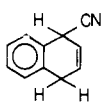
of **3** suggested a deprotonation–disproportionation of **10**/**11**. When **1**, with completely deuteriated methyl groups was used, approximately 22% of H_A in **2** was replaced by deuterium. This was consistent with the ratio of **3**:**2** being 2:10.

In order to determine what percentage of **2** was formed directly from **10**/**11** either by hydrogen atom transfer to produce **12** followed by back electron transfer from the 1-CN anion radical or by back electron transfer followed by proton transfer, an acetonitrile solution of **1-*O*-*d*** and 1-CN was irradiated. Only 34% of H_A was replaced by deuterium. These results demanded the incursion of additional mechanistic paths in order to account for the source of the other 44% of the hydrogen at H_A.

In order to establish the nature of any additional mechanisms, the non-lactone portion of the reaction mixture was carefully analyzed. This allowed the isolation of 1,4-dihydro-1-cyanonaphthalene, **13**. The reduction of 1-CN to **13** could be ra-



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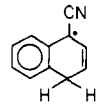

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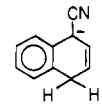

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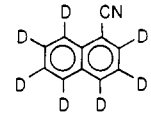

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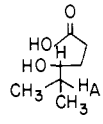
tionalized by a variety of paths, but either proton transfer or hydrogen atom transfer to the 1-CN radical anion seems most rational. This should yield **14** or **15**, respectively. Subsequent hydrogen atom transfer to **14** or proton transfer to **15** would yield **13**. Since **14** should be a significant hydrogen atom source, it would serve as a hydrogen atom carrier which would disproportionate with **10**/**11** (or their deprotonated analogues) to produce **2**. In order to test this theory, we prepared **16** from naphthalene-d_8 and used it as a photosensitizer. Two entirely different deuterium labeling experiments showed that ca. 11% of H_A of **2** was replaced by deuterium from **16**. These experiments implicate **14** as a significant reaction intermediate[15] and left the source of 33% of H_A undetermined.

A major change in the workup procedure provided part of the answer to the remaining source of H_A. Omission of the steam distillation step coupled with extraction of any free carboxylic acid and careful neutralization gave 12% of **17** and 2% of **18**.[16] Both



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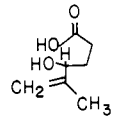

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17 and **18** resulted from the addition of water to the initially formed cation radical **9**. These compounds were readily converted into **2** and **3** under the normal reaction workup conditions,[16] providing an additional source of H_A in **2**. When an acetonitrile–deuterium oxide solution of **1-*O*-*d*** and 1-CN was irradiated and worked up under the standard conditions, 60% of H_A of **2** was replaced by deuterium. This implies that substantial amounts of **17** and **18** were converted into **2** and **3**, respectively.

Addition of the 60% deuterium at H_A, obtained from the combination of **1-*O*-*d*** with deuterium oxide, to the 22% deuterium at H_A, obtained from disproportionation with the labeled methyl groups, and 11% deuterium at H_A, obtained from exchange with **16**, accounts for the source of 93% H_A on **2**.[17] This demonstrates that at least four reaction paths are involved in the synthetically useful SET-photoconversion of γ,δ-unsaturated acids into γ-lactones.

Acknowledgment. We are indebted to the National Science Foundation for a grant which supported this investigation.

(13) Irradiation of a solution of **1** and 1-CN in acetonitrile-d_3 gave no replacement of H_A by deuterium. The use of biphenyl-d_{10} as a cosensitizer showed that none of the biphenyl deuteriums replaced H_A by deuterium.

(14) Deuterium analyses were carried out by a combination of ^2H NMR, ^{13}C NMR, and GC-CI-MS.

(15) Irradiation of an acetonitrile–deuterium oxide solution of **1-*O*-*d***, 1-CN, and biphenyl followed by isolation and spectral analysis of the 1,4-dihydro-1-cyanonaphthalene showed the 1-position of **13** to be 98% deuteriated while the 4-position was 58% deuteriated. ^2H NMR showed that only the 1- and 4-positions contained deuterium. Isolation of the 1-CN followed by spectral analysis showed that approximately 10% of the 1-CN had incorporated deuterium at C-4. Because of the possible involvement of isotope effects and the added role which 1-CN may have played in the transfer of hydrogen from either the methyls of **1** or the acidic hydrogen of **1**, the 11% transfer of a deuterium to C-5, when **16** was used, represents a minimal value for the involvement of **14** and/or **13**.

