Chemical Mechanism of DNA Scission by (1,10-Phenanthroline)copper. Carbonyl Oxygen of 5-Methylenefuranone Is Derived from Water

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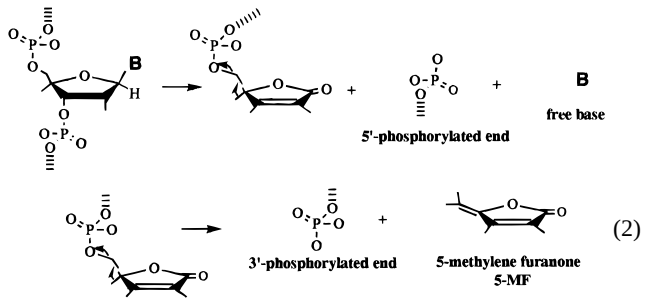
The tetrahedral 2:1 (1,10-phenanthroline)copper(I) complex ($(OP)_2Cu^+$), cleaves the phosphodiester backbone of B-DNA under physiological conditions by oxidation of the deoxyribose moiety using hydrogen peroxide as an essential coreactant (eq 1).[1,2] The principal products are free base, 5-methylenefuranone

$$(OP)_2Cu^+ + DNA \rightleftharpoons (OP)_2Cu^+ \cdots DNA \xrightarrow{H_2O_2} \text{scission products} \quad (1)$$

(5-MF),[3] and 3′- and 5′-phosphorylated ends (eq 2).[4] In this paper, we investigate the atom source of the carbonyl oxygen of 5-MF to elucidate the mechanism of this efficient nucleolytic activity. Using ^{18}O-enriched hydrogen peroxide and water, we find that the carbonyl oxygen is derived from H_2O.



(2)

Poly dA-T was used as the substrate to determine the source of the carbonyl oxygen in 5-MF because of its ready availability and the ease of preparing internally labeled phosphodiester bonds using *Escherichia coli* DNA polymerase I and (α-^{32}P)−dATP. In our initial demonstration of 5-MF as the product of DNA scission,[3] superoxide generated by ^{60}Co irradiation was used as the source of reducing equivalents to activate the nuclease activity in order to avoid possible addition of nucleophilic reducing agents, such as 3-mercaptopropionic acid (MPA), to 5-MF. Since this precaution was unnecessary in the present studies, activation by MPA was used because it is more efficient and permits the use of small (1 mL) reaction volumes. Control experiments have demonstrated that 5-MF is stable (less than 10% loss observed) in the presence of MPA at 25 and 90 °C for up to 1 h.

Aerobic reactions were initiated by the addition of a freshly prepared solution of OP (1,10-phenanthroline) and Cu^{2+} to a solution of 20 μg of poly dA-T in pH 7.5 Tris−HCl (50 mM), NaCl (25 mM), and MPA (1 mM) to a final concentration of 135 μM OP and 30 μM $CuSO_4$. Hydrogen peroxide was generated *in situ* by the $(OP)_2Cu^{2+}$-catalyzed oxidation of MPA in the presence of O_2 or included at a concentration of 1 mM when required. After a 15 min reaction at 25 °C, the reactants were heated at 90 °C for another 15 min to insure complete conversion of the metastable intermediate at the 3′ end to 5-MF,[5] which was isolated as indicated below.[6] Anaerobic reactions were performed in glass tubes, sealed with a rubber septum, and purged with argon. Following the addition of reactants via a syringe, the reaction was purged continuously for 30 min prior to heating the tube at 90 °C and isolation of 5-MF.[6] As anticipated from our previous studies,[3] we isolated 5-MF from reaction mixtures containing OP, Cu^{2+}, and MPA in the presence of oxygen. Under these conditions, H_2O_2 is generated *in situ*, although our present studies indicate that addition of 1 mM H_2O_2 generates slightly higher yields. Under anaerobic conditions, the coreactant H_2O_2 must be added to achieve scission. The simultaneous presence of OP, copper ion, and reducing agent (e.g., MPA) is essential for the isolation of 5-MF under both anaerobic and aerobic conditions.

