Efficient DNA Cleavage with an Iron Complex without Added Reductant

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Many DNA cleaving agents use metal ions and O_2 to cause oxidative damage to DNA.[1] The most prominent of these is the iron complex of the glycopeptide bleomycin (Fe(BLM)),[2] which can cause double-strand breaks in DNA and is clinically used in cancer treatment.[3] Bleomycin combines a metal binding moiety and a DNA recognition element; the latter increases the drug's affinity for DNA, thereby enhancing the efficacy of the metal binding unit to cleave DNA. Several synthetic DNA cleaving agents use this principle.[4] An important example is Fe(MPE), where MPE consists of an EDTA metal binding domain covalently attached to an intercalating methidium group via a short spacer.[5] Fe(MPE) has successfully been used in DNA footprinting studies because of its ability to afford nonselective DNA cleavage at μM concentrations.[6] However, Fe(MPE) requires the use of either H_2O_2 or O_2 with a large excess of reductant to carry out DNA cleavage. In the course of our efforts to model the iron site of bleomycin, we have characterized $[(N4Py)Fe(CH_3CN)](ClO_4)_2$ (**1**), where N4Py is a pentadentate N5 ligand like bleomycin.[7] Since **1** forms a transient low-spin Fe(III)-OOH species that has properties resembling those of "activated bleomycin",[7,8] we have investigated its DNA cleaving properties and appended an acridine tether to the complex. With this strategy, we have developed a new biomimetic DNA cleaving agent that is effective without added reductant. We detail our results below.

Treatment of Litmus29 supercoiled plasmid (0.1 μg/μL)with 10 μM **1** in the presence of air at pH 8 and 37 °C resulted in the nearly immediate formation of nicked DNA (23%). We found this to be a remarkable observation, since the cleavage chemistry was carried out in the absence of H_2O_2 or excess reductant, unlike in the case with Fe(EDTA). Following the design principles of bleomycin and the synthetic footprinting agent MPE, we sought to increase the affinity of N4Py for DNA by attaching the DNA intercalating agent acridine onto the ligand via a five-atom tether. The new ligand, L1, was synthesized according to Scheme 1. Metalation of L1 was carried out in CH_3OH/CH_3CN in the presence of 1 equiv of $HClO_4$ to protonate the basic aminoacridine moiety, and $[Fe(H-L1)(CH_3CN)](ClO_4)_3$ (**2**, Figure 1) was obtained as red crystals from CH_3CN/ethyl acetate. Its ^{1}H NMR showed **2** to be a diamagnetic low-spin Fe(II) complex like **1**, while the formulation of **2** as a trication tris(perchlorate) was established by elemental analysis[9] and ESI-MS, which exhibited signals at *m*/*z* 899 and 400, corresponding to the ions $\{[(H-L2)Fe](ClO_4)_2\}^+$ and $\{[(H-L2)Fe](ClO_4)\}^{2+}$, respectively. The complex of L2, the precursor to L1, could be prepared similarly and was formulated as $[(H-L2)Fe(CH_3CN)](ClO_4)_3$ (**3**), on the basis of its ^{1}H NMR, ESI-MS spectra and elemental analysis.[9] Due to their high pK_a values,[10] the appended moieties of both **2** and **3** would be expected to be protonated at pH 8, which should increase the affinity of the complexes for DNA.

Figure 1a compares the cleavage of supercoiled Litmus29 plasmid DNA (0.1 μg/μL) by **1**, **2**, **3**, Fe(MPE), and Fe(BLM) at 1 μM concentration under air *but with no added reductants*. The reactions were initiated by adding the metal complex to a buffered solution of DNA and quenched within 20 s by adding 10 μL of the solution to a solution of number III dye[11] and freezing with liquid nitrogen (Figure 2, Table 1). While less than 2% cleavage was observed for **1**, **3**, and Fe(MPE) (lanes 2, 4, and 5, respectively), significant DNA cleavage (>50%) was observed immediately after addition for **2** (lane 3) and Fe(BLM) (lane 6). While Fe(BLM) afforded both nicked and linear DNA, **2** produced only nicked DNA, typical for a single-strand cleavage agent.[12] Its cleavage activity was retained in the pH range from 5.7 to 9.2. We attribute the enhanced cleavage activity of **2** relative to **1** to an increase in DNA affinity arising from the introduction of the DNA-intercalating acridine moiety and, to a lesser extent, a positively charged group.[13]

As indicated in Table 1, **2** remains active after the initial burst of activity, albeit at a much slower rate. The amount of cleavage increased from 60 to 75% after 1 h. This result suggests that the complex must undergo a rate-limiting re-reduction to the active iron(II) form after the very rapid initial turnover.

