Chemical Nucleases

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Contents

I. Introduction

The first-order rate constants for the hydrolysis of a phosphodiester bond under physiological conditions have been estimated to be 6×10^{-9} and 6×10^{-10} min^{-1} for single-stranded and double-stranded DNA, respectively.[1,2] These rates can be enhanced by metal ions and pH, but if the temperature is constrained to 37 °C the half-lives are still insignificant on the physiologically relevant time scale.[3-6] The evolution of a family of phosphodiesterases or nucleases which could catalyze the cleavage of the phosphodiester bond by a factor of 10^{12} relative to the noncatalyzed rate was therefore necessary in order to use DNA as genetic material. Except for the enzymes involved in DNA repair,[7] these nucleases rely on a direct nucleophilic displacement reaction with inversion of configuration to achieve catalysis.[8] This generalization is valid for ribozymes, restriction endonucleases, as well as nonspecific nucleases (e.g. micrococcal nuclease).[9]

The mechanisms of phosphodiester bond hydrolysis, both enzymatic and nonenzymatic, have been a subject of continuing interest to physical–organic chemists.[10] Many of these studies, some of which will be summarized toward the end of this review, are biomimetic and rely on simple systems which incorporate well-known enzymatic properties such as metal coordination, general acid–base catalysis, and nucleophilic attack to hydrolyze activated phosphodiesters (and often triesters). Despite impressive strides in our conceptual understanding of catalysis, simple systems have not yet been constructed which efficiently catalyze the hydrolysis of phosphodiesters.

The only efficient chemical solutions for the design of nucleolytic agents operative at neutral pH have involved free-radical and oxidative pathways which indirectly result in phosphodiester cleavage. Generally this has taken the form of redox-active coordination complexes (e.g. 1,10-phenanthroline–copper, ferrous–EDTA) which react either via metal–oxene or diffusible hydroxyl radicals. Perhaps because low molecular weight species are inherently too simple to catalyze phosphate ester hydrolysis, nature has also evolved various natural products capable of oxidative and/or radical chemistry. For example, bleomycin, a product of streptomyces fermentation, cleaves DNA via an iron–oxo intermediate and has played a heuristic role in our understanding of the reactions of abiological coordination complexes.[11] In addition, Nature has evolved the ene–diyne family of antibiotics which are presumably triggered by cytosolic nucleophiles or reducing

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David S. Sigman received his A.B. degree in Chemistry from Oberlin College in 1960 and the A.M. (1962) and Ph.D. (1965) degrees from the Department of Chemistry, Harvard University. His Ph.D. research was carried out in Professor F. H. Westheimer's laboratory and explored the catalytic role of zinc ion at the active site of alcohol dehydrogenase. Postdoctoral research in Professor E. R. Blout focused on developing affinity labels for proteolytic enzymes. Dr. Sigman joined the Department of Biological Chemistry of UCLA School of Medicine in 1968. He has worked on model systems for metalloenzymes, the mechanism of action of the ATPase from chloroplasts, and ligand binding interactions of acetylcholinesterase and the acetylcholine receptor. He discovered the chemical nuclease activity of 1,10-phenanthroline–copper in 1978 while exploring the inhibition of *E. coli* DNA polymerase I by 1,10-phenanthroline. This DNA scission activity neatly explained the dependence of 1,10-phenanthroline inhibition of the polymerase on thiol and copper. His current research focuses on the development of scission reagents suitable for genome analysis and devising gene-specific inactivation agents.

Abhijit Mazumder was born in Bombay, India, in April, 1965. He graduated from the Johns Hopkins University with a B.A. in 1986 after majoring in the Natural Sciences. He then obtained a Ph.D. in Biochemistry in 1990 from the University of Maryland. His doctoral dissertation focused on the enzymology of DNA repair. He determined that endonuclease V from bacteriophage T_4 cleaves aldehydic abasic sites in DNA by a syn β-elimination and not an hydrolysis mechanism. This was the first demonstration of an endonuclease acting as a lyase and not as an hydrolase. He then conducted postdoctoral research with D. S. Sigman from 1990 to 1993. While at UCLA, he investigated mechanisms of gene repression (with the *E. coli* trp repressor) and transcription (with *E. coli* and eukaryotic RNA polymerases). He was a Lucille P. Markey fellow for 1990–1991 and a Giannini fellow for 1991–1992. He has continued postdoctoral research at the National Cancer Institute designing inhibitors for the human immunodeficiency virus type one integrase and antitumor drugs which trap the topoisomerase cleavable complexes for the treatment of AIDS and cancer, respectively. His research interests lie in the field of DNA structure and DNA-binding proteins and the processes in which they are involved as targets for the development of chemotherapeutic agents.

agents which in turn promote an aromatic cyclization generating a diradical species which again results in phosphodiester cleavage.[12]

This review will focus on the reactivities and applications of abiological, nucleolytic, redox-active coordination complexes. The nucleolytic activities of the coordination complexes of 1,10-phenanthroline will be emphasized since it is the central subject of the authors' research program. A recent review[13] has discussed the strategies of the targeting chemical nucleases using proteins, peptides, DNAs, and RNAs as carrier ligands with both 1,10-phenanthroline and ferrous–EDTA as scission reagents and therefore will not be considered here.

II. 1,10-Phenanthroline–Copper

A. Background

The chemical nuclease activity of 1,10-phenanthroline–copper was discovered while studying the mechanism of inhibition of *Escherichia coli* DNA polymerase I (Pol 1) by 1,10-phenanthroline. Initially, the sensitivity of this enzyme to 1,10-phenanthroline was presumed to be due to the presence of a catalytically essential zinc ion at the active site of the enzyme.[14] However, this interpretation was inconsistent with the dependence of the inhibition on thiol and copper ion.[15,16] Subsequent studies established that the inhibition resulted from a surprisingly efficient nucleolytic reaction involving the 2:1 1,10-phenanthroline–cuprous complex [$(OP)_2Cu^+$] and hydrogen peroxide as a coreactant.[17] Inhibition of Pol I results because the deoxyribooligonucleotides with 3′-phosphomonoester

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termini which are generated in the chemical cleavage reaction are effective dead-end inhibitors of DNA polymerases.[18] Under the experimental condition in which Pol I inhibition was observed, the reaction was specific for copper ion and 1,10-phenanthroline derivatives which lacked substituents ortho to the nitrogen.

