Spin-trapping Study of DNA Cleavage Induced by Enediyne C-1027 Chromophore

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We report the direct observation of radical intermediates in the course of the DNA cleavage induced by the chromoprotein antitumor agent C-1027 using spin-trapping methods. An intriguing difference of behaviors was found between MNP (2-methyl-2-nitrosopropane) and DMPO (5,5-dimethyl-1-pyrroline *N*-oxide).

C-1027 was isolated from culture broth of *Streptomyces globisporus* C-1027,[1] and was found to exert extremely potent antitumor activity.[2] This compound belongs to the family of chromoproteins[3] that contains a highly unstable chromophore (**1**, Figure 1)[4] and a carrier apoprotein.[5] While the enediyne **1** is reasonably stable when bound to the apoprotein, **1** in its free form quickly aromatizes via a Masamune-Bergman rearrangement even at room temperature (**1**→**5**).[4b] Previous studies indicated that the 9-membered enediyne **1** equilibrates with *p*-benzyne **2** in the apoprotein and is kinetically stabilized.[6] Conversely, **1** released from the apoprotein interacts with the minor groove of double strand DNA, and causes oxidative cleavage after O_2-addition with remarkable sequence selectivity.[7–9] Although the outcome of C-1027-mediated DNA damage has been studied in considerable detail, no direct observation of radical intermediates has been reported.[10] As part of our continuing interest in the chemical behavior of C-1027,[6] we conducted an ESR study on the DNA-cleaving reaction.[11] The difficulty associated with the direct ESR measurement arises from the extremely short life-time of radical species under physiologically relevant conditions. We therefore applied a spin-trapping method using MNP (2-methyl-2-nitrosopropane) and DMPO (5,5-dimethyl-1-pyrroline *N*-oxide).[12,13]

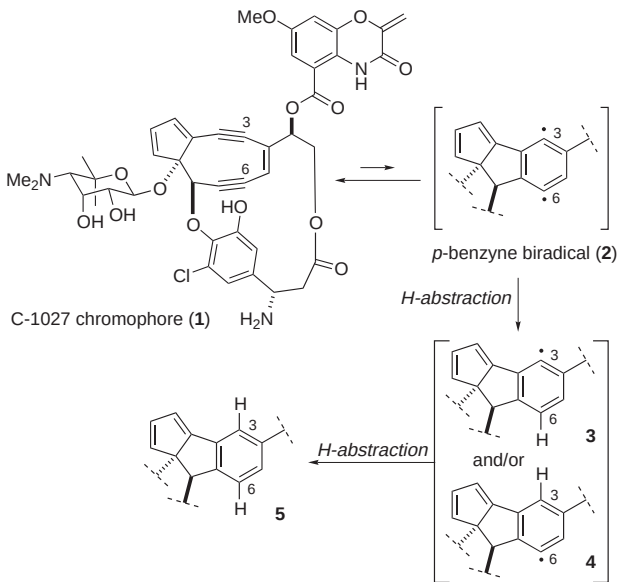

Figure 1. Structure of the C-1027 chromophore (**1**) and the aromatized product (**5**).

As a DNA substrate, we selected the dodecamer 5′-GCC**GTTA$_1$T**GCCG/5′-CGGC**ATA$_2$A$_3$C**GGC (**6**), because Goldberg showed from extensive gel electrophoresis studies that **1** abstracts hydrogen atoms of the C4′, C1′ and C5′ positions from the A_1, A_2, and A_3 nucleotide sugars of **7**, respectively, through the sequence selective binding indicated in the bold face.[8]

Firstly, the nitroso spin-trapping reagent MNP was applied to our system. Interestingly, a mixture of C-1027 and MNP in aqueous buffer did not show any signal, indicating that MNP did not trap the radicals **2–4**.[14] No spin-adduct was observed either, when the single strand DNA (5′-GCCGTTATGCCG) and C-1027 were added to MNP. In sharp contrast to these results, a combination of double strand DNA **6**, MNP and C-1027 in the buffer provided the highly anisotropic spectrum shown in Figure 2 ($g = 2.006$, $A_N = 1.5$ mT). These results indicate that **1** was bound to **6** and consequently abstracted hydrogen from **6** to generate DNA radicals trapped by MNP. An identical ESR spectrum was also observed under anaerobic conditions (data not shown),[15] from which it is speculated that the initially-formed DNA radicals[8] were trapped before reacting with molecular oxygen.

