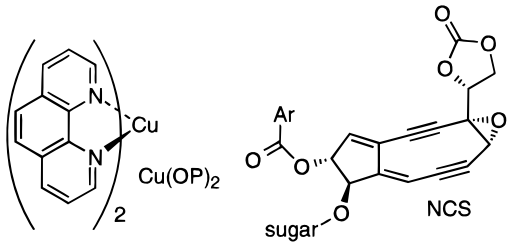
Model Studies Indicate That Copper Phenanthroline Induces Direct Strand Breaks via β-Elimination of the 2′-Deoxyribonolactone Intermediate Observed in Enediyne Mediated DNA Damage

Tongqian Chen and Marc M. Greenberg*

Department of Chemistry, Colorado State University
Fort Collins, Colorado 80523

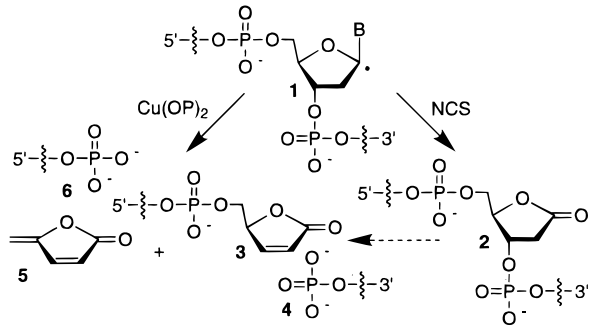
Received January 2, 1998

Oxidative damage of nucleic acids at the anomeric position of nucleotides is effected by a variety of damaging agents and can arise as a result of formal hydride abstraction, oxidation of the pendant nucleobase, or hydrogen atom abstraction (**1**).[1−7] Copper phenanthroline ($Cu(OP)_2$) and the enediynes (e.g., the neocarzinostatin chromophore, NCS) represent two of the most well studied families of DNA damaging agents that oxidize the C1′-position of nucleotides in the biopolymer. Product studies and, in the case of the enediynes, isotopic labeling experiments suggest that the initial step in damage is hydrogen atom abstraction.[5,6] Despite the formation of a common radical intermediate, $Cu(OP)_2$ and the enediynes yield different products (Scheme 1). The 2′-deoxyribonolactone (**2**), an alkaline labile lesion, is produced by the enediynes, whereas direct strand breaks result from $Cu(OP)_2$ mediated DNA damage.

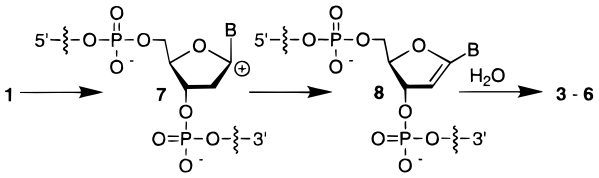


The cause for the apparent bifurcation in the reactivity of **1** has remained an open question. Recently, a mechanism was tentatively put forth to explain the disparate reactivity of **1** in the presence of these different DNA damaging agents (Scheme 2).[8] Although several pathways were considered, it was suggested that in the presence of $Cu(OP)_2$, **1** is oxidized to the carbocation (**7**), which subsequently undergoes deprotonation to the 1′,2′-dehydronucleotide (**8**). The oxidation of **1** to **7** by one or more $Cu(OP)_2$ complexes of undefined oxidation state is consistent with the incorporation of ^{18}O from $H_2{}^{18}O$.[9] The 1′,2′-dehydronucleotide (**8**) is the immediate precursor to strand break formation, and it was suggested that it gives rise to the metastable 3′-furanone (**3**) and 5′-phosphate (**6**) containing DNA fragments via solvolysis,

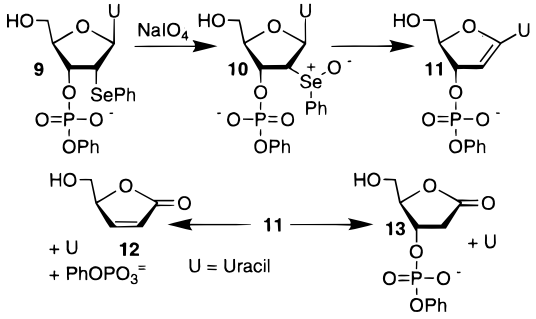
Scheme 1



Scheme 2



Scheme 3



obviating the need to proceed through **2**. We have probed the viability of the overall mechanism presented in Scheme 2 by independently generating a mononucleotide analogue of **8** (**11**). Based upon observations made using **11**, in conjunction with studies on **13** (a model for **2**), we propose an alternative explanation that accounts for the distinctive products formed by $Cu(OP)_2$ and the enediynes, such as the neocarzinostatin chromophore.