The contrast in the DNA cleavage activities of **2** and Fe(MPE) in the absence of reducing agents is striking. When a reductant like DTT (0.01−1 mM) was added in the DNA cleavage experiments, both reagents exhibited significantly more cleavage activity, affording about 90% nicked DNA at 1 μM complex.[14] Due to this increased reactivity, DNA cleavage could be obtained with as little as 0.1 μM **2**, giving 38% nicked DNA (Table 1).

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(1) (a) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089−1107. (b) Pratviel, G.; Bernadou, J.; Meunier, B. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 746−769.

(2) Abbrevations used: BLM, bleomycin; MPE, methidium propyl EDTA.; N4Py, *N*,*N*-bis(2-pyridylmethyl)-*N*-bis(2-pyridyl)methylamine; DTT, dithiothreotol.

(3) (a) Burger, R. M. *Chem. Rev.* **1998**, *98*, 1153−1169. (b) Stubbe, J.; Kozarich, J. W.; Wu, W.; Vanderwall, D. E. *Acc. Chem. Res.* **1996**, *29*, 322−330. (c) Marusak, R. A.; Meares, C. F. *Active Oxygen in Biochemistry*; Valentine, J. S., Foote, C. S., Greenberg, A., Liebman, J. F., Eds.; Chapman and Hall: Glasgow, 1995; pp 336−400. (d) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107−1136.

(4) (a) Youngquist, R. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 1382−1391. (b) Herman, D. M.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1998**, 120, 1382−1391. (c) Turner, J. M.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1997**, *119*, 7636−7644. (d) Trauger, J. W.; Baird, E. E.; Dervan, P. B. *Nature* **1996**, *382*, 559−561. (e) Ross, S. A.; Pitié, M.; Meunier, B. *Eur. J. Inorg. Chem.* **1999**, 557−563. (f) McClean, M. S. S.; Madden, B.; Wakelin, L. P. G. *J. Chem. Soc., Perkin Trans. 2* **1997**, 523−532.

(5) (a) Hertzberg, R. P.; Dervan, P. B. *Biochemistry* **1984**, *23*, 3934−3945. (b) Hertzberg, R. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 313−315.

(6) (a) Van Dyke, M. M.; Dervan, P. B. *Science* **1984**, *225*, 1122−1127. (b) Van Dyke, M. W.; Dervan, P. B. *Biochemistry* **1983**, *22*, 2373−2377.

(7) Lubben, M.; Meetsma, A.; Wilkinson, E. C.; Feringa, B.; Que, L., Jr. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1512−1514

(8) (a) Ho, R. Y. N.; Roelfes, G.; Feringa, B. L.; Que, L., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 264−265. (b) Roelfes, G.; Lubben, M.; Chen, K.; Ho, R. Y. N.; Meetsma, A.; Genseberger, S.; Hermant, R. M.; Hage, R.; Mandal, S. K.; Young, V. G., Jr.; Zang, Y.; Kooijman, H.; Spek, A. L.; Que, L., Jr.; Feringa, B. L. *Inorg. Chem.* **1999**, *38*, 1929−1936.

(9) Elemental analysis Calcd for $C_{42}H_{44}Cl_3Fe_1N_9O_{15}$ (**2**·$2H_2O$): C 46.84, H 4.12, N 11.70; found: C 46.99, H 4.17, N 11.70; calcd for $C_{29}H_{33}Cl_3Fe_1N_8O_{13}$ (**3**): C 40.37, H 3.87, N 13.00; found: C 40.26, H 3.86, N 13.05.

(10) Acheson, R. M. *The Chemistry of Heterocyclic Compounds: Acridines*, 2nd ed.; Interscience Publishers: New York, 1973; pp 352−355.

(11) Sambrook, J.; Fritsch, E. F.; Maniatus, T. *Molecular Cloning A Laboratory Manual*, 2nd ed.; Cold Spring Laboratory Press: Plainview, New York, 1989; pp B.24.

