

Paradoxical hotspots for guanine oxidation by a chemical mediator of inflammation

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Guanine in DNA is a major oxidation target owing to its low ionization potential (IP), and there is often an inverse correlation between damage frequency and sequence-dependent variation in guanine IP. We report that the biological oxidant nitrosoperoxy carbonate (ONOOCO₂) paradoxically selects guanines with the highest IP in GC-containing contexts. Along with sequence-dependent variation in damage chemistry, this behavior points to factors other than charge migration as determinants of genomic DNA oxidation.

Determinants of the location and quantity of oxidatively damaged DNA have been extensively studied in recent years for a variety of one-electron oxidants^{1–3} that selectively oxidize the 5'-guanine in runs of guanines. This behavior has been attributed to migration of the initial radical cation to the guanine with the lowest IP, with termination by trapping and product formation¹. Support for this model comes from observations of an inverse correlation between calculated IP for guanines in different sequence contexts and the reactivity of the

guanines toward riboflavin-mediated photooxidation³. These observations have led to the general conclusion that DNA oxidants produce large amounts of damage at the most readily oxidized guanines as a result of charge migration to the lowest-energy hole trap. Contrary to this model, we observed a different behavior with ONOOCO₂, a chemical mediator of inflammation arising from macrophage-derived nitric oxide⁴. Homolysis of the O-O bond in ONOOCO₂ (*t*_{1/2} < 1 ms) produces the one-electron oxidants nitrogen dioxide ([NO₂][•]) and carbonate radical anion ([CO₃]^{•-})⁴, of which only [CO₃]^{•-} is capable of oxidizing guanine⁵.

We previously observed a correlation between the quantity of ONOOCO₂-induced damage and mutations in the *Escherichia coli* *supF* gene⁶. Re-examination of this data revealed a marked selectivity of ONOOCO₂ for guanines in TGC and AGC sequences. This observation was characterized by quantitatively comparing ONOOCO₂ reactivity with guanines in all possible trinucleotide sequence contexts in the double-stranded, 30-mer oligodeoxynucleotides used in published studies of riboflavin-mediated photooxidation³. The consensus sequence 5'-CGTACTCTTTGGTX₁G₁Y₁TX₂G₂Y₂TTCTTCTAT-3' contains two variable guanine contexts (X₁G₁Y₁) and an invariant TGG (underlined) for normalizing damage. We quantified ONOOCO₂-induced damage at each guanine in 5'-³²P-labeled oligodeoxynucleotides on sequencing gels (**Supplementary Fig. 1** online) after converting guanine lesions to strand breaks with piperidine or formamidopyrimidine DNA glycosylase (Fpg; both treatments produced similar results for sequence selectivity; **Supplementary Fig. 2** online)⁶.

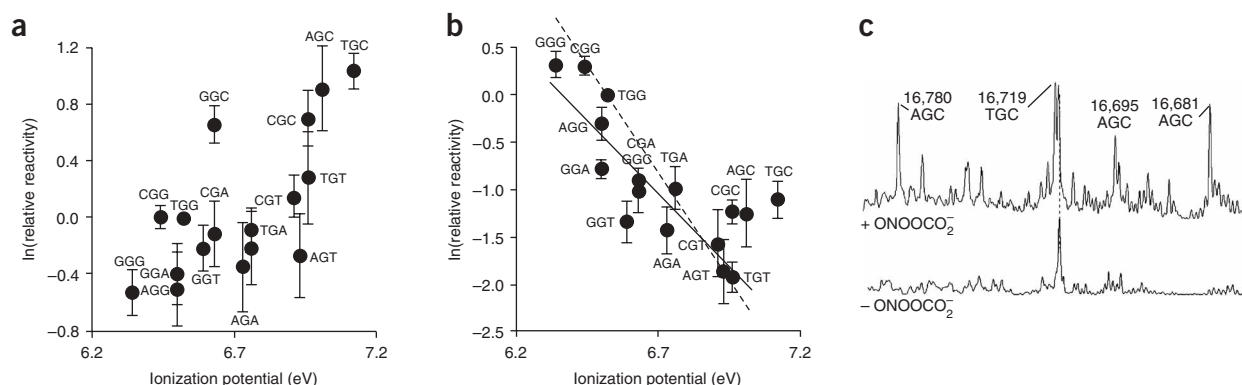


Figure 1 Sequence-selective guanine oxidation in duplex DNA by ONOOCO₂ and riboflavin-mediated photooxidation³. **(a,b)** Data we obtained for the relative reactivity of ONOOCO₂ **(a)** and riboflavin photooxidation **(b)** in XGY motifs in oligodeoxynucleotides are plotted against guanine IP (IP values obtained from ref. 3). Lines in **b** represent linear regression fits of riboflavin-mediated photooxidation data from our studies (solid line; $y = 18.5 - 2.90x$; data for TGT, CGT, CGC and TGC omitted) and from other published work³ (dashed line; $y = 30.2 - 4.62x$). Data represent mean \pm s.d. for three experiments. **(c)** Frequency of guanine oxidation by ONOOCO₂ in the *HPRT* gene in human lymphoblastoid TK6 cell genomic DNA mapped by ligation-mediated PCR (**Supplementary Methods**). Oxidized guanines noted above specific peaks in the line graphs are derived from **Supplementary Figure 4**.

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Received 13 February 2006; accepted 9 May 2006; published online 4 June 2006; corrected after print 23 January 2007; doi:10.1038/nchembio796

