DNA Sequencing with the Hydroperoxide of Tetrahydrofuran

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The development of new reagents and methods to chemically sequence DNA is of interest for both mechanistic and practical reasons.[1–8] In the present study, we describe conditions under which 2-hydroperoxytetrahydrofuran (THF-OOH), an autoxidation product of THF,[9] reacts with DNA to afford heat-labile sites selectively at either C or G and A. This novel chemistry is discussed along with its use in DNA sequencing.

The incubation of THF-OOH[10] with ^{32}P-end-labeled DNA in the absence and presence of reducing agents is shown in Figure 1. THF-OOH by itself or in the presence of 10 mM dithiothreitol (DTT) or Fe(II) shows a specificity for C (Figure 1, lanes f–h) that is time- (data not shown) and dose-dependent (Figure 2, lanes j–l). The intensity of the C cleavage bands is enhanced by the coaddition of 10 mM DTT (Figure 1, lane l), while the addition of Fe(II) without DTT has a small enhancing affect on the C cleavage (Figure 1, lane f *vs* g). The coaddition of Fe(II) and DTT provides a strong C cleavage pattern; however, the increase is less than that observed with only the coaddition of DTT (Figure 1, lanes h *vs* l). The substitution of H_2O_2 for THF-OOH generates a weak cleavage ladder (Figure 1, lane e) in the presence of Fe(II) and DTT but with no C specificity, indicating that HO• is not responsible for the C bands. Above 10 mM DTT, the cleavage specificity begins to switch from C to G > A (Figure 2, lane g), and this transition is complete at 100 mM DTT.

