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DNA Damage Emanating From a Neutral Purine Radical Reveals the Sequence Dependent Convergence of the Direct and Indirect Effects of Gamma-Radiolysis

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Abstract

Nucleobase radicals are the major intermediates generated by the direct (e.g. $\text{dA}^{\bullet+}$) and indirect (e.g. $\text{dA}^\bullet$) effects of γ -radiolysis. $\text{dA}^\bullet$ was independently generated in DNA for the first time. The $\text{dA}^{\bullet+}/\text{dA}^\bullet$ equilibrium, and consequently the reactivity in DNA is significantly shifted towards the radical cation by a flanking dA. Tandem lesions emanating from $\text{dA}^\bullet$ are the major products when the reactive intermediate is flanked by a 5'-dGT. In contrast, when $\text{dA}^\bullet$ is flanked by dA, the increased $\text{dA}^{\bullet+}$ pKa results in DNA damage arising from hole transfer. This is the first demonstration that sequence effects lead to the intersection of the direct and indirect effects of ionizing radiation.

Graphical Abstract

Nucleic acid oxidation plays an important role in genomic integrity, genetic diversity, and cell survival.¹ For instance, the cytotoxicity of γ -radiolysis and some chemotherapeutic agents is attributable to nucleic acid oxidation.^{2,3} γ -Radiolysis unselectively damages DNA by direct ionization of DNA (e.g. $\text{dA}^{\bullet+}$) and indirectly via reaction with hydroxyl radical (Scheme 1). Hydroxyl radical reacts with dA yielding 2'-deoxyadenosin-*N*6-yl radical ($\text{dA}^\bullet$), the reactivity of which is not well understood in DNA.^{2,4,5} By independently generating $\text{dA}^\bullet$ in DNA, we uncover that the reactivity of $\text{dA}^\bullet$ is sequence and pH dependent, and intersects with that of $\text{dA}^{\bullet+}$. These findings demonstrate the sequence dependent convergence of the direct and indirect effects of γ -radiolysis.

$\text{dA}^{\bullet+}$ is a key intermediate involved in DNA electron transfer, a process by which radical cations (holes) migrate through DNA.^{6–11} The deprotonation of $\text{dA}^{\bullet+}$ ($\text{pK}_a \sim 1$) generates $\text{dA}^\bullet$ (Scheme 1),¹² which attenuates hole transfer.¹³ Knowledge of $\text{dA}^\bullet$ reactivity has mostly

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Supporting Information. Experimental details for all experiments. ESI-MS of oligonucleotides containing **1** and representative autoradiograms of experiments. Supporting Information is available free of charge on the ACS Publications website.

been obtained from pulse radiolysis experiments on the nucleoside or dinucleotides containing dA.^{4,14–17} These experiments established that dA• oxidizes dG, likely via a proton coupled electron transfer in which the first step is endothermic (–0.16 V) but the overall reaction is thermodynamically favorable (0.13 V) and has been observed in similar systems in DNA (eqn. 1). The reactivity of dA• is potentially further complicated when flanked by dA, which is proposed to significantly shift the equilibrium towards dA^{•+}.^{18,19} The pK_a of the dA^{•+} is estimated to be as high as 7 at 25 °C when it is flanked by dA in DNA.¹⁸ Consequently, we anticipated that when flanked by dA, dA• will be at least partially protonated to form dA^{•+} and result in chemistry in DNA that is attributable to both species (Scheme 2). Site selective, independent dA• generation from **1**²⁰ in DNA enables us to directly probe the nucleobase radical's reactivity, including the effects of the flanking sequence. All duplexes used in this study contain a dGGG reporter group proximal to the site at which the radical is produced.^{21–23} Holes that migrate to dGGG triplets predominantly localize at the 5' - and middle-dG.²⁴ Approximately 10% of these holes react with water and O₂ to form lesions that are detected as strand breaks by denaturing PAGE, following alkaline or enzymatic treatment.²¹ In contrast, photolysis of 5' -³²P-**2** revealed O₂ dependent alkali-labile lesions predominantly at the 3' -terminal position of the dGGG triplet (dG₁₁), along with smaller amounts of damage at T₁₂ (Figure 1a, Figure S1–S4).²⁵ Treatment with formamidopyrimidine DNA glycosylase (Fpg) that cleaves DNA at a variety of oxidized purines, only yielded cleavage at dG₁₁.²⁶ Importantly, damage is not observed at dG₉ or dG₁₀ following either treatment, indicating that the DNA damage does not result from hole transfer. Hole transfer also is not detected in duplexes **3–5** (Chart 1, Figure S5–S7). These observations suggest that unlike benzotriazinyl radicals and the neutral radical derived from 2-aminopurine,^{15,17} one electron oxidation of dG to dG^{•+} by dA• does not compete with other reaction pathways in these duplexes, despite thermodynamically favorable dG oxidation by dA•.¹⁶