(16) Control experiments demonstrated that **2** and **3** were not converted into **17** and **18**, respectively, during the base extraction. In addition, steam distillation resulted in the complete conversion of **17** and **18** to **2** and **3**, respectively.

(17) Deuterium labeling experiments demonstrated that the hydrogens at C-2, C-3, and C-4 were not transferred to C-5 in the formation of **2**.

Design of a Double-Stranded DNA Cleaving Agent with Two Polyamine Metal-Binding Arms: $Ru(DIP)_2Macro^{n+}$

Lena A. Basile and Jacqueline K. Barton*

Department of Chemistry, Columbia University
New York, New York 10027

Received August 3, 1987

There is considerable interest in the design of small molecules which react at specific sites along the DNA strand as reactive models for protein–nucleic acid interactions, in developing new probes of DNA structure, as an aid to drug design, and as tools in molecular biology.[1] In our laboratory, a family of chiral metal complexes have been developed which are DNA conformation-specific cleaving molecules, and these may be targeted along the helical strand with site specificity to examine local variations in DNA secondary structure.[2] We report here the synthesis[3,4] and

(1) (a) Dervan, P. B. *Science* (*Washington, D.C.*) **1986**, *232*, 464. (b) Spassky, A.; Sigman, D. S. *Biochemistry* **1985**, *24*, 8050. (c) Sugiyama, H.; Kilkuskie, R. E.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1985**, *107*, 7765. (d) Hertzberg, R. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 313. (e) Fleisher, M. B.; Waterman, K. C.; Turro, N. J.; Barton, J. K. *Inorg. Chem.* **1986**, *25*, 3549. (f) Youngquist, R. S.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2565.

(2) (a) Barton, J. K. *Science* (*Washington, D.C.*) **1986**, *233*, 727. (b) Mei, H. Y.; Barton, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 7414.

(3) The Macro ligand is first synthesized by the reaction of 4,7-diphenyl-1,10-phenanthroline (DIP), disodium salt (GFS), with $SOCl_2$ in dimethylformamide at ~80 °C for 3–4 h. The resultant disulfonyl chloride, which is amber in color, is then reacted in situ with excess tren to form a disulfonamide (Macro ligand). After purification by gel chromatography (Sephadex G-10), Macro is then heated at ~60 °C with $Ru(DIP)_2Cl_2$ in 30% acetonitrile/70% H_2O for 1 h, which gives a color change from purple to yellow-orange, indicative of coordination of a third phenanthroline ligand to the metal center. Purification by gel chromatography (Sephadex G-15) yields a deeply colored yellow-orange, hygroscopic compound, $Ru(DIP)_2Macro^{n+}$. The racemic complex is then recrystallized from ethanol to remove excess salt. $Ru(DIP)_2Cl_2$ is synthesized in an analogous manner to $Ru(bipy)_2Cl_2$. See: Sullivan, B. P.; Salmon, D. J.; Meyer, T. J. *Inorg. Chem.* **1978**, *17*, 3334.

(4) UV-vis: ε (275 nm) = 1×10^5 M^{-1} cm^{-1}; ε (340 nm) = 6×10^4 M^{-1} cm^{-1}; ε (440 nm) = 2×10^4 M^{-1} cm^{-1}. Infrared: S=O stretch at 1170 and 1305 cm^{-1}; aliphatic C–N vibrations at 1095, 1130, 1140, and 1205; overtones at 1980 cm^{-1}; aromatic C–C stretches at 1450 and 1395 cm^{-1}; and characteristic bands at 610 and 480 cm^{-1}. ^1H NMR (in D_2O): methylene protons at δ 2.25, 2.30, 2.45, 2.65 ppm, and aromatic protons centered at δ 7.9. Integration of the methylene/aromatic regions varies substantially with solvent and acquisition parameters, consistent with the different correlations times and relaxation rates of the two distinct components of the molecule. FAB mass spectrum: 1514 ($Ru(DIP)_2Macro^{2+}$); 1492 ($Ru(DIP)_2Macro^+ - CH_2CH_2NH_2 + Na$; 1469 ($Ru(DIP)_2Macro^+ - CH_2CH_2NH_2$); 1410 ($Ru(DIP)_2Macro^{2+} - CH_2NH_2 - CH_2NH_2 - CH_2CH_2NH_2$); 1298 ($Ru(DIP)_2Macro^+ - NHCH_2CH_2N(CH_2CH_2NH_2)_2 - CH_2CH_2NH_2 - CH_2CH_2NH_2 + H_2O$); 1223 ($Ru(DIP)_2Macro^+ - NHCH_2CH_2N(CH_2CH_2NH_2)_2 - NHCH_2CH_2N(CH_2CH_2NH_2)_2$); 1181 ($Ru(DIP)Macro^+$); 1079 ($Ru(DIP)Macro^{3+} - CH_2NH_2 - CH_2NH_2 - CH_2CH_2NH_2$).

