

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.

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(11) Copper coordination sites, either on the DNA bases or phosphates can compete with Ru(DIP)₂Macroⁿ⁺ 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 Δ -Co(DIP)₃³⁺ in pBR322^{6a} are used as a means of orienting the observed fragment bands obtained by the reaction of Ru(DIP)₂Macroⁿ⁺ with DNA, molecular weights for these linear fragments are then consistent with the cleavage pattern seen with Δ -Co(DIP)₃³⁺.

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Metal-Activated Hydrolytic Cleavage of DNA

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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

Co²⁺ Zn²⁺ Cd²⁺ Cu²⁺ Pb²⁺

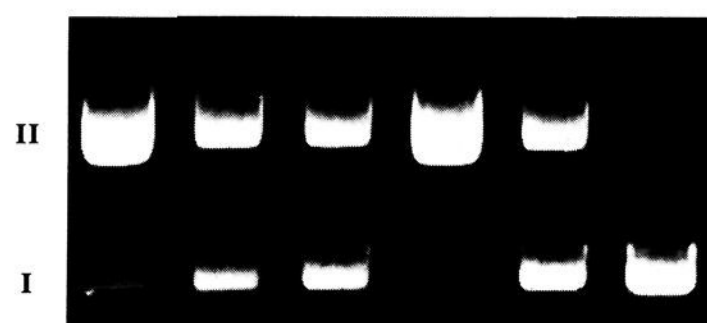


Figure 1. Cleavage of supercoiled I pBR322 DNA to the nicked II form by Ru(DIP)₂Macroⁿ⁺ in the presence of 160 μ M added metal (from left to right) Co²⁺, Zn²⁺, Cd²⁺, Cu²⁺, Pb²⁺, and DNA control.

Table I. Metal Ion Promoted Cleavage by Ru(DIP)₂Macroⁿ⁺ 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

^aMetals were added at a concentration of 160 μ M. Cleavage was conducted with 7 μ M Ru(DIP)₂Macro, 360 μ M DNA-phosphate, incubated for 5-7 h at 37 $^{\circ}$ C, pH 8.5. ^bPercent form II DNA produced after subtraction of form II DNA in the control. Relative percent efficiencies are given in parentheses. ^cPercent religation is calculated after subtraction of the percent closed circular DNA from parallel samples in the absence of ligase. ^dZero religation is apparent after cleavage with cobalt or copper phenanthroline complexes (see ref 25).

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 shows the result of cleavage of plasmid pBR322 by Ru(DIP)₂Macroⁿ⁺¹³ in the presence of a variety of added metal ions. After 5 h at 37 $^{\circ}$ C with 7 μ M Ru(DIP)₂Macroⁿ⁺, conversion of form I supercoiled (360 μ M nucleotides) to form II (nicked) DNA¹⁵ is apparent in the presence of metal ions (160 μ M) 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)₂Macroⁿ⁺. The efficiency of DNA cleavage with added metal decreases in the order Cu(II) > Co(II) > Zn(II) \approx Cd(II) \approx Pb(II).

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

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(13) DIP = 4,7-diphenyl-1,10-phenanthroline and Macro = 4,7-[(NH₂CH₂CH₂)₂NCH₂CH₂NHSO₂C₆H₄]-1,10-phenanthroline. See: Basile, L. A.; Barton, J. K., preceding paper in this issue. Ru(DIP)₂Macroⁿ⁺ has at its core the Ru(DIP)₃²⁺ DNA binding moiety but also contains two polyamine tridentate¹⁴ armlike segments to deliver metal ions to the phosphate backbone.

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(15) At high divalent metal concentrations (greater than 250 μ M), a substantial level of nonrandom form III (linear) DNA production has been observed. In experiments described here, added metal concentrations were maintained at 160 μ M 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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with hydrolysis of the phosphodiester linkages.¹⁶ The efficiency of religation after cleavage by these differing metal ions in the presence of Ru(DIP)₂Macroⁿ⁺¹⁸ is given in Table I along with cleavage efficiencies for the different added metals. As may be seen in the table, *the greatest extent of religation is apparent with the nonredox active metal ions: Zn(II), Cd(II), and Pb(II)*. The efficiency of religation¹⁹ of cleaved DNA is 40% for these metals and may be compared to the 50% yield that one would expect if hydrolysis gave the equally probable 3'- and 5'-phosphate products.^{5,20,21} Despite high levels of cleavage, only a small but significant level of religation is found both with cobalt and copper; these cleavage reactions must be operating at least in part through redox chemistry.^{22,23}

So as to determine whether Ru(DIP)₂Macroⁿ⁺ acts essentially to deliver locally high concentration of metal ion to the DNA helix for nucleophilic attack, we examined DNA cleavage by the same metal ions also in the presence of phenanthroline. The same trend with coordinated phenanthroline complexes²⁴ as was seen for Ru(DIP)₂Macroⁿ⁺ is apparent, though with lower efficiency. Relative levels of cleavage²⁵ for the phenanthroline complexes were 1.0, 0.49, 0.20, and 0.25 upon coordination with Cu(II), Co(II), Zn(II), and Cd(II), respectively, a series which resembles that seen upon activation of Ru(DIP)₂Macroⁿ⁺. Moreover a low level of religation has been detected with the phenanthroline complexes of the nonredox active metal ions, zinc and cadmium.