In order for a 1′,2′-dehydronucleotide (**8** or **11**) to account for the observed strand scission products, solvolysis must be complete on the time scale of typical $Cu(OP)_2$ cleavage reactions (minutes). It is also worth noting that **11** (**8**) can undergo hydrolysis to yield the free base and **13** (**2**) (Scheme 3).[10] The 1′,2′-dehydronucleotide (**11**) was produced from phenyl selenide **9** via oxidation to a diastereomeric mixture of selenoxides (**10**) by $NaIO_4$ at 4 °C in the probe of an NMR spectrometer (Figure 1). Upon warming to room temperature, the major diastereomer of **10** gave rise to **11**, which showed no evidence for decomposition after 48 h at 25 °C, and an additional 5 h at 50 °C.[11] Purified **11** (3 mM) was then shown to be stable in the presence and absence of $Cu(OP)_2$ (6 mM) in HEPES buffer (pH 7.4) for 48 h at 25 °C, suggesting that $Cu(OP)_2$ does not accelerate its decomposition to either **12** or **13**.[12]

Given the stability of **11** under aqueous conditions, we explored an alternative explanation for the difference between $Cu(OP)_2$

(1) Neyhart, G. A.; Cheng, C. C.; Thorp, H. H. *J. Am. Chem. Soc.* **1995**, *117*, 1463.
(2) Decarroz, C.; Wagner, J. R.; Cadet, J. *Free Rad. Res. Commun.* **1987**, *2*, 295.
(3) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor & Francis Inc.: Philadelphia, PA, 1987.
(4) (a) Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295. (b) Goyne, T. E.; Sigman, D. S. *J. Am. Chem. Soc.* **1987**, *109*, 2846.
(5) (a) Meschwitz, S. M.; Schultz, R. G.; Ashley, G. W.; Goldberg, I. H. *Biochemistry* **1992**, *31*, 9117. (b) Goldberg, I. H.; Kappen, L. S.; Wu, S. H.; Stubbe, J.; Worth, L.; Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 2797. (c) Kappen, L. S.; Goldberg, I. H. *Biochemistry* **1989**, *28*, 1027.
(6) (a) Yu, L.; Golik, J.; Harrison, R.; Dedon, P. *J. Am. Chem. Soc.* **1994**, *116*, 9733. (b) Epstein, J. L.; Zhang, X.; Doss, G. A.; Liesch, J. M.; Krishnan, B.; Stubbe, J.; Kozarich, J. W. *J. Am. Chem. Soc.* **1997**, *119*, 6731. (c) Xu, Y.; Xi, Z.; Zhen, Y.; Goldberg, I. H. *Biochemistry* **1995**, *34*, 12451.
(7) Pitié, M.; Bernadou, J.; Meunier, B. *J. Am. Chem. Soc.* **1995**, *117*, 2935.
(8) (a) Meijler, M. M.; Zelenko, O.; Sigman, D. S. *J. Am. Chem. Soc.* **1997**, *119*, 1135. (b) Zelenko, O.; Gallagher, J.; Sigman, D. S. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2776.
(9) Wayner, D. D. M.; Griller, D. *Mol. Struct. Energy* **1989**, *11*, 109.
(10) (a) Haraguchi, K.; Tanaka, H.; Maeda, H.; Itoh, Y.; Saito, S.; Miyasaka, T. *J. Org. Chem.* **1991**, *56*, 5401. (b) Robins, M. J.; Trip, E. M. *Tetrahedron Lett.* **1974**, *3369*.
(11) The product mixture observed by ^1H NMR was characterized by electrospray mass spectrometry, which confirmed the presence of **10** and **11**.