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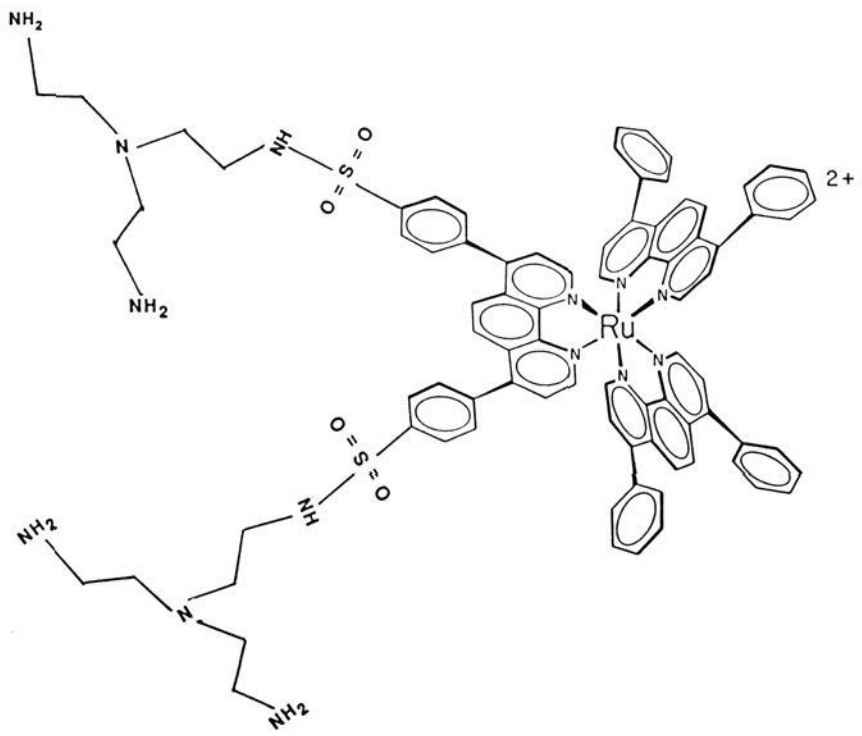
Table I. Cleavage of pBR322 Plasmid DNA

reagent	reagent/DNA (bp)	percent form[a] I	II	III
$Ru(DIP)_2Macro^{n+}$ (125 μM Cu^{2+})	0.04	0	46	54
$Ru(DIP)_2Macro^{n+}$ (100 μM Cu^{2+}, 1 h)	0.04	0	0	100
$Ru(DIP)_2Macro^{n+}$ (100 μM Cu^{2+}, 20 min)	0.04	11	47	42
$Ru(DIP)_2Macro^{n+}$ (100 μM Cu^{2+}, 5 min)	0.04	82	10	8
$Ru(DIP)_2Macro^{n+}$ (75 μM Cu^{2+})	0.04	13	70	17
$Ru(DIP)_2Macro^{n+}$ (75 μM Cu^{2+}, 120 μM H_2O_2)	0.04	13	59	28
$Ru(DIP)_2Macro^{n+}$ (125 μM Cu^{2+}, 120 μM H_2O_2)	0.04	0	26	74
$Ru(DIP)_2Macro^{n+}$ (200 μM Cu^{2+}, no mercaptoethanol)	0.1	90	10	0
$Ru(DIP)_2Macro^{n+}$ (no Cu^{2+})	0.1	100	0	0
tren (100 μM Cu^{2+})	0.08	100	0	0
diethylenetriamine (100 μM Cu^{2+})	0.08	100	0	0