At the time of the discovery of the chemical nuclease activity of 1,10-phenanthroline–copper, the ability of other redox-active coordination complexes to cleave DNA had been established. For example, Zamenhof[19] had demonstrated that ferrous–EDTA reduced the

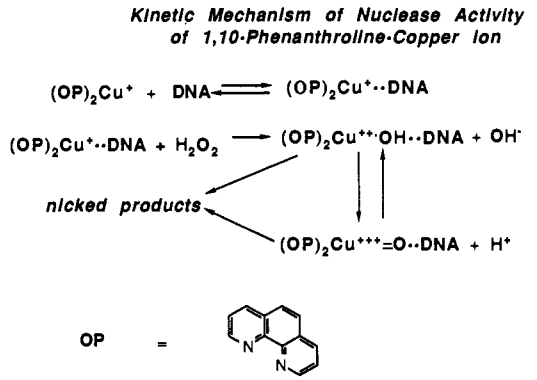


Figure 1. Kinetic mechanism of the chemical nuclease activity of 1,10-phenanthroline–copper.

transforming activity of DNA. In addition, the mechanism of action of bleomycin was the subject of intense scrutiny in a number of laboratories.[20,21] Nevertheless, the scission chemistry of 1,10-phenanthroline–copper was unexpected and prompted further studies of its mechanism. One of the striking features of the reaction was its specificity for the 1,10-phenanthroline ligand system and the absolute requirement for copper ion.

B. Kinetic Mechanism

A variety of characteristics of the reaction have led us to postulate that the first step in the nucleolytic reaction of $(OP)_2Cu^+$ with DNA involves the reversible binding of the tetrahedral cuprous complex of 1,10-phenanthroline with DNA to form an essential noncovalent intermediate[22] (Figure 1). The most compelling feature is the dependence of the nucleolytic reaction on the structure of DNA. B-DNA is the preferred substrate; Z-DNA and single-stranded DNA are not extensively cleaved under conditions where B-DNA is totally degraded.[23,24] Reactions which proceed via freely diffusible reactive species would not be expected to demonstrate conformational specificity assuming the oxidatively sensitive bonds are freely accessible to solvent. The central role of the intermediate in catalysis means that the structure of the 1,10-phenanthroline–copper complex is crucial to understanding the specificity and reactivity of the nuclease activity. Since the available evidence indicates that the 1,10-phenanthroline–cupric complex can be reduced in solution, but not when bound to DNA,[25] the chemical nuclease proceeds by an ordered reaction mechanism in which the 1,10-phenanthroline–*cuprous* complex binds first to the DNA. However, the structure of the *cupric* complex must also influence the reaction because it is formed during the redox cycling required for the reaction to proceed.

The second step is the oxidation of the DNA-bound cuprous ion by hydrogen peroxide resulting in a copper–oxo species which then attacks the DNA leading to a series of reactions resulting in strand scission. The reaction is absolutely dependent on hydrogen peroxide as demonstrated by the complete blockage of the reaction by catalase. No organic peroxide has been found which can substitute for hydrogen peroxide.

The postulated reaction scheme indicates that there are two aspects which must be addressed if we are to understand the chemical nuclease activity of 1,10-phenanthroline–copper: (a) the nature of the reactive species responsible for the scission, and (b) the forces which govern the noncovalent interaction between the complex and its DNA target. Thus, both the intrinsic redox chemistry due to the copper ion as well as the extrinsic stereoelectronic and steric properties of phenanthroline copper complexes are of equal importance in characterizing the overall reactivity.

C. Chemical Mechanism

The first aspect was addressed by the characterization of the scission products in the hope that we could infer something about the nature of the reactive intermediate. An understanding of the chemistry of scission would also provide insight into the structure of the reactive noncovalent intermediate. Using a range of techniques, we demonstrated that the predominant isolatable reaction products were nucleotides bearing 5′- and 3′-phosphomonoester termini, free bases, and 5-methylene furanone.[26] In addition, a metastable intermediate at the 3′-end could be detected when 5′-^{32}P-labeled DNA was used as the substrate.[18,27] The reaction scheme (Figure 2) is consistent with these observed products and suggests that the initial site of attack of the DNA-bound $(OP)_2Cu^+$ in the presence of H_2O_2 was the C-1 hydrogen of the deoxyribose. A minor alternative reaction pathway involves the attack of the oxidative species formed by DNA-bound $(OP)_2Cu^+$ and H_2O_2 on the C-4 hydrogen leading to the production of 3′-phosphoglycolate termini free bases and an as yet uncharacterized 3-carbon fragment. The reaction mechanism of the chemical nuclease activity *demands that $(OP)_2Cu^+$ binds to DNA in an orientation in which the copper ion is accessible to the C-1 hydrogen of the deoxyribose in the minor groove.*

The precise structure of the oxidative intermediate is not yet known. Since the reactants, $(OP)_2Cu^+$ and H_2O_2 are formally equivalent to those required in Fenton chemistry, it has been assumed that it resembles a hydroxyl radical. However, the kinetics of the reaction of $(OP)_2Cu^+$ with hydrogen peroxide have been carefully analyzed with the specific goal of determining whether

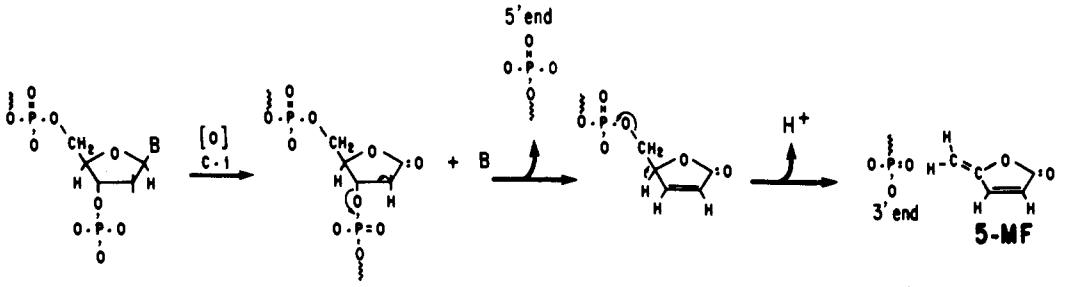


Figure 2. Reaction pathway of the scission of DNA by $(OP)_2Cu^+$. 5-MF = 5-methylenefuranone.

