Transition-Metal Complexes that Bind and Cleave DNA

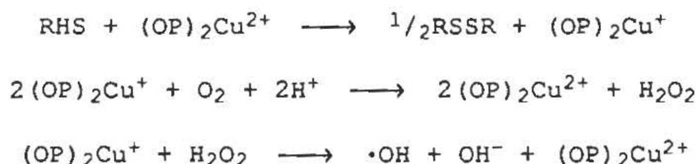
Elizabeth Wilkinson Literature Seminar April 3, 1990

DNA is a remarkably complex molecule. It is found in three main double helical forms, the right-handed-A form and B form (the Watson-Crick double helix), and the left handed Z-form [1]. Local inhomogeneities in DNA, such as cruciform sections, loops, and kinks, as well as covalent modifications, are involved in gene expression [2]. Metal complexes that cleave DNA are important because their chemistry supplements the chemistry available in naturally-occurring nucleases [3]. Metal complexes are used as footprinting reagents and a structural probes of DNA.

Unlike most naturally occurring nucleases, which cleave DNA by hydrolyzing the phosphodiester backbone, synthetic nucleases cleave DNA by damaging the deoxyribose moiety through metal-mediated redox chemistry [4]. These metal complexes bind to DNA and cleave the sugar-phosphate backbone by delivering reactive oxygen species to the sugar, or through photochemical oxidation of the sugar [3b,5].

Methidium-propyl-EDTA Fe(II) (MPE•Fe(II)) is a non-specific DNA cleaving reagent that is used in footprinting experiments [1,3b]. This complex contains a DNA intercalator, methidium, covalently bound to EDTA by a short hydrocarbon tether [6]. In the presence of dioxygen and a reducing agent, the iron-MPE complex produces single-strand breaks in double-helical DNA at every base position [7]. This reaction has been thought to involve the hydroxyl radicals, although recent work has suggested the intermediacy of an iron-oxo species in oxidations by ferrous chelates [8].

In 1979 Sigman, while doing inhibition studies of *E. coli* DNA polymerase I, discovered that the cuprous complex of 1,10-phenanthroline ($(OP)_2Cu^+$) shows nuclease activity [9]. Subsequent work demonstrated that $(O)_2Cu^+$ exhibits secondary structure specificity [10], *i.e.*, $(OP)_2Cu^+$ preferentially cleaves the B form of DNA. As does iron-MPE, $(OP)_2Cu^+$ requires dioxygen and a reducing agent for nuclease activity. Hydroxyl radicals are thought to be the reactive species and the following mechanism has been proposed for the generation of OH• in the presence of dioxygen and a thiol reducing agent [10b,12]:



$$RHS + (OP)_2Cu^{2+} \longrightarrow {}^1/_2RSSR + (OP)_2Cu^+$$

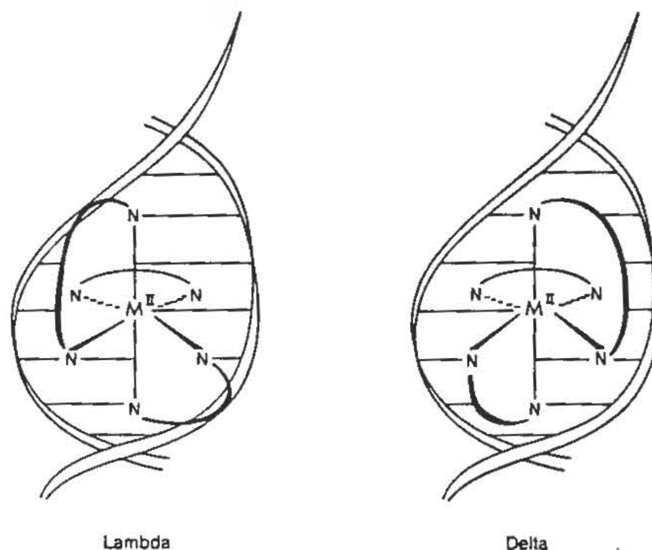
$$2(OP)_2Cu^+ + O_2 + 2H^+ \longrightarrow 2(OP)_2Cu^{2+} + H_2O_2$$

$$(OP)_2Cu^+ + H_2O_2 \longrightarrow \cdot OH + OH^- + (OP)_2Cu^{2+}$$

The cleavage patterns observed in recent work done on this system indicates minor groove binding rather than the intercalative binding observed for iron-MEP [13].