[a] All samples were reacted at 37 °C for 30 min and contained mercaptoethanol unless otherwise noted. Ruthenium concentrations varied from 4–7 μM. Percent forms reported were obtained by subtraction from the DNA controls with copper.

application of a novel double-stranded cleaving agent, $Ru(DIP)_2Macro^{n+}$.[5] The complex, given below, resembles in its



central structure its analogue $Co(DIP)_3^{3+}$, shown earlier to cleave DNA at conformationally distinct sites such as Z-DNA.[6] $Ru(DIP)_2Macro^{n+}$ contains in addition two polyamine armlike segments which, in the presence of metal ions, may direct chemistry to each strand of duplex DNA for double-stranded cleavage.

$Ru(DIP)_2Macro^{n+}$ efficiently cleaves form I DNA to produce a mixture of nicked form II and linear form III DNA upon activation with copper. Table I shows the conditions used to obtain DNA cleavage. As with $Cu(phen)_2^+$,[7] DNA cleavage by this

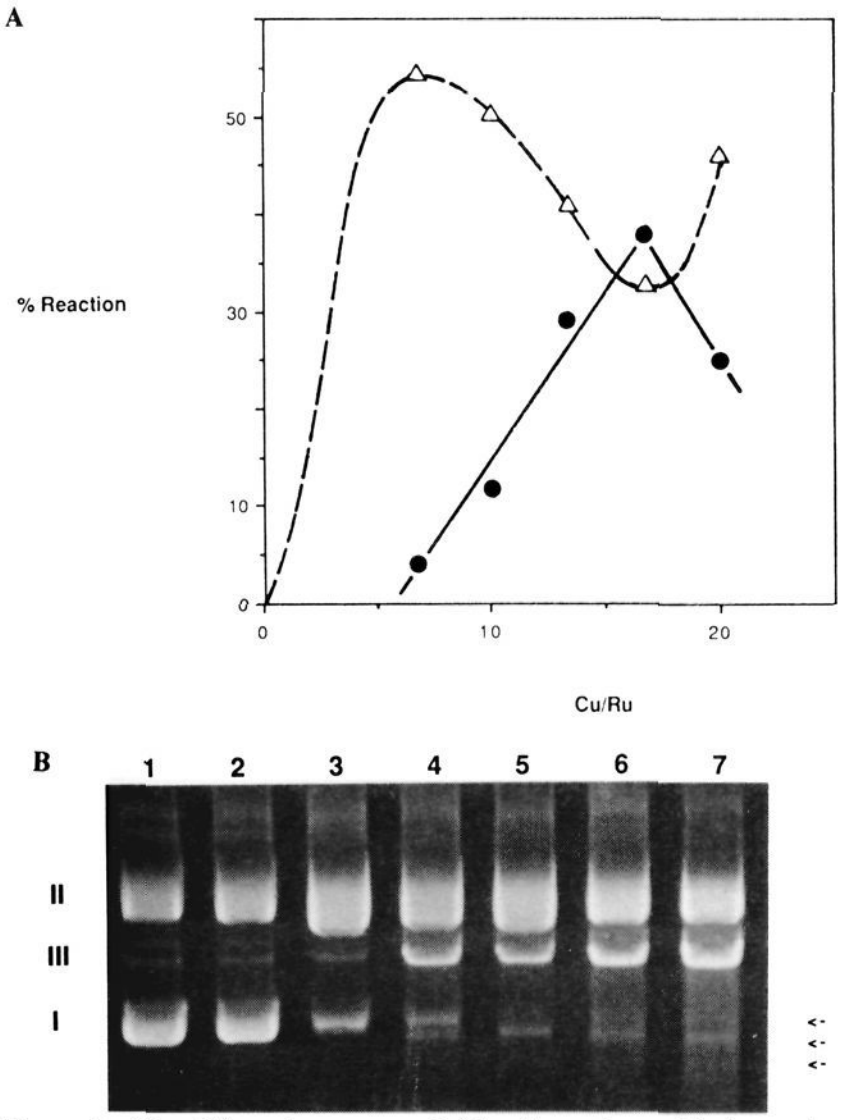


Figure 1. Plot (A) and agarose gel (B) of reactions with increasing $Cu^{2+}/Ru(DIP)_2Macro^{n+}$ ratios showing the variation in form II(Δ) and form III(●) percentages as a function of occupancy by copper of the tethered polyamine arms. (A) The reaction mixture contained 170 μM bp DNA, 7.5 μM $Ru(DIP)_2Macro^{n+}$, and 0.5 mM mercaptoethanol in buffer (20 mM Tris, 2.5 mM sodium acetate at pH 7.8). In some samples, lower molecular weight bands were found (see Figure 1B), and after quantitation by densitometry were added to the percent form III. The percent form II plotted represents the percent reaction over the DNA controls with copper. The reaction was initiated by the addition of mercaptoethanol and incubated for 30 min at 37 °C in a 20-μL volume. Samples were quenched by the addition of EDTA and bromphenol blue, electrophoresed on a 1% agarose gel, and then stained with ethidium bromide. (B) The agarose gel shows reaction with 130 μM bp DNA, 5 μM $Ru(DIP)_2Macro^{n+}$, and 0.5 mM mercaptoethanol in buffer. Lanes 1–7 show cleavage with increasing Cu^{2+} concentrations (left to right) of 20, 40, 60, 100, 125, 150, and 200 μM.

complex, containing simple tethered amines, requires copper ion and a reducing agent, and the reaction is made more efficient by the addition of H_2O_2.[8] The form II/form III ratio is time-dependent, and form III production is seen to be linear with time.[9]

Figure 1 illustrates the dependence of form II and form III production on copper/ruthenium ratios. Molecular modeling studies, assuming a binding mode similar to $Ru(DIP)_3^{2+}$,[10] reveal the two arms poised to target reactivity to each of the two DNA strands. This notion may be illustrated in cleavage experiments where the copper/ruthenium ratio is varied. At low copper/ru-