S0002-7863(98)00007-9 CCC: $15.00
Published on Web 04/04/1998

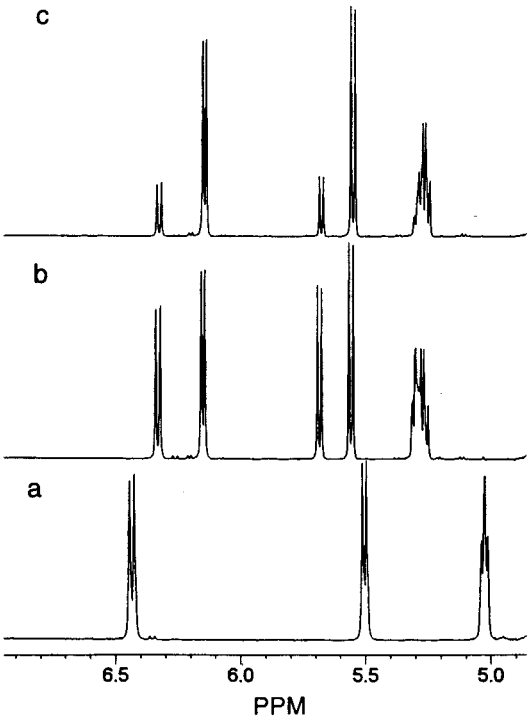


Figure 1. ^{1}H NMR spectra describing the generation of **11** from **9** (20 mM) via **10** in phosphate buffer (0.1 M, pD 7.4). (a) **9** prior to the addition of $NaIO_4$ (4 °C). (b) After the addition of 1 equiv of $NaIO_4$ (2 h at 4 °C, then 22 min at 25 °C). (c) After 48 h at 25 °C. ^{1}H NMR assigments: **9**; δ 6.45 (C5), 5.51 (C1′), 5.04 (C3′). **10**; δ 6.33 (C5), 5.69 (C1′), 5.29 (C3′). **11**; δ 6.15 (C5), 5.56 (C2′), 5.26 (C3′).

and enediyne reactivity. We considered the possibility that **2** is formed by both the enediynes and $Cu(OP)_2$ but that the noncovalently bound copper complex catalyzes its β-elimination to **3**. Such a mechanism is consistent with the observed ^{18}O-incorporation in the lactone from $H_2{}^{18}O$ by including the proposed one-electron oxidation of the initially formed radical.[8a] Consequently, the rate of decomposition of **13** was examined in HEPES buffer over a range of $Cu(OP)_2$ concentrations (Figure 2).[13] The rate of disappearance of **13** was quantitatively accounted for by the appearance of phenyl phosphate and obeyed first-order kinetics for two half-lives. Higher conversion of **13** led to a deviation from first-order decay, which was attributed to inhibition by coordination of $Cu(OP)_2$ with the product phenyl phosphate. The inhibition observed by added phenyl phosphate (3 mM) was consistent with this hypothesis. The observed rate constant for the disappearance of **13** varied linearly with $Cu(OP)_2$ concentration but was unaffected by $CuSO_4$ (which precipitates at pH 7.4) or phenanthroline by themselves. The observed rate constant for the disappearance of **13** approximately doubles between 0 and 6 mM $Cu(OP)_2$, and the significance of this increase is evident when one considers the potential effective molarity of $Cu(OP)_2$ bound

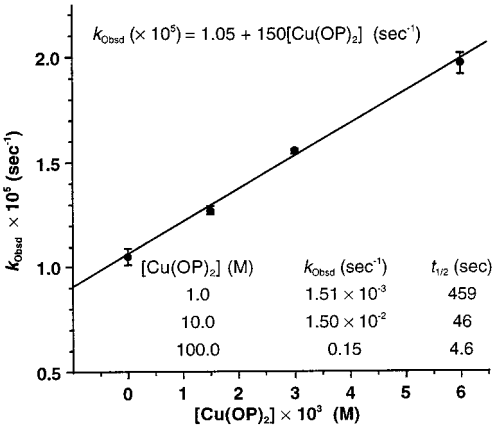


Figure 2. Plot of observed rate constant for the disappearance of **13** (3 mM) as a function of $Cu(OP)_2$ concentration. Inset: Extrapolated k_{obsd} and half-life as a function of $Cu(OP)_2$ concentration.

