$]$^\bullet$···$^\bullet NHCH_3$	ΔH/TS = -0.71 / -0.78 eV
[9MG – H$_{N2}$ + H$_{N7}$]$^\bullet$···$^\bullet NHCH_3$	ΔH/TS = -0.70 / -0.77 eV

3. Compared to a common DPC mechanism mediated by G$^{\bullet+}$

1) **Intermediacy of HA_CH$_3$**
2) **Intermediacy of HA_NH$_2$** (as HA product-like complexes may ultimately rearrange to various DPCs)
3) **C2- and C8-addition**



Conclusions

Distinctive reactivities of guanine nucleosides during oxidative transformations

	Thermal energy			High energy (0.5 – 1 eV)		
	yield%	mechanisms	probable adducts	yield%	mechanisms	probable adducts
OG$^{\bullet+}$	19	direct addition (major)	X-$^+NH_2CH_2$[9MOG]$^\bullet$ (C = C2, C4)	0.5–10	async add. + PT	C6-NHCH$_2$[9MOG + H$_{N7}$]$^{\bullet+}$
		HA_NH$_2$-mediated	[9MOG + H$_2$]$^+$···$^\bullet NHCH_3$ (X = O6, O8)		HA_NH$_2$-mediated	C6-NHCH$_2$[9MOG + H$_{O6}$]$^{\bullet+}$
					HA_CH$_3$-mediated	X-CH$_2$NH$_2$[9MOG + H$_{O6}$]$^{\bullet+}$ (X = C2, C4, C5,C6) X-CH$_2$NH$_2$[9MOG + H$_{O8}$]$^{\bullet+}$ (X = C5, C8)
G$^{\bullet+}$	3	HA_CH$_3$-mediated (major)	C8-CH$_2$NH$_2$[9MG+H$_{N7}$]$^{\bullet+}$	none	N/A	N/A
		direct addition	X-NH$_2$CH$_2$[9MG]$^{\bullet+}$ (X = N2, C8)			
[G – H]$^+$	9.3	direct addition (major)	X-$^+NH_2CH_2$[9MG – H$_{N2}$] (major, X = N2, N3, C5, C8)	1.5	direct addition	X-$^+NH_2CH_2$[9MG – H$_{N2}$] (X = N2, N3, C5, C8)
		HA_CH$_3$	X-CH$_2$NH$_2$[9MG – H$_{N2}$ + H$_{O6}$]$^+$ (minor, X = N2, N3, C5, N7, C8)			

OG$^{\bullet+}$ Direct N-terminal methylamine addition to C2 and C4 of OG$^{\bullet+}$ is nearly barrierless. NHCH$_2$[9MOG + H]$^{\bullet+}$ forms via intramolecular PT, concerted addition and PT, and/or crosslinking accompanying or following HA_NH$_2$. HA_CH$_3$ and H$^\ominus$A_CH$_3$ can form non-covalently bonded products, and crosslinking is possible within these non-covalent complexes.

G$^{\bullet+}$ Alongside the direct formation of X-NH$_2$CH$_3$[G]$^{\bullet+}$ (X = C2, C8), C8-CH$_2$NH$_2$[G + H$_{N7}$]$^+$ was discovered as a new crosslink resulting from methyl-H abstraction of CH_3NH_2 by the N7 of 9MG$^{\bullet+}$, followed by adding $^\bullet CH_2NH_2$ to [G + H$_{N7}$]$^+$.

Crosslinking is enhanced for **[G – H]$^+$**, yielding X-$^+NH_2CH_2$[G – H$_{N2}$] (X = N2, N3, C5, C8) which form from direct CH_2NH_2 addition to [G – H]$^+$, and X-CH$_2$NH$_2$[G – H$_{N2}$ + H$_{O6}$]$^+$ (minor, X = N2, N3, C5, N7, C8) which arise from the combination of HA_CH$_3$ products.

Investigation of DPCs for **[OG – H]$^+$** is undergoing.

1. Y. Fang and J. Liu, *J. Phys. Chem. A*, 2009, ***113***, 11250-11261 (***Instrumentation***)
2. S. Wee; R. A. J. O'Hair; W. D. McFadyen, *Rapid Commun. Mass Spectrom.* **2005**, *19*, 1797; and P. Cheng; D. K. Bohme, *J. Phys. Chem. B* **2007**, *111*, 11075 (***Formation of nuclobase radical cations***)
3. M. M. Moe, J. Benny, V. Lee, M. Tsai, J. Liu, *Nucleic Acids Res.* **2025**, *53*, gkaf071; and W. Zhou, L Feng, M.M. Moe, J. Ceballos, M. Tsia, and J . Liu, *Chem. Eur. J.* **2026**, DOI 10.1002/chem.20253540 (***DPCs***)

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