(5) $Ru(DIP)_2Macro^{n+}$ has an intrinsic +2 charge at the ruthenium(II) center and may contain an additional +2–4 positive charges owing to protonation of the tethered amines. The total charge of the complex is, therefore, pH dependent, and we represent it as $n+$.

(6) (a) Barton, J. K.; Raphael, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 6460. (b) Muller, B. C.; Raphael, A. L.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1764.

(7) See: Graham et al. (Graham, D. R.; Marshall, L. E.; Reich, K. A.; Sigman, D. S. *J. Am. Chem. Soc.* **1980**, *102*, 5419) or Goldstein et al. (Goldstein, S.; Czapski, G. *J. Am. Chem. Soc.* **1983**, *105*, 7276) for mechanistic details. Whether the reactive moiety is a metal–oxo species or the dissociated hydroxyl radical is in some debate; see: Kremer (Kremer, M. L. *Israel J. Chem.* **1971**, *9*, 321) and Walling (Walling, C. *Acc. Chem. Res.* **1975**, *8*, 125.

(8) In the presence of H_2O_2, the form III/form II DNA ratio is noticeably increased. If H_2O_2 is a limiting reagent in the cleavage reaction, then its increase in concentration should lead to an increase in form III production, corresponding to simultaneous oxidative scission of the two strands.

(9) Form III production is evident even at short reaction times (see Table I), indicating that linear DNA formation is not a result of two random single-strand cleavage events within 12–16 base pairs.

(10) Goldstein, B. M.; Barton, J. K.; Berman, H. M. *Inorg. Chem.* **1986**, *25*, 842.

thenium ratios, with copper only in one arm, single-stranded scission is observed. With increasing copper/ruthenium ratios, both arms may be filled with copper, and form III production becomes clearly evident; concomitantly, the form II yield decreases since few ruthenium complexes contain copper bound only to one polyamine arm. At still higher Cu/Ru ratios, the form II yield increases again, likely as the result of increased levels of free copper ion reacting with DNA.[11,12]

Attachment of two polyamine armlike segments to a DNA binding moiety provides a novel route to double-stranded cleavage.[13] Moreover, these results illustrate a first application of copper oxidative chemistry with saturated amines. Tethering of primary amines, such as tren, therefore, to a DNA binding agent provides a synthetically simple route to deliver metal-activated chemistry to one or both DNA strands.

Acknowledgment. We are grateful for the financial support of the NIH (GM33309) and NSF (Alan T. Waterman Award, CHE-85-17354).

