Double-Strand DNA Cleavage by Copper·Prodigiosin

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The prodigiosins[1−3] (i.e. **1**) are the red pigments produced by microorganisms such as *Streptomyces* and *Serratia* that possess promising anticancer,[4] antimicrobial,[2] and immunosuppressive[5] activities. The methoxy group is critical for their anticancer properties,[4b] where they cause apoptosis[4c] and exhibit selective activity against liver cancer cell lines (50% inhibitory concentration (IC_{50}) = 0.276−0.592 μM[4c]).[6]

While the biological receptor for **1** has yet to be determined, we recently demonstrated that the related natural product tambjamine E (**2**) binds DNA effectively[7] and facilitates single-strand DNA (ssDNA) cleavage in the presence of Cu(II) and molecular O_2.[8] This implied that Cu(I) was formed reductively through the concomitant oxidation of **2** to a π-radical cation in analogy to the oxidation of pyrrole by Cu(II) salts.[9] We now report that prodigiosin (**1**) is more potent than **2** in copper-mediated DNA cleavage and has the ability to perform oxidative double-strand DNA (dsDNA) cleavage. Agents that facilitate dsDNA cleavage, such as the bleomycins[10] and the enediyne antibiotics,[11] are known to be cytotoxic, as dsDNA cleavage creates damage that is considered much more difficult for the cell to repair than ssDNA cleavage.[12] Since **1** is more cytotoxic than **2**,[13] DNA may represent a therapeutic target for the prodigiosins.

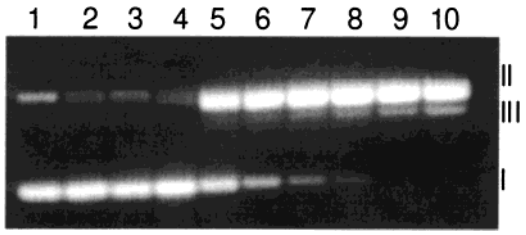
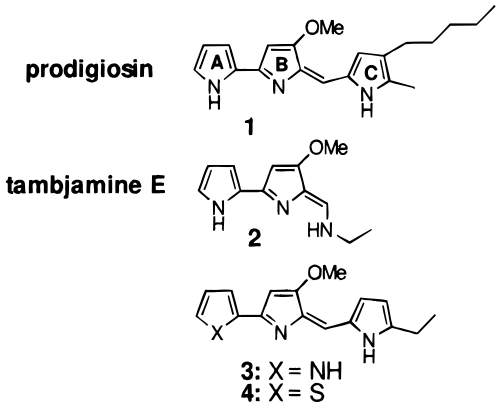


Figure 1. Relaxation of supercoiled plasmid DNA (Form I) by 30 μM CuProd at 37 °C. Reaction mixtures (20 μL total volume) contained 800 ng of Form I DNA in 10 mM MOPS buffer, pH 7.4, 75 mM NaCl, and 10 vol % CH_3CN. Lanes 1−3 were incubated for 90 min at 37 °C, while no heat with immediate quenching were conditions for 0 time (lane 4). Lane 1, DNA alone; lane 2, + 30 μM **1**; lane 3, + 30 μM Cu(II); lanes 4−10, cleavage after 0, 10, 20, 30, 40, 60, and 90 min incubation with CuProd, respectively.



The ability of **1** to perform DNA cleavage in the presence of a redox-active transition metal was carried out using agarose gel electrophoresis and supercoiled plasmid DNA (Form I). As with **2**,[8] strand-scission by **1** was effected by admixture of Cu(II), while admixture of Fe(III) and Ni(II) failed to initiate cleavage under the same conditions (data not shown). Figure 1 shows representative data for DNA cleavage by **1** in the presence of equimolar Cu(II). Following 20 to 40 min of incubation at 37 °C (lanes 6−8) all three forms of the DNA were visible on the gel. This observation is classical evidence for a dsDNA cleavage event,[10a,11c,14]

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(1) Prodigiosin (**1**) was a gift from the Natural Products Division of the National Cancer Institute (NCI) and was received as a dark red powder.