a free hydroxyl radical is formed.[28] These studies showed that the oxidative intermediate formed from $(OP)_2Cu^+$ and hydrogen peroxide is quenched by a scavenger alcohol at a rate that is 10^4 times less rapid than the rate for the quenching of free hydroxyl radicals generated by γ-irradiation. Thus, the intermediate favored by these studies is a hydroxyl radical coordinated to Cu(II).[28]

In agreement with these physical-organic studies, the nucleic acid chemistry also argues against the formation of a diffusible hydroxyl and in favor of a hydroxyl radical coordinated to Cu(II). One line of evidence is that equivalent amounts of 3′-phosphomonoester and 3′-phosphoglycolates are **not** formed as would be expected if hydroxyl radicals were generated.[27] The former predominates over the latter in a ratio of 8:1. Were free hydroxyl radicals the reactive species, this preference should not be observed and instead a 1:1 ratio of the two possible products, as has been observed with ferrous–EDTA, would be expected.[29] The second is that 5-succinamido-1,10-phenanthroline–cuprous ion, which does not bind to DNA because of its negative charge, does not cause DNA cleavage although it is redox-active.[25] If free hydroxyl radicals were generated in solution by the 1,10-phenanthroline–copper system, DNA cleavage would be observed as is the case for ferrous–EDTA.[30,31] Currently, we favor a copper oxene or a copper-coordinated hydroxyl radical as the reactive species directly responsible for initiating the cleavage reaction.

This underscores an important difference between the nucleolytic activity of the 2:1 phenanthroline–cuprous complex and that of other metal chelates such as ferrous–EDTA: *The reactive species remains tightly coordinated to the copper ion and its association and reactivity with DNA is mediated by noncovalent interactions between the phenanthrolines and the DNA.*

D. Dependence of the Reaction on Secondary Structure

Since the intrinsic chemical reactivity of the deoxyribose is not altered by the conformation of DNA, the reaction specificity must reflect binding affinity of the coordination complex and not the covalent bond-making/bond-breaking steps. The chemical nuclease activity exhibits specificity for different secondary structures of DNA and for primary sequence within a B-DNA format. B-DNA is the most susceptible secondary structure to $(OP)_2Cu^+$. However, A-DNA is 14–17% as reactive as B-DNA depending on the polynucleotides used for the assay; Z-DNA is at least <2% as reactive as B-DNA.[23,24] These rate estimates were made by comparing the scission of the B-DNA, poly(dA-T), in 3 M NaCl to that of the Z-DNA, poly(dG-dC) also in 3 M NaCl. Single-stranded DNA is not a substrate if poly dT is used in the assay. However, if M-13 DNA is the substrate, cleavage can be observed but the reaction clearly depends on residual secondary structure that this single-stranded DNA of heterogeneous sequence can form.[32] Consistent with this view, cleavage of unmodified single-stranded M-13 DNA shows an inverse temperature effect, reacting more rapidly at lower temperatures most likely because transiently formed B-DNA subdomains provide a binding site for $(OP)_2Cu^+$. In contrast, if the M-13 DNA is reacted with formaldehyde, which blocks the generation of these snap-back structures, no nicking is observed.

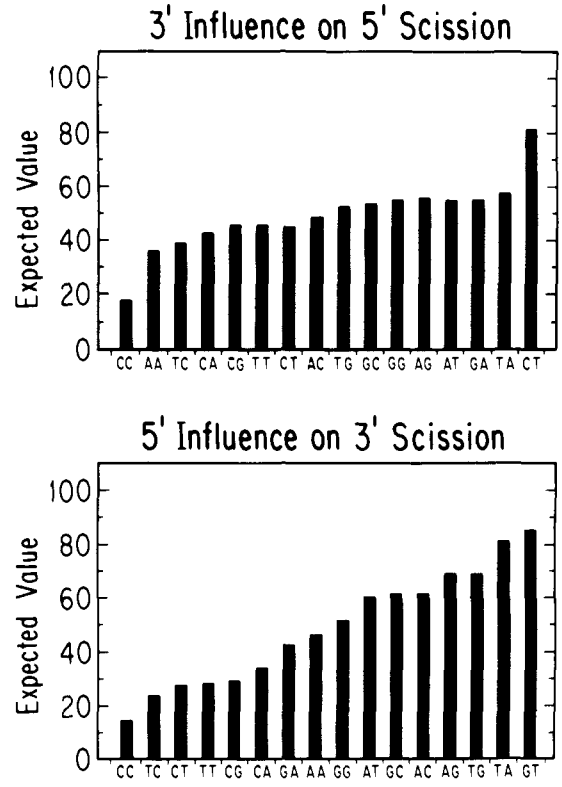


Figure 3. Primary sequence dependence of the scission reaction by $(OP)_2Cu^+$: (upper panel) influence on scission rate by the 3′-nucleotide on its 5′-neighbor (e.g. AG indicates the influence of G on the scission at A); (lower panel) influence on scission rate by the 5′-nucleotide on its 3′-neighbor (e.g. AG indicates the influence of A on the scission at G).

E. Dependence of the Reaction on Primary Sequence

The primary sequence dependence of the chemical nuclease activity of $(OP)_2Cu^+$ is readily apparent with any restriction fragment.[24,32–35] Certain sequences show intense scission; others are cleaved only slightly. There is no correlation between nucleotide structure and cleavage preference.[36,37] For example, it is just as likely for a guanosine to be a site of strong scission as one of weak scission. The cleavage pattern shows some resemblance to that of DNase I in that scission by both nucleolytic agents depends on sequence. An important difference, however, is that DNase I cuts are very discrete (i.e. scission sites may be as far away from one another as 4 sequence positions). In contrast, $(OP)_2Cu^+$ generally will generate an envelope of scission sites in any sequence domain. Ferrous–EDTA and methidium propyl–EDTA cleavage patterns show some variation in intensity but the gradations are much more subtle than those observed with $(OP)_2Cu^+$.[30,38]