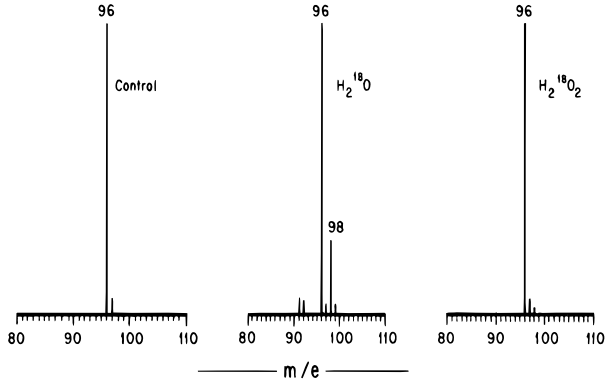


Figure 1. Mass spectra of 5-methylenefuranone (5-MF) isolated from reactions containing ^{18}O-H_2O and H_2O_2. Left panel: authentic 5-MF. Middle panel: 5-MF isolated from an anaerobic reaction mixture containing 22% $H_2{}^{18}O$ and 1 mM $H_2{}^{16}O_2$. Right panel: 5-MF isolated from a reaction mixture containing $H_2{}^{18}O_2$ and $H_2{}^{16}O$.

The oxygen source of the carbonyl in 5-MF was examined by performing the reaction under (a) anaerobic conditions in the presence of 1 mM $H_2{}^{18}O_2$, (b) anaerobic conditions using 22% $H_2{}^{18}O$ and 1 mM $H_2{}^{16}O_2$, (c) aerobic conditions in the presence of 20 and 30% $H_2{}^{18}O$, and (d) aerobic conditions with $^{18}O_2$ as the source of molecular oxygen. Since the carbonyl group of 5-MF is not exchangeable with solvent, the possibility that oxygen is incorporated during the isolation of the product can be excluded.[7] In Figure 1, the mass spectra of synthetic 5-MF (left panel) is compared to that of 5-MF isolated from an anaerobic reaction mixture containing 22% $H_2{}^{18}O$ and 1 mM $H_2{}^{16}O_2$ (middle panel) and to that of 5-MF isolated from an anaerobic reaction mixture containing 1 mM $H_2{}^{18}O_2$(right panel). The results clearly indicate that $H_2{}^{18}O$ is the exclusive source

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(6) After the sample was cooled to room temperature, the 5-MF was extracted into dichloromethane, concentrated, and dissolved either in 10% acetonitrile for HPLC analysis or acetone for GC/MS analysis. The dichloromethane soluble reaction products were separated on a Phenomex Nucleosil C-18 column (5 μM, 4.6 × 0.250 mm) and detected at 260 nm using a Waters LC spectrophotometer. The mobile phase consisted of 14% acetonitrile and 86% water; results were plotted with a Shimadzu Chromatopac integrator. Calibration of the HPLC analyses from 200 nM to 2 μM was accomplished using authentic 5-MF prepared according to Grundmann and Kober.[17] The GC-MS system was composed of a Finnigan 9610 capillary GC fitted with a J & W DB-5MS 30 m × 0.28 mm i.d. fused silica capillary column interfaced directly to a Finnigan 4000 with an Incos 2300 data system. Fragments generated by electron impact ionization at 70 eV were scanned from 38 to 500 *m*/*z* in 1 s intervals. The source temperature was 250 °C.

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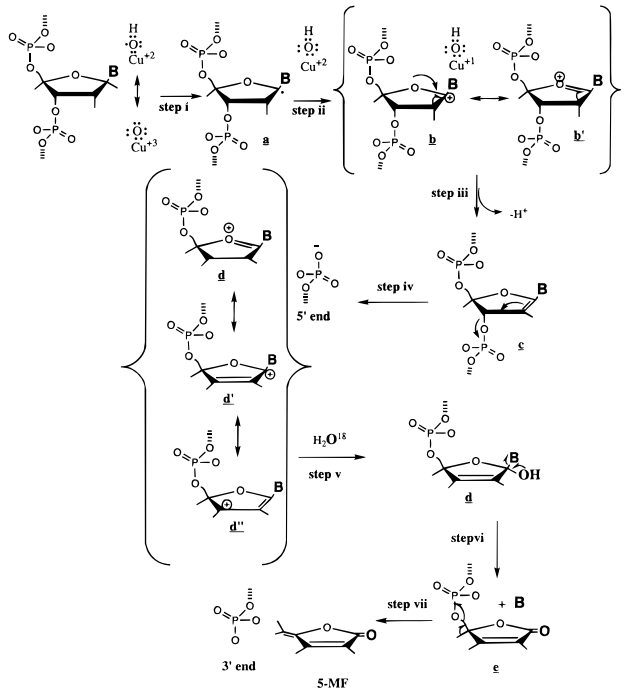
Scheme 1