(11) Copper coordination sites, either on the DNA bases or phosphates can compete with $Ru(DIP)_2Macro^{n+}$ for metal ions (DNA nucleotide/metal ratio ~3).

(12) As long as sufficient copper ion is available for occupation of both polyamine arms, lower molecular weight linear DNA fragments are observed (see Figure 1B). If the cleavage sites of Λ-$Co(DIP)_3^{3+}$ in pBR322[6a] are used as a means of orienting the observed fragment bands obtained by the reaction of $Ru(DIP)_2Macro^{n+}$ with DNA, molecular weights for these linear fragments are then consistent with the cleavage pattern seen with Λ-$Co(DIP)_3^{3+}$.

(13) See: Schultz, P. G.; Dervan, P. B. *J. Am. Chem. Soc.* **1983**, *105*, 7748. Schultz, P. G.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6834. Helene, C. ASBC Symposium 1986 for alternative schemes for double-stranded cleavage.

Metal-Activated Hydrolytic Cleavage of DNA

Lena A. Basile, Adrienne L. Raphael, and Jacqueline K. Barton*

Department of Chemistry, Columbia University New York, New York 10027

Received August 3, 1987

DNA strand scission reactions are of considerable interest both in understanding the ubiquitous phosphate ester hydrolysis reactions carried out in nature and in designing new artificial restriction enzymes. Many of the enzymes which carry out nucleophilic displacement reactions on phosphate esters require metal cations for activity,[1-4] and several model systems have elucidated the importance of an intramolecular attack of hydroxide coordinated to a metal cation on the phosphate ester to achieve nucleophilic substitution.[5-9] Small molecular DNA cleaving agents have been developed by coupling instead redox active metal ions to a DNA binding moiety.[10-12] We report here the metal-activated cleavage of DNA by small molecules through phosphodiester hydrolysis, yielding products which may be religated enzymatically.

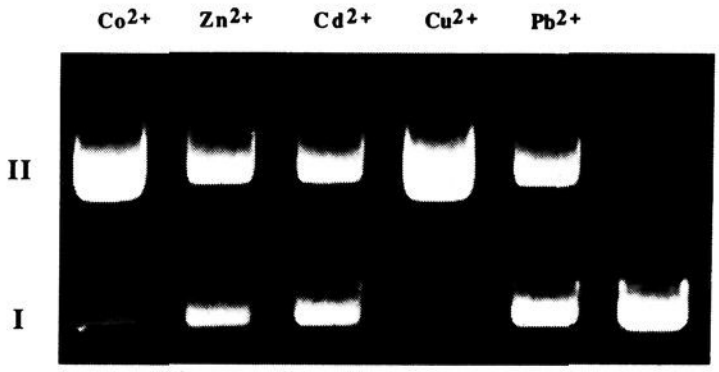


Figure 1. Cleavage of supercoiled I pBR322 DNA to the nicked II form by $Ru(DIP)_2Macro^{n+}$ in the presence of 160 μM added metal (from left to right) Co^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, and DNA control.

Table I. Metal Ion Promoted Cleavage by $Ru(DIP)_2Macro^{n+}$ and Religation of pBR322 DNA

added metal ion	cleavage[a,b]	% religated/cleaved[c,d]
Cu(II)	73 (100)	14
Co(II)	64 (88)	9
Zn(II)	30 (41)	38
Cd(II)	29 (40)	39
Pb(II)	29 (40)	39

[a] Metals were added at a concentration of 160 μM. Cleavage was conducted with 7 μM $Ru(DIP)_2Macro$, 360 μM DNA-phosphate, incubated for 5–7 h at 37 °C, pH 8.5. [b] Percent form II DNA produced after subtraction of form II DNA in the control. Relative percent efficiencies are given in parentheses. [c] Percent religation is calculated after subtraction of the percent closed circular DNA from parallel samples in the absence of ligase. [d] Zero religation is apparent after cleavage with cobalt or copper phenanthroline complexes (see ref 25).