The method that we chose to analyze the sequence dependent variability was to examine the $(OP)_2Cu^+$ cleavage pattern of a variety of DNAs and then score how frequently any one of the 16 possible dinucleotide sequences were in the first to fourth quartile as intense sites of cleavage.[37] Statistical analysis provided the reaction profiles presented in Figure 3. These data led to the interesting conclusion that an important deter-

minant governing the scission reaction at a given sequence position was the nucleotide 5′ to the site of scission (Figure 3). Given the antiparallel structure of double-stranded DNA, it can also be stated that the nucleotide 3′ on the complementary strand influences scission. Although the structural basis for this empirical correlation is not yet apparent, these results provide general support for the notion of sequence-dependent conformational variability of DNA advanced by Dickerson and colleagues based on their pioneering single-crystal X-ray studies of deoxyoligonucleotides.[39]

Although the 5′ neighboring nucleotide is of primary significance, it is obvious from the bar graph that the 3′ neighbor also has some influence on the scission pattern. In one study, the cleavage preference of $(OP)_2Cu^+$ in restriction fragments derived from a pUC9 plasmid was examined.[40] The most sensitive sequence to scission was the deoxyribose of the adenosine in TAT triplet. Related sequences TGT, TAAT, TAGPy, and CAGT were moderately preferred.[40] Up to 2 sequence positions in either direction (a range of 5 sequence positions) of the oxidized ribose may play a role in the reaction preference of $(OP)_2Cu^+$. In this screening of the reaction specificity, it was also apparent that polypyrimidine and polypurine stretches were cleaved with low frequency. In all studies of the reaction specificity, strong scission sites on one strand were associated with the preferred scission sites on the opposing strand as well. However, owing to the antiparallel orientation of the two strands of DNA, the scission site positions of opposite strands are displaced with respect to each other in the 3′ direction. It is this so-called 3′ stagger which provides further evidence that the minor groove is the site of binding and attack by the chemical nuclease.[41,42]

F. Structure of Reactive Intermediate

1. Pertinent Crystal Structures

The crystal structures of various 1,10-phenanthroline–copper complexes are relevant to the structure of the reactive noncovalent intermediate. The crystal structure of monoaquobis(1,10-phenanthroline)copper(II) nitrate $(Cu(H_2O)(phen)_2)(NO_3)_2$ has demonstrated that the two phenanthroline ligands deviate from coplanarity owing to the steric repulsion between the ortho 2 and 9 hydrogens. The structure of $Cu(H_2O)(phen)_2)(NO_3)_2$ is presented in Figure 4a and demonstrates that the cupric complex with a water molecule as a fifth ligand can be considered a distorted trigonal bipyramid with the axial positions occupied by N(1) and N(1′) and cupric ion, N(2), N(2′), and O4 lying in the trigonal plane.[43] The N(1)–Cu–O(4) angle is 85.5°. The angles of O(4)–Cu–N(2) and N(2)–Cu–N(2′) are 110° and 139.6°, respectively. The N–Cu–N angles are 83°. This structure obviously is relevant to the structures of the complexes that may be formed during the nucleolytic reaction but it cannot be assumed that they will have an identical geometry.

The structures of the cuprous complexes of 1,10-phenanthroline have demonstrated variability. In the complex with 2,9-dimethyl-1,10-phenanthroline (DMOP), the dihedral angle formed by the two phenanthroline rings has varied from 94.6° to 107.6°.[44] For a perfect tetrahedral complex, this angle would be

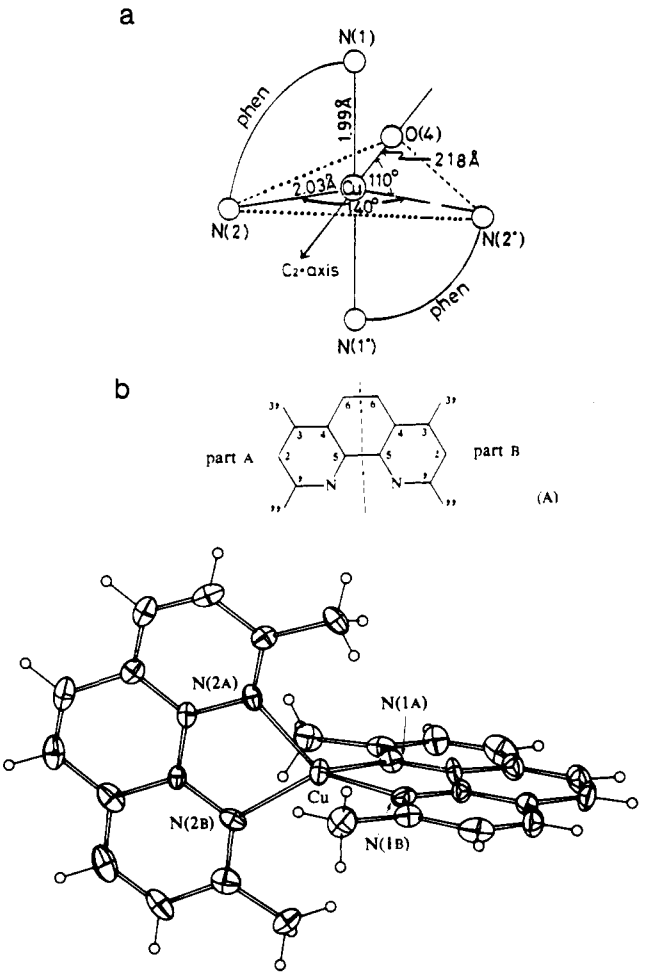


Figure 4. Crystal structures of 1,10-phenanthroline–copper complexes: (a) monoaquabis(1,10-phenanthroline)copper(II) nitrate $Cu(H_2)(phen)_2(NO_3)^{2+}$;[43] (b) structure of the 2,9-dimethyl-1,10-phenanthroline–cuprous complex bromide.[44]

90°. In contrast, the dihedral angles for the crystal structure of $(OP)_2Cu^+ClO_4^-$ and $(OP)_2Cu^+CuBr_2^-$ are 49.9° and 76.8° [45] (Figure 4b). An important conclusion of these crystallographic studies is that packing forces exert a major influence on the molecular geometry of these complexes and are responsible for their deviation from idealized symmetry.