to DNA. The effective molarity of noncovalent complexes can be as high as 10^5-10^7 M.[14] Extrapolation of the observed rate constant to between 10 and 100 M effective concentration of $Cu(OP)_2$ would result in a half-life for elimination from **13** of less than 1 min and, if extrapolable to **2** in DNA, would explain the formation of direct strand breaks by $Cu(OP)_2$. Although the oxidation state of the copper phenanthroline complex (or the stoichiometry) responsible for this catalysis is unknown, the stability of **11**, a likely solvolysis candidate, in the presence of $Cu(OP)_2$ concentrations that accelerate elimination from **13**, suggests that the complex is acting as a general base catalyst.

While the monomeric compounds described above cannot unequivocally model the reaction between $Cu(OP)_2$ and DNA, their reactivity leads us to propose that the differences in the effects of the enediynes and $Cu(OP)_2$ on nucleic acids can be explained by applying Ockham's razor or the principle of mechanistic economy.[15] We propose that the structurally distinct DNA damaging agents referred to above produce **2** as a common intermediate (possibly via different mechanisms), which when formed in the presence of noncovalently bound $Cu(OP)_2$ undergoes subsequent elimination. These results suggest that similar catalysis may be operating in other nucleic acid damage processes mediated by coordination complexes where possible alkaline labile lesions are not observed.[16]

Acknowledgment. We thank Professor David Sigman (UCLA) for helpful discussions and Dr. Chris Rithner (CSU) for assistance with the NMR experiments. We are grateful for support of this research by the National Institutes of Health (GM-54996). Mass spectra were obtained on instruments supported by the National Institutes of Health shared instrumentation grant (GM-49631). M.M.G. thanks the Alfred P. Sloan Foundation for a fellowship.

Supporting Information Available: Electrospray mass spectrum of the mixture of **10** and **11**, ^{1}H NMR spectra shown in Figure 1 from δ 10.0−0.0, ^{1}H NMR spectra of **12** and **13** in D_2O (pD 7.4, 0.1 M, 25 °C), and experimental procedure for the synthesis of **13** (8 pages). See any current masthead page for ordering information and Web access instructions.

JA9800075

(12) The dehydronucleotide proved to be stable to column chromatography in the presence of Et_3N. Analysis was carried out by HPLC using a Rainin Microsorb-MV C_{18} column (5 μm, 4.6 × 250 mm). Eluent A: NH_4Cl (0.1 M, pH 7.2), 5% MeOH. Eluent B: NH_4Cl (0.1 M, pH 7.2), 70% MeOH. Gradient: 0−40% B linearly over 15 min; 40−100% B linearly over 5 min, followed by 100% B for 30 min. Ret. time: **11**, 26.6 min; uridine (internal standard), 6.6 min.

(13) Analysis was carried out by HPLC using a Waters Spherisorb S10 SAX column (5 μm, 4.6 × 250 mm). Eluent A: KH_2PO_4 (50 mM, pH 4.5). Eluent B: KH_2PO_4 (0.1 M, pH 3.0) + KCl (0.1 M); Gradient: 0% B 7 min; 0−100% B linearly over 12 min, followed by 100% B for 30 min. Ret. time: GMP (internal standard), 6.4 min; **13**, 7.6 min; phenyl phosphate, 9.6 min.

(14) For a recent example, see: Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *J. Am. Chem. Soc.* **1997**, *119*, 9578. For an overview see: Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman and Co.: New York, 1985.

(15) (a) Hoffmann, R.; Minkin, V. I.; Carpenter, B. K. *Bull. Soc. Chim. Fr.* **1996**, *133*, 117. (b) Berson, J. A. In *Rearrangements in Ground and Excited States*; de Mayo, P., Ed.; Academic Press: New York, 1980; Vol. 1.

(16) (a) Cook, G. P.; Greenberg, M. M. *J. Am. Chem. Soc.* **1996**, *118*, 10025. (b)Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303.