(12) Cowan, R.; Collis, C. M.; Grigg, G. W. *J. Theor. Biol.* **1987**, *127*, 229−245.

(13) Complex **3** is more effective than **1** in DNA cleavage after 1 h (62% scission vs 37%). The larger extent of cleavage for **3** suggests that introducing a positive charge on the complex increases its interaction with the negatively charged phosphate backbone of the DNA, as also reported for iron tpen complexes substituted with a quaternary ammonium group. See: Mialane, P.; Nivorojkine, A.; Pratviel, G.; Azéma, L.; Slany, M.; Godde, F.; Simaan, A.; Banse, F.; Kargar-Grisel, T.; Bouchoux, G.; Sainton, J.; Horner, O.; Guilhem, J.; Tchertanova, L.; Meunier, B.; Girerd, J.-J. *Inorg. Chem.* **1999**, *38*, 1085−1092.

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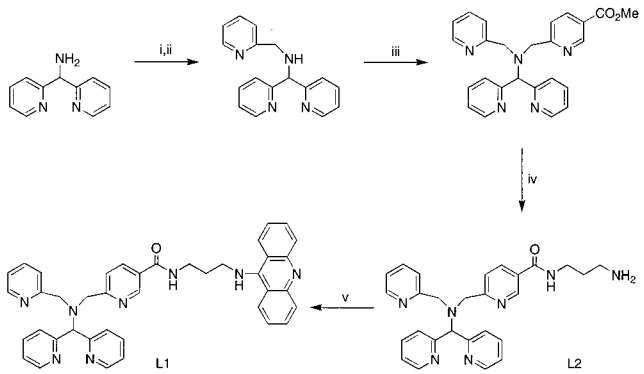
Scheme 1. Synthetic Route to Ligands L1 and L2[a]



[a] i, 2-pyridinecarboxaldehyde, 91 %; ii, $NaBH_4$, MeOH, 89 %; iii, methyl 6-(chloromethyl)nicotinate, iPr_2EtN, CH_3CN, 84 %; iv, 1,3-diaminopropane, NaCN, MeOH, 81 %; v, 9-chloroacridine, phenol, 99%.

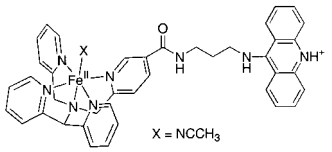


Figure 1. Structure of $[Fe(H-L1)(CH_3CN)]^{3+}$ (cation of **2**), based on the X-ray structure of **1**.[7]

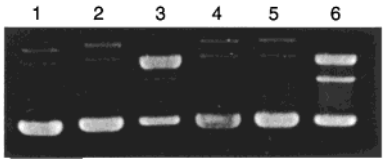


Figure 2. Cleavage of Litmus29 plasmid DNA with 1 μM reagent, cleavage at $t = 0$ h. Reactions performed in Tris buffer (10 mM, pH 8.0) at 37 °C. Lane 1, DNA control; lane 2, **1**; lane 3, **2**; lane 4, **3**; lane 5, Fe(MPE); lane 6, Fe(BLM).

Table 1. Cleavage of the Litmus29 Plasmid (0.1 μg/μL)[a]

reagent	time (h)	form I[b]	form II	form III
control	0	1.00	0	0
1	0	0.99	0.01	0
	1	0.63	0.37	0
2	0	0.40	0.59	0.01
	1	0.25	0.74	0.01
2/DTT[c]	0	0	0.85	0.15
2 / DTT[c,d]	0	0.62	0.38	0
3	0	0.98	0.02	0
	1	0.38	0.61	0.01
Fe(MPE)	0	0.99	0.01	0
Fe(MPE)/DTT[c]	0	0.07	0.92	0.01
Fe(BLM)	0	0.45	0.39	0.16

[a] Reactions were carried out with 1 μM complex in Tris buffer (10 mM, pH 8.0) at 37 °C. Forms I, II, and III were analyzed with agarose gel electrophoresis and quantified by ethidium bromide staining and densitometry. [b] Results for form I are not corrected for reduced ethidium bromide uptake capability. [c] Using 1 mM DTT concentration. [d] Using 0.1 μM complex concentration.