The unique reactivity of copper ion in the 1,10-phenanthroline-dependent chemical nuclease activity may also be due to the requirement for an approximately tetrahedral complex in this system. Only coordination complexes of this geometry may bind with high enough affinity in the oxidatively sensitive minor groove. For example, the cuprous complex with the tridentate 2,2′,2″-terpyridine will be planar and may not bind productively even if it could form a reactive copper–oxene intermediate. While the iron and cobalt complexes of 1,10-phenanthroline are redox-active and therefore might be anticipated to be competent for DNA cleavage, they have an octahedral geometry and therefore do not bind with the same affinity and orientation as the cuprous complex. In addition, the redox potentials of $(OP)_3Fe^{2+}$ will not favor the generation of hydrogen peroxide from oxygen or its reaction with hydrogen peroxide by an inner-sphere mechanism even if the octahedral complex did bind to DNA. In fact, cuprous ion is unique in its ability to form stable "tetrahedral" complexes with 1,10-phenanthroline de-

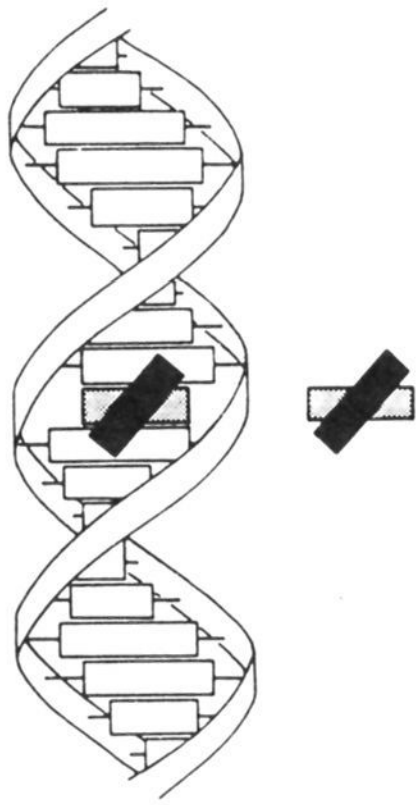


Figure 5. Postulated model of intercalative binding of the 1,10-phenanthroline–cuprous complex with DNA.[49]

rivatives as can be illustrated by the specificity of 2,9-dimethyl-1,10-phenanthroline as an analytical and colorimetric reagent for the metal ion. An impressive number of metal ions (56) do not interfere with the copper analysis presumably because they cannot form stable tetrahedral complexes and therefore are unable to compete with cuprous ion.[46]

2. Solution and Spectroscopic Studies Relevant to the Structure of the Reactive Intermediate

Partial intercalation by one of the 1,10-phenanthroline moieties has been suggested by a series of thoughtful studies by Veal and Rill (1991)[47,48] in which the interaction of the $(OP)_2Cu^+$ was monitored by spectroscopic and viscometric experiments carried out anaerobically to avoid the extensive degradation of the DNA. A concentration-dependent increase in the prominent 454-nm absorption maximum of $(OP)_2Cu^+$ was observed to be indicative of its binding to DNA. As would be expected from the binding of a symmetric complex to the asymmetric surface of DNA, a strong Cotton effect in the 454-nm band of $(OP)_2Cu^+$ was apparent. Both of these spectroscopic techniques are consistent with an intercalative component in the formation of the intermediate complex but not proof of that interaction.

The viscometry studies provide a stronger argument for intercalation. This hydrodynamic parameter reflects the increase in contour length associated with the separation of base pairs caused by intercalation. The 1:1 phenanthroline–copper complex increased the viscosity the most but the 2:1 complex was also effective. A partial intercalative model has been further supported by the crystal structure of $(OP)_2Cu^+ClO_4^-$ which reveals that the phenanthroline rings define a 49.9° dihedral angle.[45] If one phenanthroline moiety is intercalated, then the second could reside neatly in the minor groove[49] (Figure 5). However, minor groove accommodation of the other phenanthroline is only one condition which must be satisfied. The copper ion must also be accessible to hydrogen peroxide and there must be no hindrance to the configurational change accompanying oxidation to the higher valent (cupric) state.

Prior to the availability of crystal structures of both (2,9-dimethyl-$OP)_2Cu^+$ and $(OP)_2Cu^+$, it was assumed that both coordination complexes shared the same tetrahedral geometry.[50] As noted above, subsequent studies have indicated that this is only true to a first approximation.[45] Since (2,9-dimethyl-$OP)_2Cu^+$, unlike $(OP)_2Cu^+$, is stable to oxidation, its binding to DNA was studied and the results of these findings had been extrapolated to its nucleolytically active isostere. Equilibrium dialysis demonstrated that (2,9-dimethyl-$OP)_2Cu^+$ binds reversibly to DNA in the 20 μM concentration range.[50] The binding was assumed to be nonintercalative because the complex did not increase the viscosity of the DNA in the same concentration range, an observation which has been confirmed by Rill and colleagues.[47,48] In addition, they have shown that DNA is unable to induce any spectral perturbation in the strong 454-nm absorption band of (2,9-dimethyl-$OP)_2Cu^+$.[47,48]

The apparently different binding modes of (2,9-dimethyl-$OP)_2Cu^+$ and $(OP)_2Cu^+$ may be attributable to the pronounced difference in dihedral angles of the ligands in the two complexes—46° for $(OP)_2Cu^+$ and about 100° for (2,9-dimethyl-$OP)_2Cu^+$. The smaller dihedral angle may allow intercalation. Nevertheless, the results with (2,9-dimethyl-$OP)_2Cu^+$ do indicate that the complexes with a larger dihedral angle can bind with equivalent affinity without any contribution from intercalation. Photophysical studies by McMillin and colleagues have further emphasized that (2,9-dimethyl-$OP)_2Cu^+$ binds at the surface of DNA in a nonintercalative mode.[51,52] For example, no luminescence was induced in this complex suggesting that it was never sequestered from solvent. This would be the expected result for minor groove binding. In contrast, spectroscopic studies indicate that the phenyl groups of the 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline–cuprous complex are remote from solvent when this chelate binds to DNA. This observation is consistent with some intercalative binding of the phenyl group with B-DNA. Approach of the coordination complex from the major groove is suggested by its failure to bind to T-4 DNA, a bacteriophage DNA in which the major groove is sterically hindered the glycosylated hydroxymethyl groups on the 5-position of the cytosine. In contrast, the binding of (2,9-dimethyl-$OP)_2Cu^+$ with the bacteriophage DNA and calf thymus is similar as would be expected for a ligand which binds to the minor groove and therefore is not influenced by steric features associated with the major groove.