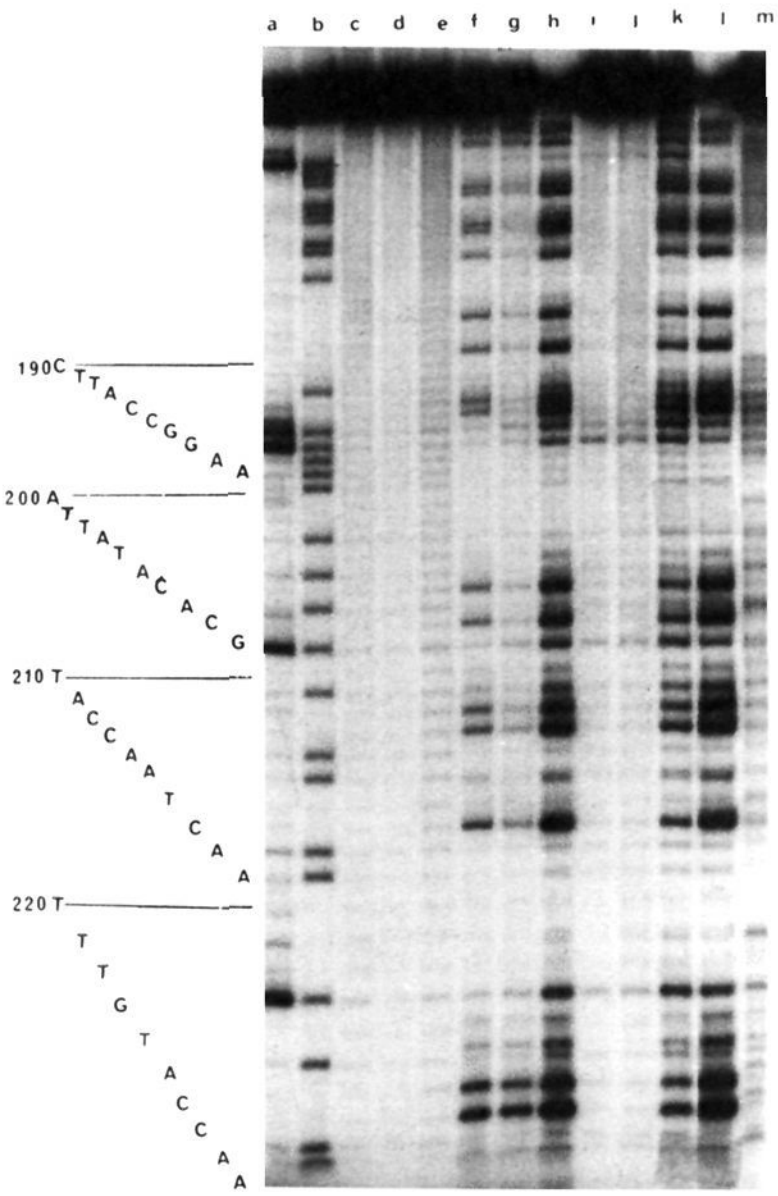

Figure 1. Cleavage pattern induced by THF-OOH in the absence and presence of reducing agents. A 5′-[^{32}P]-labeled, 85-bp restriction fragment previously described [Church, K. M.; Wurdeman, R. L.; Zhang, Y.; Chen, F.-X.; Gold, B. *Biochemistry* **1990**, *29*, 6827–6838] was prepared using standard procedures.[2] The ^{32}P-labeled DNA and sonicated calf thymus DNA (final concentration 100 μM nucleotide) were dissolved in 10 mM sodium cacodylate buffer (pH 7.6) containing, when specified, DTT and/or Fe(II). The reactions were initiated by the addition of THF-OOH followed by incubation in 10 mM sodium cacodylate buffer (pH 7.6) at 37 °C for the 24 h. Strand breaks in the reacted DNA were generated by heating at 90 °C for 15 min (except for lane m) followed by treatment with hot piperidine. After purification and *in vacuo* removal of the piperidine, the DNA was suspended in loading buffer,[2] denatured, and loaded onto a 12% polyacrylamide (7.8 M urea) denaturing gel that was run at 75 W (~55 °C): lane a, G; lane b, G + A; lane c, control; lane d, 10 mM DTT; lane e, 1 mM H_2O_2, 10 mM DTT + 200 μM $Fe(NH_4)_2(SO_4)_2$; lane f, 1 M THF-OOH + 200 μM $Fe(NH_4)_2(SO_4)_2$; lane g, 1 M THF-OOH; lane h, 1 M THF-OOH + 200 μM $Fe(NH_4)_2(SO_4)_2$ + 10 mM DTT; lanes i–l, 7.6, 77, 230, and 1000 mM THF-OOH with 10 mM DTT; lane m, 1 M THF-OOH with 10 mM DTT without neutral thermal treatment but with piperidine treatment.

Neither 2-hydroxy-THF nor γ-butyrolactone (decomposition products of THF-OOH[11]) shows any DNA cleaving activity under a variety of conditions (data not shown). The damage induced by THF-OOH at C or G + A is only exposed as strand breaks after heating the DNA at 90 °C at pH 7.0 followed by treatment with base. If the DNA is either not heated at pH 7.0 (data not shown) or not treated with piperidine (Figure 1, lane m), no cleavage is observed. This implies that the initial damage must first be transformed into an abasic site, which in turn is converted to a strand break by alkali.

ESR analysis of the decomposition of THF-OOH in the presence of the spin trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) shows the presence of three radical species (Figure 3,

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(10) Over a 45-min period, 2,3-dihydrofuran (26 g, 0.37 mol) was slowly added to an ice-cold solution of H_2O_2 (30%, 57 g, 0.50 mol) and concentrated H_2SO_4 (0.1 mL) [based on a method described by Milas, N. A.; Peeler, R. L., Jr.; Mageli, O. L. *J. Am. Chem. Soc.* **1954**, *76*, 2322–2325]. After the addition, the solution was stirred for an additional 45 min with the temperature maintained below 10 °C. The reaction mixture was then saturated with solid NH_4Cl and extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were then extracted with 20% aqueous NaOH, and the aqueous layer was washed with CH_2Cl_2 and neutralized to slightly above pH 7.2 with HOAc while the temperature was maintained below 10 °C. This aqueous solution was saturated with NH_4Cl and extracted with CH_2Cl_2, and the combined extracts were washed with cold, 10% $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield 14.7 g (38% yield) of a clear liquid: ^{1}H NMR ($CDCl_3$) δ 1.83 (m, 2, CH_2), 1.95 (m, 2, CH_2), 3.91 (m, 2, O-CH_2), 5.55 (m, 1, O-C*H*-OOH) and 8.69 (s, 1, OOH); ^{13}C NMR ($CDCl_3$) δ 23.92 and 29.13 (CH_2's), 67.73 (O-CH_2), 107.9 (O-CH-OOH).