Figure 1 shows the result of cleavage of plasmid pBR322 by $Ru(DIP)_2Macro^{n+}$[13] in the presence of a variety of added metal ions. After 5 h at 37 °C with 7 μM $Ru(DIP)_2Macro^{n+}$, conversion of form I supercoiled (360 μM nucleotides) to form II (nicked) DNA[15] is apparent in the presence of metal ions (160 μM) that are redox active and those that are not. A nonredox cleavage mechanism is therefore needed to account, at least in part, for these strand scission reactions. Also addition of ZnM magnesium ion yields no appreciable DNA cleavage and ferrous ion promotes extensive cleavage. Little cleavage was found under comparable conditions by the metal ions alone, in the absence of $Ru(DIP)_2Macro^{n+}$. The efficiency of DNA cleavage with added metal decreases in the order Cu(II) > Co(II) > Zn(II) ≅ Cd(II) ≅ Pb(II).

An enzymatic assay was performed by using T4 DNA ligase to determine whether the cleaved products were those consistent

(1) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman: San Francisco, 1979.

(2) *Metal Ions in Biology*; Spiro, T. G., Ed.; 1983; Vol. 5.

(3) (a) Coleman, J. E.; Gettins, P. *Adv. Enzym.* **1983**, *55*, 381–453. (b) Wu, F. Y.-H.; Wu, C.-W. *Met. Ions Biol. Sys.* **1983**, *15*, 157–192. (c) Barton, J. K.; Basile, L. A.; Paranawithana, S. R. *J. Biol. Chem.* **1982**, *257*, 7911–7914.

(4) Westheimer, F. H. *Science (Washington, D.C.)* **1987**, *235*, 1173–1178.

(5) Eichhorn, G. L.; Shin, Y. A. *J. Am. Chem. Soc.* **1968**, *90*, 7322–7328.

(6) (a) Milburn, R. M.; Gautam-Basak, M.; Tribolet, R.; Sigel, H. *J. Am. Chem. Soc.* **1985**, *107*, 3315–3321. (b) Scheller-Krattinger, V.; Sigel, H. *Inorg. Chem.* **1986**, *25*, 2628–2634. (c) Sigel, H.; Hofstetter, F.; Martin, R. B.; Milburn, R. M.; Scheller-Krattinger, V.; Scheller, K. H. *J. Am. Chem. Soc.* **1984**, *106*, 7935–7946. (d) Sigel, H.; Amsler, P. E. *J. Am. Chem. Soc.* **1976**, *98*, 7390–7400.

(7) (a) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 7807–7819. (b) Kenley, R. A.; Fleming, R. H.; Laine, R. M.; Tse, D. S.; Winterle, J. S. *Inorg. Chem.* **1984**, *23*, 1870–1876.

(8) Gellman, S. H.; Petter, R.; Breslow, R. *J. Am. Chem. Soc.* **1986**, *108*, 2388–2394.

(9) Brown, R. S.; Dewan, J. C.; Klug, A. *Biochemistry* **1985**, *24*, 4785–4801.

(10) (a) Dervan, P. B. *Science (Washington, D.C.)* **1986**, *232*, 464–471. (b) Wade, W. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1987**, *109*, 1574–1575.

(11) (a) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180–186. (b) Chen, C.-H. B.; Sigman, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 7147–7151.

(12) (a) Barton, J. K. *Science (Washington, D.C.)* **1986**, *233*, 727–734. (b) Muller, B. C.; Raphael, A. L.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1764–1768. (c) Mei, H.-Y.; Barton, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 7414–7415.

(13) DIP = 4,7-diphenyl-1,10-phenanthroline and Macro = 4,7-$[(NH_2CH_2CH_2)_2NCH_2CH_2NHSO_2C_6H_4]$-1,10-phenanthroline. See: Basile, L. A.; Barton, J. K., preceding paper in this issue. $Ru(DIP)_2Macro^{n+}$ has at its core the $Ru(DIP)_3^{2+}$ DNA binding moiety but also contains two polyamine tridentate[14] armlike segments to deliver metal ions to the phosphate backbone.

(14) There is some possibility of tetradentate coordination involving the deprotonated sulfonamide nitrogen, but the probability of such coordination will vary substantially with the metal ion and pH. See: Sigel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 385–426.

(15) At high divalent metal concentrations (greater than 250 μM), a substantial level of nonrandom form III (linear) DNA production has been observed. In experiments described here, added metal concentrations were maintained at 160 μM to facilitate analysis of religation experiments. At lower metal concentrations, where the polyamine arms are not fully occupied, only form II DNA is observed.

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