These results illustrate how DNA strand scission may be accomplished nonoxidatively. In our model, a DNA binding moiety may be coupled to a metal cation so as to deliver its coordinated nucleophile to the phosphate backbone for hydrolysis of the anionic

(16) DNA samples were cleaved by Ru(DIP)₂Macroⁿ⁺ with added metals to the same extent (to provide a constant form II substrate concentration) and allowed to react with T4 DNA ligase, which requires 5'-phosphate and 3'-hydroxyl termini for ligation.¹⁷ After reaction with 5-7 units of T4 DNA ligase for 6 h at 4 °C, samples in the presence or absence of ligase were treated with proteinase K containing 0.4% SDS and 3 mM EDTA and then ethanol precipitated at -20 °C. All samples were then resuspended in buffer containing 50% glycerol and bromophenol blue and electrophoresed on 1% agarose gels containing 2 μM ethidium in the gel and gel buffer. After electrophoresis at 50 V for 2 h, the gels were illuminated from below with ultraviolet light and photographed. DNA-bound ethidium in the gel unwinds and therefore resolves relaxed closed, religated DNA from the nicked circular form. The mobilities of each DNA form were determined by comparison to religated controls (DNA nicked initially by a restriction enzyme and then religated). Levels of religation were quantitated by densitometry.

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(18) DNA with bound Ru(DIP)₂Macroⁿ⁺ and added metal inhibits ligase to a varied extent. Bound ruthenium may be removed to the greatest extent by DNA treatment with DMSO or tren. DNA nicked by a restriction enzyme, incubated with Ru(DIP)₂Macroⁿ⁺ and metal, and followed by wash with 0.3 M NaCl, EDTA, and then several ethanol precipitations, inhibits the conversion to the relaxed closed form by as much as 50% after treatment with ligase.

(19) It should be noted that because the assay measures ligation by the conversion of form II DNA to the completely closed circle, if a mixture of hydrolytic and redox cleavage products occur on the same circle, religation of the hydrolytic species would not be detected; the assay therefore indicates religation only if cleavage is solely through a hydrolytic path.

(20) In the absence of a stereochemical bias imposed by the cleaving moiety, nucleophilic attack on the phosphodiester could yield both the 5'-phosphate, 3'-hydroxyl and 3'-phosphate, 5'-hydroxyl termini, despite even small differences in acidity (see, for example, ref 8). We have on occasion observed with the nonredox active metal ions religation efficiencies as high as 89%. Such a stereochemical bias for cleavage to the 5'-phosphate may be rationalized based upon binding and reaction from the major groove.

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(23) HPLC analysis showed high levels of free bases released for the cobalt-promoted reaction, consistent with oxidative cleavage of the sugar moiety in the presence of cobalt.

(24) A stoichiometry of 2.5:1 phenanthroline per metal ion was employed in these reactions to maintain some level of unsaturation in coordination in these labile complexes. A mixture of mono-, bis-, and tris-substituted phenanthroline complexes is most likely to be present.

(25) For these reactions metals were added at a concentration of 33 μM. Cleavage was conducted with 33 μM DNA-phosphate, incubated for 7 h at 50 °C, pH 9.0. After this incubation, percent form II DNA produced was 81, 39, 16, and 20 for Cu(II), Co(II), Zn(II), and Cd(II), respectively.

diester. The reactions described here are not efficient, especially in comparison to oxidative chemistry, and the parameters to optimize efficiency, e.g., coordination number, geometry, and ligand set, need still to be determined. Yet this work may be usefully applied to elucidate the mechanisms of natural endonucleases and in the development of artificial restriction enzymes.

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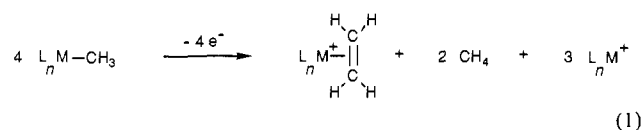
A New Method for the Activation of Metal-Bound Methyl Groups. Oxidative Disproportionation to Coordinated Ethylene and Methane

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An efficient catalytic conversion of methane to a less volatile fuel would be of considerable practical importance.² Recently, several homogeneous transition-metal complexes have been found to oxidatively insert into the methane carbon-hydrogen bond.³ However, this addition is reversible, and only a limited number of reactions of the coordinated methyl group have been found to date.^{3b} In this communication, we describe a new transformation of coordinated methyl groups, illustrated schematically in eq 1. This oxidative disproportionation converts half of the methyl groups to coordinated ethylene, which is readily displaced from metals.²ⁱ



Methyl complex $(\eta^5\text{-C}_5\text{H}_5)\text{Re}(\text{NO})(\text{PPh}_3)(\text{CH}_3)$ (1),⁴ ferricinium cation $[(\eta^5\text{-C}_5\text{H}_5)_2\text{Fe}]^+\text{PF}_6^-$ (1.0 equiv), an internal standard, and CD₃CN were combined at -78 °C and then warmed (eq 2). Gas evolution occurred when the CD₃CN melted (ca. -46 °C). The reaction was monitored by ¹H and ³¹P NMR, which showed (-15 °C) the formation of methylidene complex $[(\eta^5\text{-C}_5\text{H}_5)\text{Re}(\text{NO})(\text{PPh}_3)(=\text{CH}_2)]^+\text{PF}_6^-$ (2, 47%),⁵ deuterioacetone nitrile complex $[(\eta^5\text{-C}_5\text{H}_5)\text{Re}(\text{NO})(\text{PPh}_3)(\text{NCCD}_3)]^+\text{PF}_6^-$ (3-d₃, 51%),⁵ and methane (δ 0.18). We have previously reported that

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