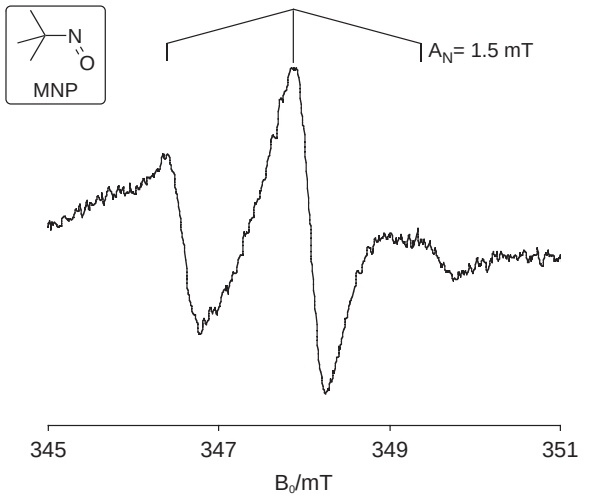

Figure 2. ESR spectrum of MNP spin adducts. C-1027 with the double strand DNA (**6**) and MNP in NTE buffer (100 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, pH 7.5) under aerobic conditions.

Next, we performed ESR experiments using DMPO, which possesses a nitrone function. Surprisingly, when only C-1027 was added to DMPO in buffer solution, an ESR signal emerged

(Figure 3A, $g = 2.006$, $A_N = 1.51$ mT, $A_H = 2.25$ mT). DMPO clearly showed a different reactivity from MNP. The same hfc values were observed even in the presence of double strand DNA **6**, while the spectrum broadened somewhat, presumably due to increased viscosity caused by the addition of **6** (Figure 3B). When the isolated chromophore **1**[16] was treated with DMPO in MeCN-H_2O, an ESR spectrum ($A_N = 1.48$ mT, $A_H = 2.09$ mT, data not shown) similar to that shown in Figures 3A and B was also exhibited. The possibility of trapped radicals derived from apoprotein **6** was thus ruled out.[6] The observed hfc values agree well with those reported for phenyl radicals[17] and no doubly trapped spin-adduct of biradical **2** was detected. Consequently, spin adducts were assigned as **7** and/or **8** (Figure 4), which can arise from single trapping of the *p*-benzyne biradical **2** or from the trapping of phenyl radicals **3** or **4**. In addition, the presence of **7** and/or **8** in the reaction mixture was supported by MALDI-TOF MS spectroscopy [Calcd for $C_{49}H_{56}O_{14}N_4Cl$ 959.348 ($M^- + 2H^+$), found 959.350].

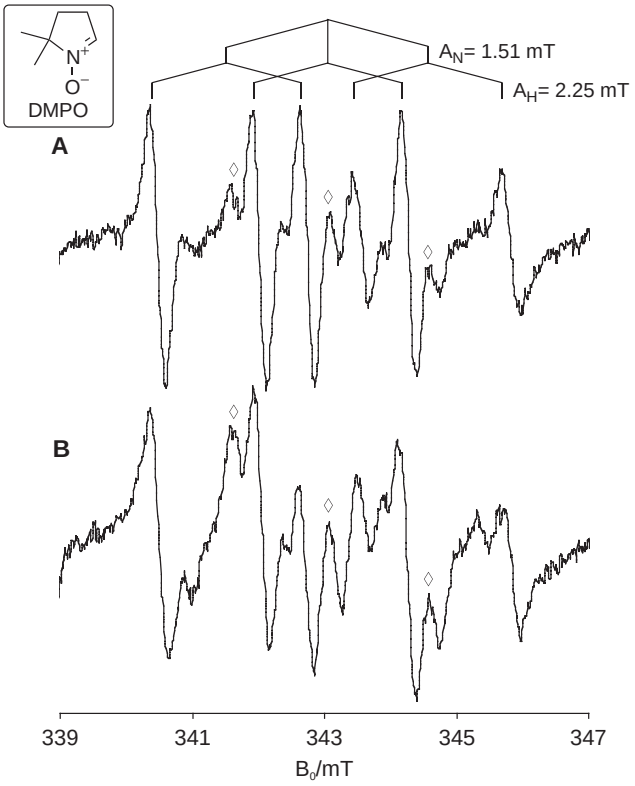

Figure 3. ESR spectra of DMPO adducts. (A) C-1027 and DMPO in NTE buffer (100 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, pH 7.5). (B) C-1027 with double strand DNA (**6**) and DMPO in NTE buffer. The triplet signal (◊, $A_N = 1.53$ mT) was tentatively assigned as 2,2-dihydroxy-5,5-dimethyl-1-pyrrolidinyloxy, an oxidized product of DMPO (Makino, K.; Hagi, A.; Ide, H.; Murakami, A. *Can. J. Chem.* **1992**, *70*, 2818–2827.).

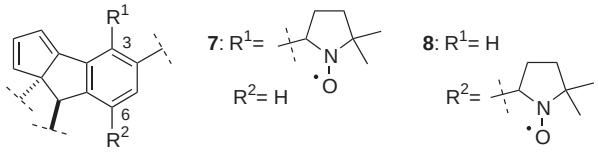

Figure 4. Possible DMPO-adducts of **1**.

In conclusion, the radical mechanisms of C-1027-induced DNA cleavage were clearly demonstrated using spin-trapping methods. Furthermore, an intriguing difference of behaviors was observed between MNP and DMPO. The present method will be useful for analyzing other radical-mediated biological processes.

References and Notes

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