The nature of the termini of the DNA fragments was analyzed by 5′-^{32}P-end-labeling of a 172-bp restriction fragment. Identical results with **2** were obtained both in the presence (Figure 3) and the absence of DTT (Figure S1). No sequence or base pair selectivity was observed in the cleavage reaction. This was expected since 9-aminoacridine is known to intercalate into DNA in a sequence-neutral fashion.[15] The reaction of **2** with DNA yields two distinguishable DNA products (Figure 3a, lane 6), which match well with the 3′-phosphate and 3′-phosphoglycolate products in the Fe(BLM) and Fe(MPE) lanes but do not match at all with the 3′-OH products in the DNase I lane. The distinction among these products is most evident in the bottom section of the gel. The observed 3′-end products are typical of cleavage by a complex that generates reduced oxygen species,[1a] which strongly suggests the involvement of O_2. These results show that DNA cleavage by **2** is oxidative in nature and not hydrolytic.

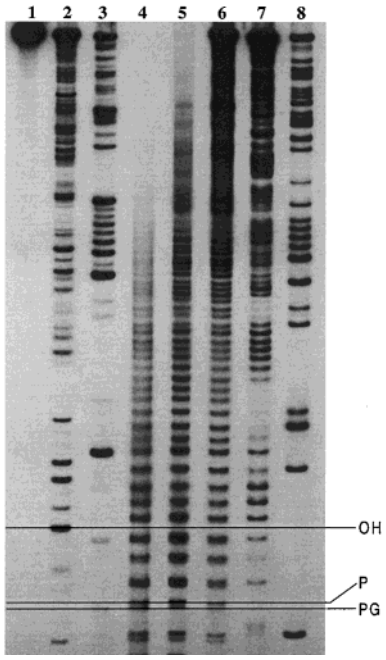


Figure 3. High-resolution denaturing PAGE gel of cleavage products of a 5′-^{32}P-end-labeled 172-bp restriction fragment after 1 h reaction. Cleavage reactions performed in Tris buffer (10 mM, pH 8.0) at 37 °C in the presence of 1 mM DTT. P denotes 3′-phosphate, PG denotes 3′-phosphoglycolate, and OH denotes 3′-OH. Lane 1, control; lane 2, DNaseI; lane 3, Fe(BLM) (10 μM); lane 4, Fe(MPE) (10 μM); lane 5, **2** (10 μM); lane 6, **2** (1 μM); lane 7, C & T; lane 8, G.

The novel feature of **2** is that it can oxidatively cleave DNA instantaneously without added reductant, unlike Fe(MPE). The latter has a carboxylate-rich environment, which stabilizes the high-spin Fe(III) state, and thus requires reductive activation. In contrast, **2** is an air-stable low-spin Fe(II) complex. Upon dissolution, we propose that the coordinated CH_3CN is labilized and can be displaced by solvent water or the phosphodiester of the DNA, thereby converting the low-spin Fe(II) center to a high-spin form that is capable of binding O_2 and activating it for DNA cleavage. This notion is supported by NMR studies of **1** and **2** in D_2O which showed the appearance of paramagnetically shifted peaks with relatively sharp signals in the 0−80 ppm range, typical for a high-spin Fe^{II} complex.[8b]

In conclusion, we have synthesized an Fe^{II}(N4Py) complex that is covalently attached via a spacer to an acridine unit. Like bleomycin, this complex instantaneously cleaves DNA using O_2, without the necessity of adding external reducing agents. Efforts toward increasing sequence selectivity by replacing the acridine by a selective DNA binder are in progress.

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Supporting Information Available: Experimental procedures for the DNA cleavage experiments and the synthesis and characterization of L1, L2, and complexes **2** and **3**, Figure S1 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Under these conditions **2** is still more efficient than Fe-MPE immediately after addition. However, it appears that Fe-MPE gives higher yields of cleavage products after 1 h compared to **2**, based on the increased degradation of 5′-^{32}P-end-labeled DNA.

(15) (a) Sakore, T. D.; Reddy, B. S., Sobell, H. M. *J. Mol. Biol.* **1979**, *135*, 763−785. (b) Crenshaw, J. M.; Graves, D. E.; Denny, W. A. *Biochemistry* **1995**, *34*, 13628−13687.