Strand scission at dG₁₁ in photolyzed **2** was reduced ~10-fold when the photolysate was treated with piperidine containing γ-mercaptoethanol, indicating that 7,8-dihydro-8-oxo-2'-deoxyguanosine (OG) is the major product at this position (Figure S2).²⁵ In addition, alkali-labile cleavage at T₁₂ was greater than at dG₁₁ when 3' -³²P-**2** was photolyzed (Figure S3), suggesting both nucleotides are damaged in at least some duplex molecules. The observations on **2** are indicative of tandem lesions, an important form of DNA damage, defined as two or more contiguously damaged nucleotides in a DNA strand.^{27–29} Tandem lesion formation by pyrimidine nucleobase radicals are well described, but this is the first report in which damage is initiated by a purine nucleobase radical.^{30–33} Although additional studies are needed, we propose that dA• reacts with the 5' -adjacent thymidine (T₁₂ in **2**) to transfer spin to that nucleotide (Figure 1b). Under aerobic conditions, the resulting thymidine peroxy radical(s) transfers damage to dG₁₁.

Much less strand damage was detected in 5' -³²P-**3**, despite the presence of the same flanking sequence, albeit in the 3' -direction. No cleavage was observed in the dGGG triplet

embedded within the 3'-flanking sequence relative to dA•, and only modest damage was detected at T₁₂ ($1.7 \pm 0.1\%$, Figure S5). The difference in reactivity between **2** and **3** is attributed to the helical DNA structure, which places dA• in different proximity with the adjacent nucleotides. In contrast, hole transfer readily proceeds in the 5'- and 3'-directions.^{34,35}

After eliminating the possibility of hole injection by dA• in **2–5** (Figure 1, S1–S7), we investigated the neutral radical's reactivity when it is flanked by dA (Chart 2). In marked contrast to photolyses of **2–5**, the major sites of strand scission following piperidine or Fpg treatment of 5'-³²P-**6** photolysates are at the 5'- and middle-dG (dG₉ and dG₁₀) of the dGGG sequence, the characteristic damage pattern for hole transfer (Figure 2, 3). The cleavage pattern indicates that as predicted by Sevilla, π -stacking with the adjacent dA increases the dA^{•+} pK_a such that the radical cation is produced from initially formed dA• at pH 7.2.¹⁸ The extent of strand damage increases upon reducing the pH from 7.2 to 6 to 5.1, but the general cleavage pattern is unchanged (Figure 2a, S8, S16). Importantly, this pH effect is not observed in the corresponding duplex (**2**, Figure 1a, 3) lacking a flanking dA. However, more favorable hydration compared to deprotonation of G^{•+} at lower pH may also contribute to increased strand damage.³⁶ The absolute amount of strand cleavage detected in **6** ($9.1 \pm 1.2\%$, Figure 3b) corresponds to a high level of hole transfer when one accounts for the ~10% efficiency for G^{•+} conversion to alkaline-labile lesions at pH 5.²¹ When combined with UPLC analysis following nuclease digestion, which showed that ~80% of **1** is consumed upon photolysis (Figure S19, Table S2), these experiments indicate that dA• produced from **1** in **6** results in almost quantitative hole transfer at pH 5.1. A small amount (~3.3%) of strand cleavage is observed at the position where dA• is generated (Figure 2) but the source of this damage is unknown.