3. Geometry of the Productive Complex/ Structure–Reactivity Correlations

The geometry of the complex which leads directly to scission is the crucial issue—not the geometry of a complex which may be in rapid equilibrium with the central complex, but is not itself on the central reaction pathway. Reaction specificity and not equilibrium studies must play the central role in defining the structure of the intermediate especially since the structures of the tetrahedral complexes determined crystallographically vary and fail to provide definitive constraints on the structure of the intermediate. Structure–reactivity studies with a family of redox-active 1,10-phenanthroline–cuprous complexes (i.e. those complexes that do not have a substituent ortho to

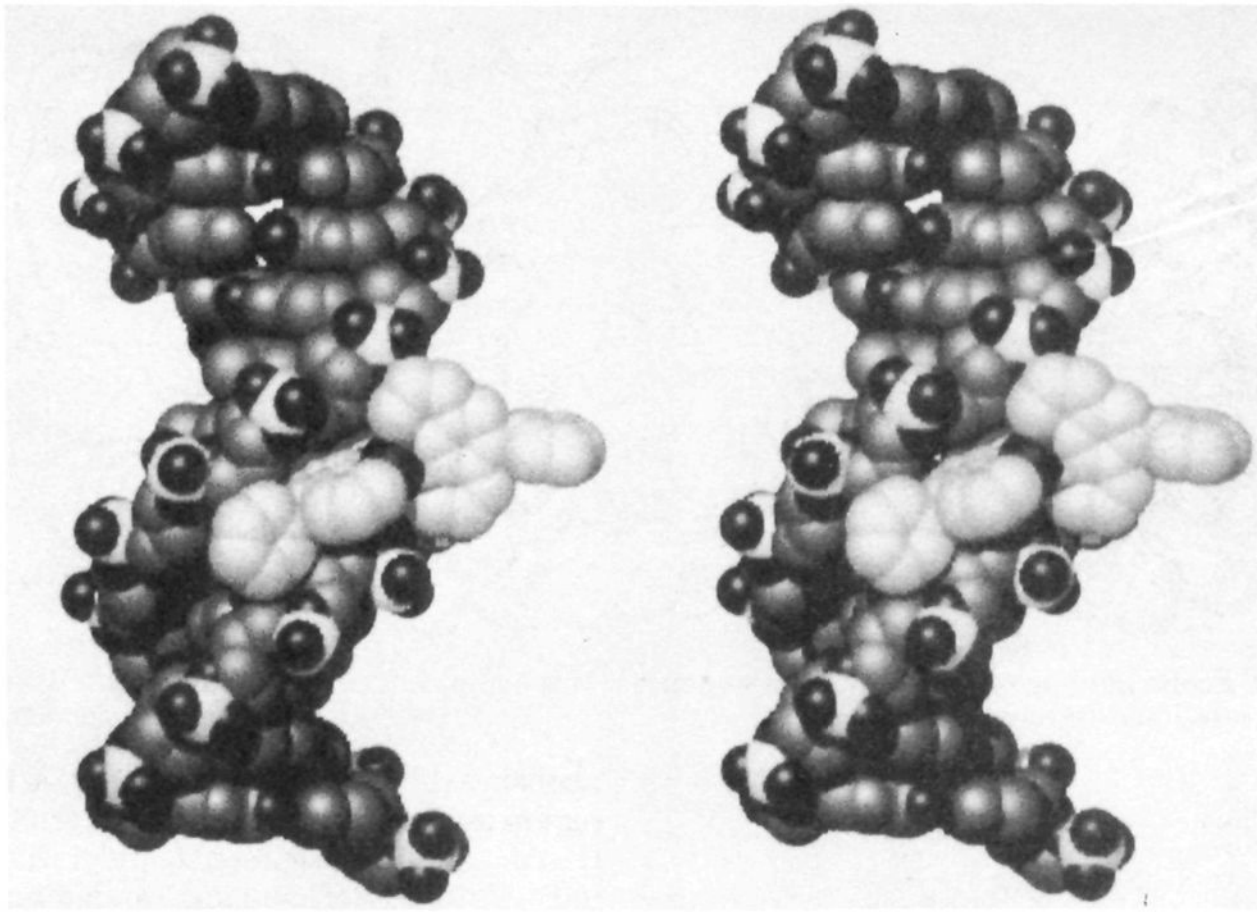


Figure 6. Postulated model for the nonintercalative binding of the 1,10-phenanthroline–cuprous complex with DNA.[25]

the liganding nitrogens) do not require that the essential noncovalent intermediate have an intercalative component even though the physical studies of the binding do not disallow that possibility. In fact, the similarity of the cleavage pattern for both the copper complexes of 1,10-phenanthroline, 5-methyl-1,10-phenanthroline, and 5-phenyl-1,10-phenanthroline suggest that substituents on 5-position of the central ring avoid intercalative interaction as illustrated in the docking model presented in Figure 6.

This model makes an interesting prediction which has been borne out by experiment. Since the symmetrical 3,4 and 7,8 positions of 1,10-phenanthroline approach the floor of the minor groove, derivatization of the ligand at these sites would be expected to alter the scission pattern of the corresponding copper complex. In fact, the sequence-dependent reactivity of the cuprous complex of 3,4,7,8-tetramethyl-1,10-phenanthroline [(3,4,7,8-tetramethyl-OP)$_2$Cu$^+$] strongly contrasts with that of (OP)$_2$Cu$^+$. Rather than generating a characteristic pattern of hyper- and hyposensitive sites, (3,4,7,8-tetramethyl-OP)$_2$Cu$^+$ is a weak scission reagent presumably because of the instability of its noncovalent intermediate with DNA. Although the copper complex of 4,7-dimethyl-OP reacts similarly to (OP)$_2$Cu$^+$, the copper complex of 4,7-diphenyl-OP behaves comparably to (3,4,7,8-tetramethyl-OP)$_2$Cu$^+$. Perhaps, the bulky 4,7-diphenyl-OP complex remains at the periphery of the minor groove or possibly reacts from the major groove with an entirely different covalent chemistry from (OP)$_2$Cu$^+$. This alternative would be fully consistent with the photophysical studies of McMillin and colleagues discussed above.[51] As will be discussed below, *octahedral* complexes of 4,7-diphenyl-1,10-phenanthroline bind to and react from the major groove.