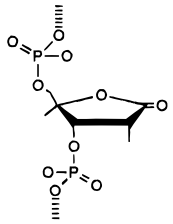


of the carbonyl oxygen of 5-MF. This conclusion is further reinforced by the finding that 5-MF isolated from aerobic reaction mixtures containing 20 and 30% $H_2{}^{18}O$ is enriched in ^{18}O to the corresponding extent (condition c above) and the finding that 5-MF isolated from a reaction mixture containing $^{18}O_2$ as the source of molecular oxygen (condition d above) is not ^{18}O enriched.

5-MF is also the product of the oxidative degradation of DNA by the manganese complex of the cationic porphyrin derivative *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin using the water-soluble potassium monopersulfate as oxidant.[7,8] In this case, the carbonyl oxygen in 5-MF is derived from the monopersulfate and not $H_2{}^{18}O$, consistent with the reaction proceeding by an oxygen rebound mechanism.[9] In addition to the different source of oxygen of the carbonyl group of 5-MF, the reactions of (1,-10-phenanthroline)copper and the manganese porphyrin with DNA also differ because the latter chelate reacts with guanosine of DNA when tethered to an oligonucleotide, generating alkaline labile sites which require piperidine treatment for strand scission.[9] In contrast, the reaction of (1,10-phenanthroline)-copper with the deoxyribose moiety of DNA leads to strand scission without the requirement of either alkaline or heat treatment.[4,10]

Several possible reaction pathways are consistent with the oxygen of water as the source of the carbonyl oxygen of 5-MF. We favor the pathway presented in Scheme 1 in which the copper−oxo species formed by the oxidation of the DNA bound $(OP)_2Cu^+$ by hydrogen peroxide and denoted as a resonance hybrid of a copper(II)−hydroxyl radical and a putative copper(III)−oxo species generates a deoxyribose-centered radical by hydrogen abstraction (drawn here C-1 localized) (**a**, step i) . The DNA bound cupric ion then oxidizes **a** to form the carbocation (**b**, step ii) which is stabilized by partial double-bond formation from the furanose oxygen. Species **c**, formed by loss of the C-2 proton (stereochemistry unknown, step iii), eliminates the 3′-phosphate resulting in strand scission, a new 5′-phosphorylated terminus and resonance-stabilized **d** (step iv). Attack by water on **d** leads to the observed incorporation of ^{18}O into the C-1 position of the deoxyribose (step v). Elimination of the purine/pyrimidine from **d** would lead to **e** (step vi), which has properties consistent with a metastable intermediate trapped in previous studies that decomposes into the new 3′-phosphorylated end and the ^{18}O-labeled 5-methylenefuranone (step vii).[3,5,11]

An important feature of the mechanism of Scheme 1 is that the phosphodiester backbone is cleaved prior to attack of water. This avoids the formation of a 2-deoxyribonolactone prior to strand scission. This intermediate has been proposed both in the scission of DNA by a manganese porphyrin[7,9] or enediynes[12,13] and in the photoreaction of a deoxyoligonucleotide containing 2′-iododeoxyuridine.[14] It is stable in the absence of heat or base treatment, two steps which are ***not*** necessary for



2-deoxyribonolactone

the cleavage of the phosphodiester backbone by $(OP)_2Cu^+$. Although the present study has focused on the products formed by the tetrahedral $(OP)_2Cu^+$, the results are probably applicable to the targeted scission of DNA by (1,10-phenanthroline)copper directed with proteins and oligonucleotides.[15,16]

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