The formation of dA^{•+} and consequential hole transfer observed in **6** indicates that the indirect (dA•) and direct (dA^{•+}) effects of ionizing radiation converge in a sequence dependent manner. The effect of dA stacking on dA^{•+} pK_a is believed to be unique; a similar effect is not observed for dG.^{11,37} The absence of hole migration in **4** or **5** in which dG is adjacent to the radical site, is consistent with this. The generality of this sequence dependent phenomenon was explored using established guidelines governing hole transfer in DNA with respect to directionality and sequence/distance effects.^{6,38,39} For instance, a dGGG triplet is more easily oxidized than a single dG, which provides a driving force for holes to hop short distances from dG^{•+} to dGGG triplets. Indeed, photolysis (pH 5.1) of 5'-³²P-**7** (Figure 3, S9, S17) produced ~10-fold greater strand damage at the dGGG triplet than at the single dG adjacent to the site at which dA• was generated (dG₁₂). In the corresponding sequence (**4**) lacking dA stacked with dA•, cleavage was detected only at dG₁₂, and in much lower yield than in **7** (Figure S6). In another example, generating dA• in 5'-³²P-**8** (Figure 3, S10) illustrates the expected ability of hole transfer to proceed in the 3'-direction, unlike tandem lesion formation. Damage in **8** was detected predominantly at the 5'- and middle dG's of the dGGG triplet; whereas irradiation of the corresponding sequence lacking a stacking dA (5'-³²P-**5**) produced a small amount of damage at the 5'-T₁₂ and none within the dGGG sequence (Figure S7). These observations are consistent with the well-established paradigm of hole migration.

To further support the formation of $\text{dA}^{\bullet+}$, we took advantage of $\text{dA}^{\bullet+}$ delocalization over multiple dAs within poly•dA sequences.⁴⁰ Delocalization of positive charge leads to efficient hole transfer with weak distance dependence.^{10,41} Generating $\text{dA}^{\bullet}$ within dA_5 sequences (**9**, **10**) again yielded strand damage at the 5'- and middle-dGs of the dGGG triplet (Figure 3). The damage detected in dGGG triplets in **9** and **10** (Figure S11, S12) is the same within experimental error, despite the distances between initial hole injection site and the reporter sequence differing by several angstroms (2 base pairs). This is consistent with the intermediacy of $\text{dA}^{\bullet+}$.

Finally, we examined strand damage when $\text{dA}^{\bullet}$ was generated in a sequence where hole transfer and tandem lesion formation are possible (**11**). dG_{11} was the site of greatest damage (Figure 3a, 4a) following photolysis of 5'-³²P-**11** at pH 7.2. In addition, strand scission is observed at T_{12} in 3'-³²P-**11** (Figure S13, S14), as expected for tandem lesion formation. At pH 5.1, the preference switched to G_9 and G_{10} , and the damage pattern was consistent with that of hole transfer (Figure 3b, 4a, S18). These data suggest that $\text{dA}^{\bullet}$ and $\text{dA}^{\bullet+}$ contribute to strand damage in **11** (Figure 4b) in a manner proportional to their relative amounts. Furthermore, the differences observed between **11** and **6** suggest that at pH 7.2 tandem lesion formation from $\text{dA}^{\bullet}$ competes with radical cation formation. However, we cannot rule out that $\text{dA}^{\bullet}$ is initially formed in a conformation from which tandem lesions are formed more rapidly than $\text{dA}^{\bullet+}$.

In conclusion, independent generation of $\text{dA}^{\bullet}$, a species produced from the indirect effect of ionizing radiation, demonstrates that the nucleobase radical does not initiate hole transfer but yields tandem lesions in 5'-dGT sequences. A flanking dA dramatically shifts the $\text{dA}^{\bullet+}/\text{dA}^{\bullet}$ equilibrium and results in formation of the radical cation ($\text{dA}^{\bullet+}$) that is formally produced from the direct effect (Scheme 2). Support for $\text{dA}^{\bullet+}$ formation is based on well-precedented DNA hole transfer chemistry. These data provide the first experimental evidence in DNA that π -stacking increases the pK_a of $\text{dA}^{\bullet}$ at room temperature. It is believed that the direct and indirect effects of γ -radiolysis contribute approximately equally to DNA damage.^{2,42,43} By independently generating $\text{dA}^{\bullet}$ for the first time in DNA, we demonstrate that when flanked by another dA, $\text{dA}^{\bullet}$ generation leads to DNA hole transfer, a process induced by the direct effect of ionizing radiation.^{44,45} Our results demonstrate that the indirect and direct effects γ -radiolysis are closely intertwined, and converge in a sequence dependent manner.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Tubbs A, Nussenzweig A. Cell. 2017; 168:644–656. [PubMed: 28187286]
2. von Sonntag, C. Free-Radical-Induced DNA Damage and Its Repair. Springer-Verlag; Berlin: 2006.