Chiral tris-chelate transition metal complexes which can discriminate between left-handed and right-handed DNA helices have been the subject of intensive studies in Barton's laboratory [14]. Optically pure tris(phenanthroline)ruthenium(II) complexes have been prepared, and their absolute configurations have been determined. These complexes show enantiomeric selectivity, with the delta isomer binding preferentially to right-handed helices. The tris(phenanthroline) complexes bind with one ligand inter-

calating, and the other two ligands fitting along the major groove. The selectivity of binding of these enantiomers of DNA is a direct consequence of their structures. The non-intercalating ligands of the lambda isomer are opposed to the direction of the right-handed groove (as depicted below [15]:



Lambda Delta

Tris-chelates of the bulkier 4,7-diphenylphenanthroline (DIP) ligand show greater stereoselectivity than the tris-phenanthroline complexes. The lambda isomer of the tris(DIP)ruthenium(II) complex shows a strong preference for left-handed (Z) DNA and thus could be used as a probe for left-handed helicity. These ruthenium(II) complexes, as well as Co(III) and Rh(III) tris-chelate complexes prepared by Barton and coworkers, cleave DNA by photoactivated redox processes [5].

Sequence-specific DNA cleaving molecules have been synthesized by attaching a metal chelator to a sequence-specific DNA binding molecule. This approach has been dubbed "affinity cleaving" [1]. Dervan and coworkers prepared one such molecule, distamycin-EDTA (DE) [16]. De•Fe(II), in the presence of dioxygen and dithiothreitol as reductant, cleaves the DNA strand next to four-base-pair A•T rich regions. This chemistry has been extended in the synthesis of several other affinity-cleaving reagents [17].

References

1. Saenger, W. *Principles of Nucleic Acid Structure;* Springer, New York, 1984.

2. (a) Barton, J. K. "Recognizing DNA Structures" *Chem. Eng. News* **1984**, *66* (39), 30.
   (b) Dervan, P. B. "Design of Sequence-Specific DNA-Binding Molecules" *Science* ***1986***, *232*, 464.

3. (a) Tullius, T. D. In *Metal-DNA Chemistry*: Tullius, T. D., Ed.; ACS Symposium Series 402, American Chemical Society: Washington, D.C., 1989.
   (b) Stubbe, J.; Kozarich, J. W. "Mechanism of Bleomycin-Induced DNA Degradation" *Chem. Rev.* **1987**, *87*, 1107.

4. Barton, J. K. In *Metal Ions in Biological Systems*: Sigel, H.; Sigel, A., Eds.; Marcel Dekker: New York, 1989; Vol. 25, Chapter 2.

5. Barton, J. K. "Light-Induced Cleavage of DNA by Metal Complexes *Inorg. Chem.* **1986**, *25* (20), 3549.

6. Dervan, P. B.; Hertzberg, R. P. "Cleavage of Double Helical DNA by (Methidiumpropyl-EDTA)iron(II)" *J. Am. Chem. Soc.* **1982**, *104*, 313.

7. Van Dyke, M. M.; Dervan, P. B. "Echinomycin Binding Sites on DNA" *Science* **1984**, *225*, 1122.

8. Rahhal, S.; Richter, H. W. "Reduction of Hydrogen Peroxide by the Ferrous Iron Chelate of Diethylenetriamine-N,N,N',N",N"-pentaacetate" *J. Am. Chem. Soc.* **1988**, *110*, 3126.

9. Sigman, D.S.; Graham, D. R.; D'Aurora, V.; Stern, A. M. "Oxygen-dependent Cleavage of DNA by the 1,10-Phenanthroline·Cuprous Complex" *J. Biol. Chem.* **1979**, *254*, 12269.