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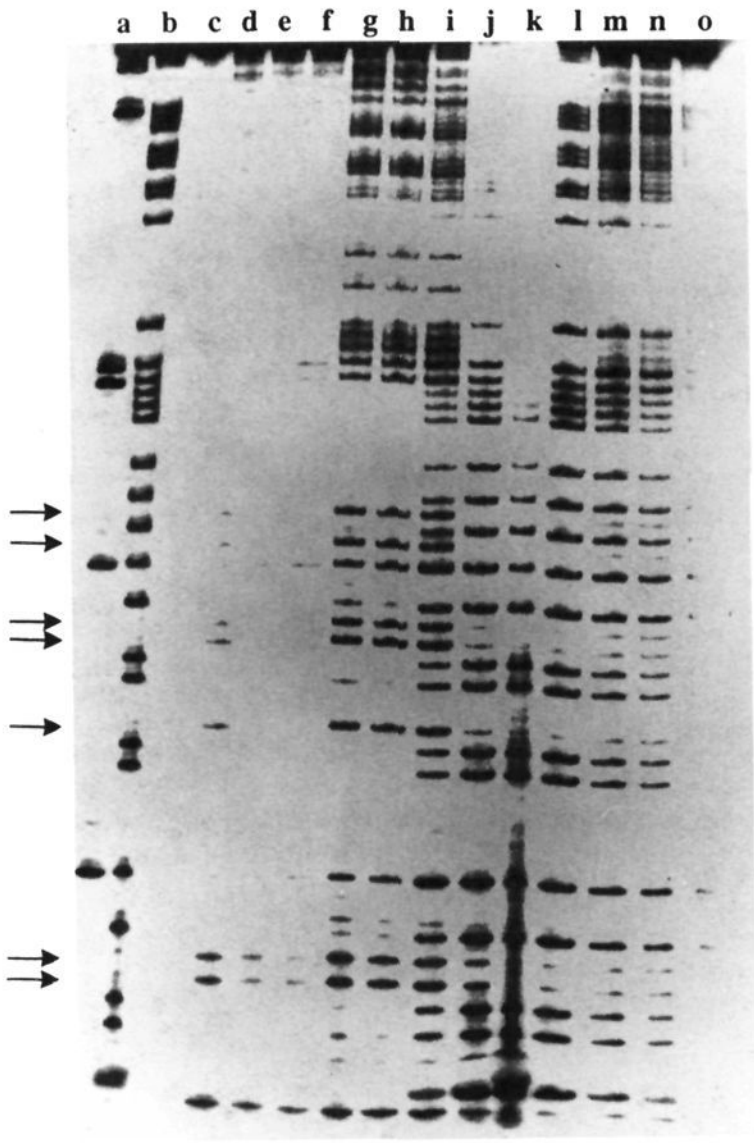

Figure 2. Effect of DTT concentration of the cleavage pattern induced by THF-OOH (reaction conditions the same as described in Figure 1): lane a, G; lane b, G + A; lane c, control; lane d, 1.0 M THF-OOH; lanes e–n, 1.0 M THF-OOH with 0.001, 0.005, 0.01, 0.02, 0.05, 0.10, 0.25, 0.50, 0.75, and 1.0 M DTT, respectively; lane o, 1.0 M DTT.

top). The magnitude of the coupling constants obtained by computer simulation allows tentative assignment of these radicals as follows: carbon-centered, R$^\bullet$ (a(N) = 15.9 G, a(H) = 24.9 G); alkoxy, RO$^\bullet$ (a(N) = a(H) = 15.2 G); and hydroperoxy, ROO$^\bullet$ (a(N) = 14.0 G, a(H) = 11.0 G).[12] The species identified as RO$^\bullet$ is not HO$^\bullet$ because the line intensities attributed to it are insensitive to the addition of H_2O_2 or EtOH. The intensities of the lines assigned to ROO$^\bullet$ increase as the concentration of DMPO increases and are absent when the reaction is run under Ar. The addition of THF-OOH to DTT in the presence of DMPO (Figure 3, bottom) caused an increase in the intensity of R$^\bullet$ relative to RO$^\bullet$, although the spectrum is complicated by the trapping of DTT$^\bullet$ (a(N) = 15.02 G, a(H) ≈ 16.6 G).[13] At high ratios of DTT to THF-OOH (≫1), no EPR signal is observed, presumably because of rapid reduction of RO$^\bullet$ by DTT. Based on these data and a previous product study,[11] we suggest the following scheme for the formation of the different species:

$$\text{THF-OOH} \rightarrow \text{THF-O}^\bullet \rightarrow \text{HC(=O)O(CH}_2\text{)}_3^\bullet \rightarrow \text{HC(=O)O(CH}_2\text{)}_3\text{O-O}^\bullet$$

The nature of the THF-OOH-mediated C-lesion that affords a heat-labile product is not known, but both O^2- and N3-alkylC are released from DNA upon neutral thermal hydrolysis.[14,15] It has also been reported that carbon-centered free radicals, e.g., $CH_3^\bullet$, react with nucleosides to afford N^4- and N3-methylC.[16] Since the ESR results show that the C-centered radical is the