3. Galm U, Hager MH, Van Lanen SG, Ju J, Thorson JS, Shen B. *Chem Rev.* 2005; 105:739–758. [PubMed: 15700963]
4. Bamatraf MMM, O'Neill P, Rao BSM. *J Phys Chem B.* 2000; 104:636–642.
5. Dizdaroglu M, Jaruga P. *Free Rad Res.* 2012; 46:382–419.
6. Genereux JC, Barton JK. *Chem Rev.* 2010; 110:1642–1662. [PubMed: 20214403]
7. Giese B. *Acc Chem Res.* 2000; 33:631–636. [PubMed: 10995201]
8. Schuster GB. *Acc Chem Res.* 2000; 33:253–260. [PubMed: 10775318]
9. Sartor V, Boone E, Schuster GB. *J Phys Chem B.* 2001; 105:11057–11059.
10. Giese B, Amaudrut J, Kohler AK, Spormann M, Wessely S. *Nature.* 2001; 412:318–320. [PubMed: 11460159]
11. Harris MA, Mishra AK, Young RM, Brown KE, Wasielewski MR, Lewis FD. *J Am Chem Soc.* 2016; 138:5491–5494. [PubMed: 27082662]
12. Steenken S. *Chem Rev.* 1989; 89:503–520.
13. Steenken S. *Biol Chem.* 1997; 378:1293–1297. [PubMed: 9426189]
14. Candeias LP, Steenken S. *J Am Chem Soc.* 1993; 115:2437–2440.
15. Shinde SS, Maroz A, Hay MP, Anderson RF. *J Am Chem Soc.* 2009; 131:5203–5207. [PubMed: 19320486]
16. Steenken S, Jovanovic SV. *J Am Chem Soc.* 1997; 119:617–618.
17. Shafirovich V, Dourandin A, Huang W, Luneva NP, Geacintov NE. *J Phys Chem B.* 1999; 103:10924–10933.
18. Adhikary A, Kumar A, Khanduri D, Sevilla MD. *J Am Chem Soc.* 2008; 130:10282–10292. [PubMed: 18611019]
19. Kobayashi K. *J Phys Chem B.* 2010; 114:5600–5604. [PubMed: 20369809]
20. Zheng L, Griesser M, Pratt DA, Greenberg MM. *J Org Chem.* 2017; 82:3571–3580. [PubMed: 28318253]
21. Meggers E, Michel-Beyerle ME, Giese B. *J Am Chem Soc.* 1998; 120:12950–12955.
22. Saito I, Nakanura T, Nakatani K, Yoshioka Y, Yamaguchi K, Sugiyama H. *J Am Chem Soc.* 1998; 120:12686–12687.
23. Hall DB, Holmlin RE, Barton JK. *Nature.* 1996; 382:731–735. [PubMed: 8751447]
24. Yoshioka Y, Kitagawa Y, Takano Y, Yamaguchi K, Nakamura T, Saito I. *J Am Chem Soc.* 1999; 121:8712–8719.
25. Cullis PM, Malone ME, Merson-Davies LA. *J Am Chem Soc.* 1996; 118:2775–2781.
26. Tchou J, Kasai H, Shibutani S, Chung MH, Laval J, Grollman AP, Nishimura S. *Proc Natl Acad Sci U S A.* 1991; 88:4690–4694. [PubMed: 2052552]
27. Patrzyk HB, Dawidzik JB, Budzinski EE, Freund HG, Wilton JH, Box HC. *Radiat Res.* 2012; 178:538–542. [PubMed: 23106212]
28. Yuan B, Jiang Y, Wang Y, Wang Y. *Chem Res Toxicol.* 2009; 23:11–19.
29. O'Neill P, Wardman P. *Int J Radiat Biol.* 2009; 85:9–25. [PubMed: 19205982]
30. Hong IS, Carter KN, Sato K, Greenberg MM. *J Am Chem Soc.* 2007; 129:4089–4098. [PubMed: 17335214]
31. Hong IS, Carter KN, Greenberg MM. *J Org Chem.* 2004; 69:6974–6978. [PubMed: 15471441]
32. Carter KN, Greenberg MM. *J Am Chem Soc.* 2003; 125:13376–13378. [PubMed: 14583031]
33. San Pedro JMN, Greenberg MM. *J Am Chem Soc.* 2014; 136:3928–3936. [PubMed: 24579910]
34. Hess S, Götz M, Davis WB, Michel-Beyerle ME. *J Am Chem Soc.* 2001; 123:10046–10055. [PubMed: 11592883]
35. O'Neill MA, Barton JK. *Proc Natl Acad Sci U S A.* 2002; 99:16543–16550. [PubMed: 12486238]
36. Giese B. *Long-Range Charge Transfer in DNA I.* Schuster, GB., editor. Springer Berlin Heidelberg; Berlin, Heidelberg: 2004. p. 27–44.
37. Kumar A, Sevilla MD. *J Phys Chem B.* 2011; 115:4990–5000. [PubMed: 21417208]
38. Giese B. *Annu Rev Biochem.* 2002; 71:51–70. [PubMed: 12045090]
39. Joy A, Schuster GB. *Chem Comm.* 2005:2778–2784. [PubMed: 15928756]