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(3) For synthesis see: (a) Wasserman, H. H.; McKeon, J. E.; Smith, L.; Forgione, P. *J. Am. Chem. Soc.* **1960**, *82*, 506. (b) Rapoport, H.; Holden, K. G. *J. Am. Chem. Soc.* **1962**, *84*, 635. (c) Hearn, W. R.; Elson, M. K.; Williams, R. H.; Medina-Castro, J. *J. Org. Chem.* **1970**, *35*, 142. (d) Boger, D. L.; Patel, M. *Tetrahedron Lett.* **1987**, *28*, 2499. (e) Wasserman, H. H.; Lombardo, L. J. *Tetrahedron Lett.* **1989**, 1725 (f) D'Alessio, R.; Rossi, A. *Synlett* **1996**, 513. (g) Fürstner, A.; Szillat, H.; Gabor, B.; Mynott, R. *J. Am. Chem. Soc.* **1998**, *120*, 8305. (h) Wasserman, H. H.; Petersen, A. K.; Xia, M.; Wang, J. *Tetrahedron Lett.* **1999**, *40*, 7587. (i) Fürster, A.; Grabowski, J.; Lehmann, C. W. *J. Org. Chem.* **1999**, *64*, 8275. (j) Fürstner, A.; Krause, H. *J. Org. Chem.* **1999**, *64*, 8281.

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(6) The NCI (Bethesda) has also shown that **1** possesses an average IC_{50} of 2.1 μM against a panel of 57 different human-cancer cells. This information is available on the Internet at www.dtp.nci.nih.gov, the NSC number for prodigiosin is 47147-F.

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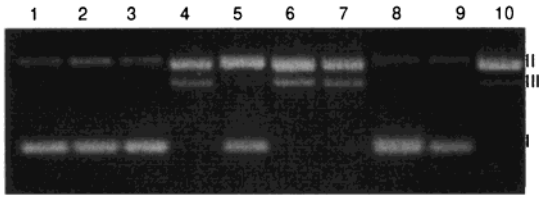

Figure 2. Inhibition studies on cleavage of Form I DNA by CuProd (40 μM). Reactions contained 400 ng of Form I DNA and were carried out for 30 min as described in the caption below Figure 1. Lane 1, DNA alone; lane 2, + 40 μM **1**; lane 3, + 40 μM Cu(II); lane 4, + CuProd; lane 5, + 100 mM NaN_3; lane 6, + 1 M *tert*-butyl alcohol; lane 7 + 1 M DMSO; lane 8 + 100 mM EDTA; lane 9, + 1000 units/mL catalase; lane 10, + 1000 units/mL SOD.

Table 1. Statistical Efficiency of Single-Strand and Double-Strand Break Formation by CuProd[a]

[CuProd] (μM)	[NaCl] (mM)	time (min)	n_1[b]	n_2	n_1/n_2
10	25	30	0.440	0.053	8.4
20	25	30	0.858	0.099	8.6
30	25	30	2.637	0.179	14.7
30	75	10	0.752	0.078	9.7
30	75	20	1.106	0.144	7.7
30	75	30	1.689	0.165	10.2
30	75	40	2.644	0.175	15.1
40	100	10	0.554	0.052	10.7
40	100	20	1.527	0.083	18.5
40	100	30	1.592	0.101	15.8
40	100	40	1.960	0.134	14.7

[a] 800 ng of Form I DNA, 100 mM MOPS, pH 7.4, 37 °C. [b] The number of ss-breaks (n_1) and ds-breaks (n_2) were determined using the statistical test of Povirk et al. (ref 10a) that assumes a Poisson distribution of strand cuts.