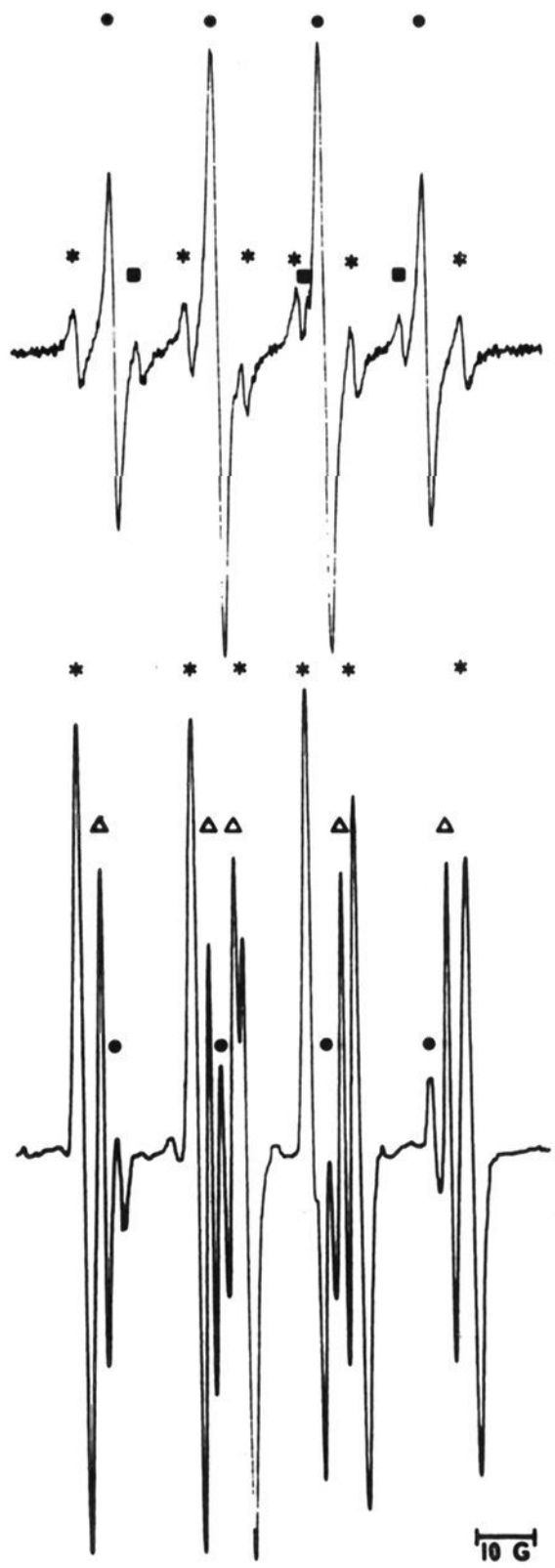

Figure 3. ESR spectra of the DMPO-trapped THF-OOH degradation products (*, R$^\bullet$; ●, RO$^\bullet$; ■, ROO$^\bullet$; △, DTT$^\bullet$): (top) THF-OOH (130 mM); (bottom) THF-OOH (130 mM) + DTT (10 mM). ESR spectra were recorded on a Varian E-3 1 min after reaction initiation. All reactions (300 μL final volume) contained DMPO (60 mM) and calf thymus DNA (20 μg/mL) in 10 mM sodium cacodylate buffer (pH 7.4) and were initiated by addition of 50 μL of an aqueous solution of THF-OOH (final concentration, 130 mM). Instrument settings: receiver gain (a) 2.5 × 10^5, (b) 1.25 × 10^3; time constant, 1 s; modulation amplitude, 1 G; scan time, 16 min; magnetic field, 3470 ± 100 G.

dominant species trapped during the decomposition of THF-OOH in the presence of DTT (Figure 3b), we *tentatively* suggest that a lesion at N3–C is involved in the formation of the apyrimidinic site, although the specificity for C differentiates THF-OOH from other radical precursors.

In conclusion, because of the uniform band intensities for all C with no trace of strand breaks at T, THF-OOH in the presence of low concentrations of reducing agent is suitable as a C sequencing reagent.

Warning: *the autoxidation products of THF can undergo detonation upon heating. In our hands, THF-OOH, even at >1 M* concentrations, *undergoes smooth and nonviolent decomposition in the presence of reducing agents.*

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