40. Renaud N, Berlin YA, Lewis FD, Ratner MA. *J Am Chem Soc.* 2013; 135:3953–3963. [PubMed: 23402652]
41. Kawai K, Takada T, Tojo S, Majima T. *J Am Chem Soc.* 2003; 125:6842–6843. [PubMed: 12783517]
42. Jones GDD, Boswell TV, Lee J, Milligan JR, Ward JF, Weinfeld M. *Int J Radiat Biol.* 1994; 66:441–445. [PubMed: 7983429]
43. Krisch RE, Flick MB, Trumbore CN. *Radiat Res.* 1991; 126:251–259. [PubMed: 1850853]
44. Sharma KKK, Swarts SG, Bernhard WA. *J Phys Chem B.* 2011; 115:4843–4855. [PubMed: 21473599]
45. Kumar A, Sevilla MD. *Chem Rev.* 2010; 110:7002–7023. [PubMed: 20443634]

Figure 1. dA• induced strand damage in 5'-³²P-2. (a.) Denaturing PAGE analysis (b.) Proposed mechanism for tandem lesion formation.

Figure 2. dA^{•+} induced hole transfer in 5'-³²P-6. (a.) Denaturing PAGE analysis (b.) Proposed mechanism for sequence dependent dA^{•+} formation, and subsequent hole transfer.

Figure 3. Sequence and pH influence on strand damage. (a) pH 7.2 (b) pH 5.1. G_5' , G_M , and $G_{3'}$ refer to the dGs in the dGGG triplet of each respective duplex. Values are the average $\pm$ std. dev. of 3 replicates.

Figure 4. pH Dependent strand damage in 5'-³²P-**11**. (a.) Denaturing PAGE analysis. (b.) Proposed mechanism of pH dependent switching of strand damage induced by dA[•] and dA^{•+}.

Scheme 1. Reactive intermediate formation from dA by γ -radiolysis.

Scheme 2.
Proposed reaction pathways of $\text{dA}^\bullet$ in DNA.

Chart 1.
DNA duplexes lacking A-stacks.

5'-d(GTC CAT AT G₉ G₁₀ G₁₁ T ₁₂ A₁ T ATA CGT GCA G)	6
3'-d(CAG GTA TA C C C A TTA TAT GCA CGT C)	
5'-d(TCC ATA T G₈ G₉ G₁₀ T G₁₂ A₁ T ATA CGT GCA G)	7
3'-d(AGG TAT A C C C A C TTA TAT GCA CGT C)	
5'-d(GTC GCG CTA TAT 1A G₁₅ G₁₆ G₁₇ T ATA GCT G)	8
3'-d(CAG CGC GAT ATA TT C C C A TAT CGA C)	
5'-d(GTC GAT AT G₉ G₁₀ G₁₁ T A₁A AAT ATA GCT G)	9
3'-d(CAG CTA TA C C C A TTT TTA TAT CGA C)	
5'-d(GTC GAT AT G₉ G₁₀ G₁₁ T AAA 1A T ATA GCT G)	10
3'-d(CAG CTA TA C C C A TT T TTA TAT CGA C)	
5'-d(GTC CAT AT G₉ G₁₀ G₁₁ T ₁₂ 1A T ATA CGT GCA G)	11
3'-d(CAG GTA TA C C C A TTA TAT GCA CGT C)	

Chart 2.
DNA duplexes containing A-stacks.