The use of (OP)$_2$Cu$^+$ as a footprinting reagent to visualize the binding of *Eco*R1 to its recognition sequence has provided strong evidence for nonintercalative binding of the tetrahedral 1,10-phenanthroline–cuprous complex within the minor groove in the kinetically competent reaction intermediate. The cocrystal structure of *Eco*R1 in the absence of Mg^{2+} shows that this restriction enzyme binds to its recognition sequence solely by interactions in the major groove[53] (Figure 7). The minor groove is freely accessible to solvent. The major groove interactions are substantiated by the fact that neither DNase nor ferrous–EDTA can cut the DNA recognition sequence when bound to *Eco*R1.[27] In contrast, (OP)$_2$Cu$^+$ smoothly cleaves the DNA bound to *Eco*R1 with a specificity that is virtually indistinguishable from the free DNA.[27] *If intercalation were essential for the binding of the chemical nuclease, this result would not be observed.*

G. Scission of RNA

The phosphodiester backbone of RNA is also cleaved by (OP)$_2$Cu$^+$.[54] A direct comparison of the chemical susceptibility of the phosphodiester backbone of RNA and DNA to attack by (OP)$_2$Cu$^+$ is possible by preparing a heteroduplex of poly(rA)–poly(dT) and using it as a substrate for the scission reaction.[24] The advantage of this approach is that the cleavage chemistry proceeds from a binding site that is equally accessible to both phosphodiester backbones. The rates of acid solubilization of both strands are identical suggesting that both the deoxyribose– and ribose–phosphodiester backbones are equivalently susceptible to the oxidative chemistry of (OP)$_2$Cu$^+$.[24]

The experimental conditions necessary for the scission of RNA are more severe than those required for the scission of DNA. Generally, exogenous hydrogen peroxide must be added in order for the rate to proceed at a useful rate. However, the most surprising observation in studying the reaction of (OP)$_2$Cu$^+$ with RNA was that single-stranded regions were much more susceptible to cleavage than double-stranded stem structures. This was exactly opposite to our experience with DNA. As with DNA, however, there is some

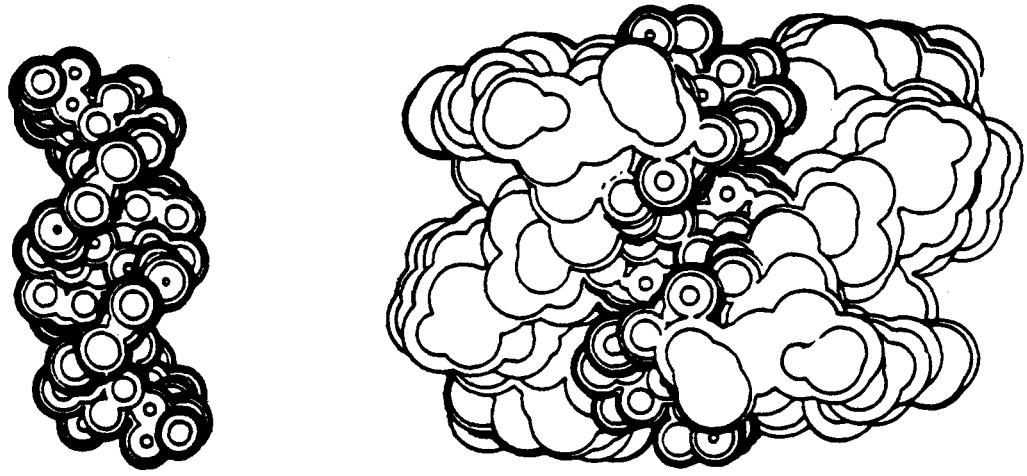


Figure 7. Model of *Eco*RI binding to its recognition sequence. This complex is readily cleaved by $(OP)_2Cu^+$ but protected from scission by DNase I and ferrous–EDTA.[27]

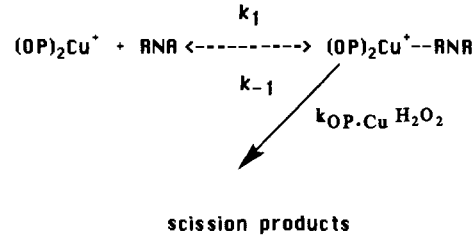


Figure 8. Kinetic mechanism for the scission of RNA.

dependence of the rates of scission on the substituent. The increased contribution of hydrogen peroxide essential for cleavage may relate to the lower stability of the noncovalent intermediate. A higher concentration of hydrogen peroxide will trap the low-affinity complex and drive the reaction in the direction of cleavage. The reaction scheme for the cleavage for RNA is therefore directly parallel to that for DNA except the productive intermediate involves interaction of the coordination complex with single-stranded regions of RNA (Figure 8). The chemical mechanism for the scission of RNA has not yet been established, but the oxidative attack on the C–1H of the ribose may also be central to the chemistry of RNA scission.

The specificity for single-stranded regions is sufficiently stringent that the reaction can be used to differentiate between double- and single-stranded regions of a postulated RNA secondary structure. For example, the stem loop structure of the transactivating region (TAR) of HIV RNA is the binding site for the transactivating (*tat*) protein essential for efficient transcription of HIV mRNA and for two cellular proteins present in Hela nuclear extracts (Figure 9).[55] It was disrupted by mutations at position 40 (U to G), 42 (U to G), and 43 (G to A) (Figure 1).[56] As a consequence of these changes, sequence positions 19–22 and 40–43, which were involved in a stable duplex structure in the wild-type RNA, became single-stranded. Consistent with this single-stranded preference of $(OP)_2Cu^+$, these sequence positions, which were marginally reactive to the chemical nuclease in wild-type RNA, became reactive in the mutant.[57] Enhanced reactivity of sequence positions 19–22 is more apparent in Figure 9 because of its position in the gel. The restoration of the integrity of the stem by compensatory mutations at positions 19 (C to U), 20 (A to C), and 22 (A to G) abolishes the reactivity toward $(OP)_2Cu^+$ of the mutated region and reestablishes the pattern for the wild-type RNA. These experiments underscore the ability of $(OP)_2Cu^+$ to detect single-stranded regions. The specificity of $(OP)_2Cu^+$ for single-stranded loops also makes this chemical nuclease activity an effective footprinting reagent for visualizing the binding of ligands in these regions. For example, the hybridization of an oligonucleotide to a loop structure will interfere with the binding site of the coordination complex and block scission.[57]