10. (a) Pope, L. S.; Sigman, D. S. "Secondary Structure Specificity of the Nuclease Activity of the 1,10-Phenanthroline-Copper Complex" *Pro. Natl. Acad. Sci. USA* **1984**, *81*, 3.
(b) Sigman, D. S. "Nuclease Activity of 1,10-Phenanthroline-Copper Ion" *Acc. Chem. Res.* **1986**, *19*, 180.

11. Kuwabara, M.; Yoon, C.; Goyne, T.; Thederahn, T.; Sigman, D. S. "Nuclease Activity of 1,10-Phenanthroline-Copper Ion: Reaction with CGCGAATTCGCG and Its Complexes with Netropsin and *Eco*RI" *Biochem.* **1986**, *25*, 7401.

12. (a) Que, B. G.; Downey, K. M.; So, A. G. "Degradation of Deoxyribonucleic Acid by a 1,10-Phenanthroline-Copper Complex: The Role of Hydroxyl Radicals" *Biochem.* **1980**, *19*, 5987.
(b) Graham, D. R.; Marshall, L. E.; Reich, K. A.; Sigman, D. S. "Cleavage of DNA by Coordination Complexes. Superoxide Formation in the Oxidation of 1,10-Phenanthroline-Cuprous Complexes by Oxygen-Relevance to DNA-Cleavage Reaction" *J. Am. Chem. Soc.* **1980**, *102*, 5419.

13. (a) Thederahn, T. B.; Kuwabara, M. D.; Larsen, T. A.; Sigman, D. S. "Nuclease Activity of 1,10-Phenanthroline-Copper: Kinetic Mechanism" *J. Am. Chem. Soc.* **1989**, *111*, 4941.
(b) Spassky, A.; Sigman, D. S. "Nuclease Activity of 1,10-Phenanthroline-Copper Ion. Conformational Analysis and Footprinting of the *lac* Operon" *Biochem.* **1985**, *24*, 8050.

14. (a) Barton, J. K.; Raphael, A. L. "Photoactivated Stereospecific Cleavage of Double-Helical DNA by Cobalt(III) Complexes" *J. Am. Chem. Soc.* **1984**, *106*, 2466.
(b) Barton, J. K. "Metals and DNA: Molecular Left-Handed Complements" *Science* **1986**, *233*, 727.
(c) Barton, J. K.; Goldberg, J. M.; Kumar, C. V.; Turro, N. J. "Binding Modes and Base Specificity of Tris(phenanthroline)-ruthenium(II) Enantiomers with Nucleic Acids: Tuning the Stereoselectivity" *J. Am. Chem. Soc.* **1986**, *108*, 2081.

(d) Mei, H.; Barton, J. K. "Tris(tetramethylphenanthroline)-ruthenium(II): A Chiral Probe That Cleaves A-DNA Conformations" *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 1339.

(e) Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. "Mixed-Ligand Complexes of Ruthenium(II): Factors Governing Binding to DNA" *J. Am. Chem. Soc.* **1989**, *111*, 3051.

15. Barton, J. K. "Tris(phenanthroline) Metal Complexes: Probes for DNA Helicity" *J. Biomol. Struct. Dyn.* **1983**, *1*, 621.

16. Schultz, P. G.; Taylor, J. S; Dervan, P. B. "Design and Synthesis of a Sequence-Specific DAN Cleaving Molecule. (Distamycin-EDTA)iron(II)" *J. Am. Chem. Soc.* **1982**, *104*, 6861.

17. (a) Youngquist, R. S.; Dervan, P. B. "Sequence-Specific Recognition of B DNA by Bis(EDTA-distamycin)fumaramidide" *J. Am .Chem. Soc.* **1985**, *107*, 5528.

(b) Wade, W. S.; Dervan, P. B. "Alteration of the Sequence Specificity of Distamycin on DNA by Replacement of an *N*-Methylpyrrolecarboxamide with Pyridine-2-carboxamide" *J. Am. Chem. Soc.* **1987**, *109*, 1574.