as Cu·prodigiosin (CuProd) generates linear (Form III) DNA before converting all of Form I to nicked circular (Form II) DNA through a ss-break. The cleavage was oxidative, as the enzyme catalase, which lowers solution concentrations of hydrogen peroxide (H_2O_2), completely inhibiting the cleavage reaction (Figure 2, lane 9).[15] Superoxide dismutase (SOD) had little effect (Figure 2, lane 10), indicating that $O_2^{\bullet-}$ is not required for the reduction of Cu(II),[15b] and nor is $O_2^{\bullet-}$ responsible for strand scission.[16] The hydroxyl radical scavengers *tert*-butyl alcohol (lane 6) and DMSO (lane 7) also failed to inhibit cleavage, which argued against participation of the freely diffusible hydroxyl radical. As noted for copper-mediated damage by hydroquinone systems, the singlet oxygen scavenger NaN_3 inhibited formation of Form III DNA (lane 5).[17] DsDNA cleavage by CuProd was also more sensitive than ss-cleavage to the concentration of NaCl (Figure S1, Supporting Information), as observed for bleomycin[18] and synthetic copper systems.[19]

Quantitation of gels[20] similar to Figure 1 afforded the data in Table 1 for the number of ds- (n_2) and ss-breaks (n_1) by CuProd. The n_1:n_2 values ranged from 8 to 19, which was significantly greater than that expected from coincidences of random ss-breaks (~120),[21] confirming that dsDNA cleavage by CuProd is a non-random process. Figure S2 (Quantitation of Figure 1, Supporting Information) illustrates the kinetics of DNA cleavage by 30 μM CuProd where half-lives ($t_{1/2}$) of 10 min for ss-breaks and 26 min for ds-breaks were acquired.[22] Figure S3 (Supporting Information) shows representative data for the number of ds-breaks (n_2) as a function of CuProd concentration. Good linear fits were obtained, which argued in favor of a single CuProd species in the dsDNA cleavage event.[10,19]

Table 2. In Vitro Cytotoxicity[a]

compd	IC_{50} (μM)
1	6.6 (2.1[b])
3	10.0
4	27.0

[a] Determined in HL-60 cancer cells. [b] Average value determined by the NCI (see ref 6).

Additional insight was provided by comparing the copper-nuclease activities of **1** with the synthetic analogues **3** and **4**.[23] Figure S4 (Supporting Information) shows that, unlike **1** and **3**, the thiophene analogue **4** (20 μM) lacked the ability to promote strand-scission of Form I DNA in the presence of equimolar Cu(II). Compared to **1** and **3**, the thiophene analogue **4** also possessed diminished cytotoxicity against HL-60 (leukemia) cancer cells (Table 2).[24] These studies demonstrated that the A-pyrrole ring plays a key role in both the copper-nuclease activities and cytotoxic potency for the prodigiosins.

Further studies are underway to determine the nature of the interaction of prodigiosin with copper and the specificity of DNA cleavage by the CuProd system.

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Supporting Information Available: Experimental details and Figures S1−S4 described in the text (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Gels were quantified using a Microtek Scanmaker E6 and the software program NIH Image 1.59. The amount of supercoiled plasmid DNA (Form I) was multiplied by a factor of 1.22 to account for reduced ethidium bromide intercalation into the supercoiled DNA.

(21) The Freifelder−Trumbo equation ($n_2 = n_1^2(2h + 1)/4L$, Freifelder, D.; Trumbo, B. *Biopolymers* **1969**, *7*, 681) was used to determine n_2 expected from coincidences of random ss-breaks (n_1), where h is the maximum separation in base pairs between two cuts on complementary strands that produces a linear DNA molecule ($h = 16$) and L is the number of phosphoester bonds per DNA strand in the plasmid ($L = 2663$).

(22) Reactions of CuProd (10−50 μM) with Form I DNA were carried out at 37 °C in 10 mM MOPS buffer, pH 7.4, and were quenched by addition of loading buffer after the required reaction time. The percent of Forms I, II, and III was quantified and first-order rate constants were determined using the ENZFITTER program. Average errors in rate constants were ±20%.

(23) The synthetic analogues **3** and **4** were prepared from 5-ethyl-1*H*-pyrrole-2-carboxaldehyde using a procedure similar to that described by Rossi for other prodigiosin analogues (ref 3f). Full experimental details are provided in the Supporting Information.

(24) Clonogenic survival assays were used to assess the cytotoxicity of prodigiosin (**1**) and the synthetic analogues **3** and **4** in HL-60, a model of human promyelocytic leukemia, cancer cells. Details are provided in the Supporting Information.