A structural model for the intermediate complex responsible for the single-stranded reactivity is difficult to envision at present. However, recent crystallographic studies have indicated that unexpected hydrogen-bond patterns appear in RNAs.[58,59] Therefore, all loops may have some residual structure which might generate a more favorable binding site for the tetrahedral 2:1 1,-10-phenanthroline–cuprous complex than the A-helix of the stem structure. The high affinity of Cibracon Blue and Reactive Blue 4 that can be demonstrated in RNAs provides an additional indication of the binding specificity that can be generated in loops regions for aromatic ligands.[60]

H. Scission at Transcription Start Sites

The nuclease activity of 1,10-phenanthroline–copper exhibits one further reaction preference which was first noted when its potential as a footprinting reagent was investigated. In comparing the abilities of $(OP)_2Cu^+$ with DNase I in visualizing the binding of *E. coli* RNA polymerase to the cyclic AMP-independent lac UV-5 promoter, it was noted that a triplet of strong scission sites on the template strand was evident at positions −6 to −4 upstream of the start of transcription.[35] These sites were within the domain of protection defined by DNase I and $(OP)_2Cu^+$. The nontemplate strand is protected from scission in this region from both DNase I and $(OP)_2Cu^+$ and shows no corresponding set of hypersensitive sites. Previous studies had demonstrated that positions −6 to −4 were within a single-stranded region formed at the active site of the RNA polymerases. The melting of the double-stranded DNA is an essential step in the formation of the "open-complex", a kinetically competent intermediate essential for productive transcription[61] (Figure 10). Like all

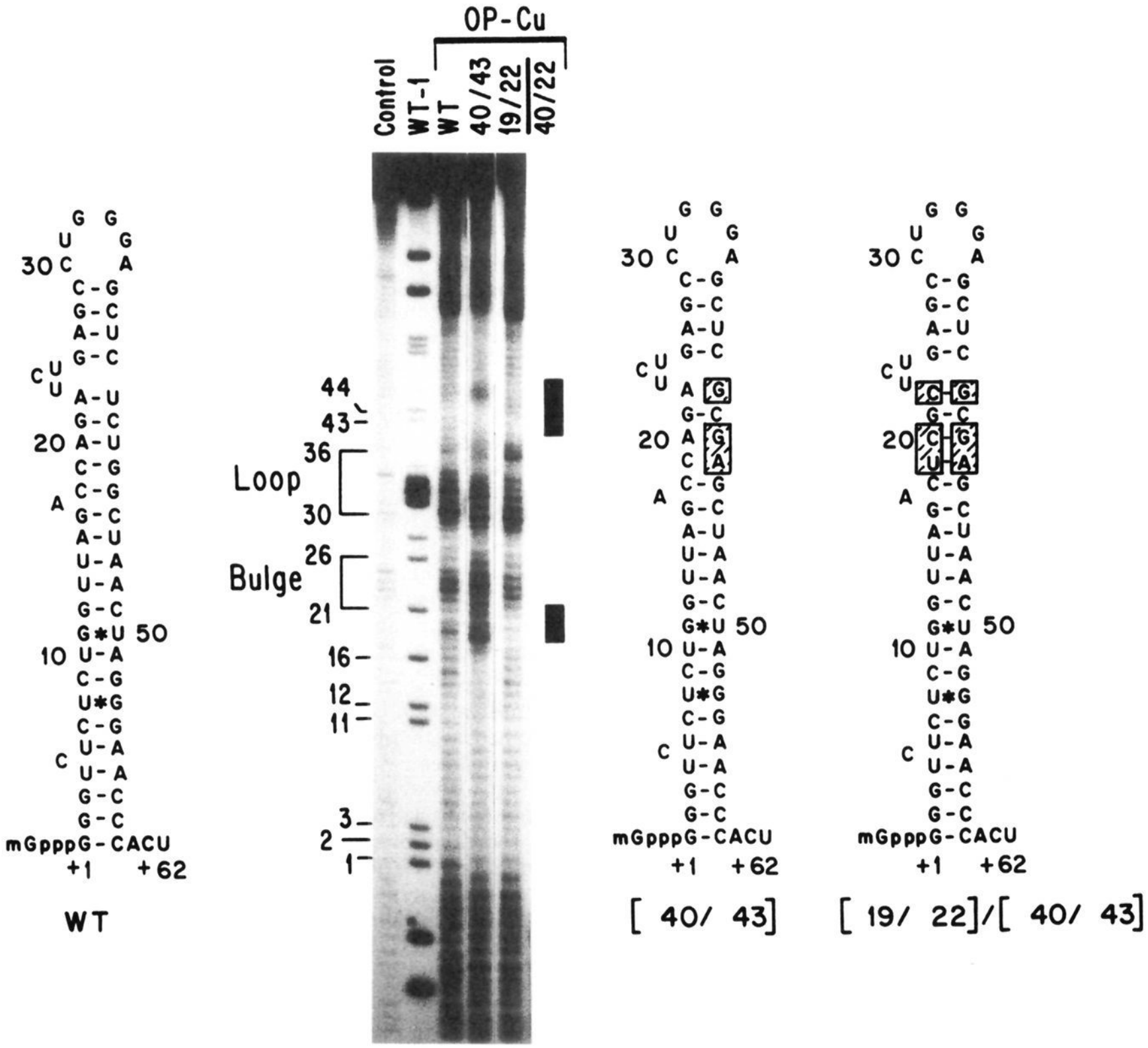


Figure 9. Scission of the TAR region of RNA by $(OP)_2Cu^+$. Mismatches in the stem caused by site-directed mutagenesis can be detected by 1,10-phenanthroline–copper. Complementary mutations which restore stem abolish reactivity.[56]

examples of reaction specificity, the selectivity depends on the binding of the coordination complex near an oxidatively sensitive site on the deoxyribose. In this case, the binding determinants may be provided by the protein, the DNA, or both. The nontemplate strand must be displaced from the binding site since no cleavage on this strand is observed. Incidentally, this result provides further evidence against $(OP)_2Cu^+$ generating diffusible hydroxyl radicals.

The preferential binding of the "tetrahedral" 2:1 1,-10-phenanthroline–cuprous complex at transcriptional start sites suggests that its redox-inactive isostere 2:1 2,9-dimethyl-1,10-phenanthroline–cuprous ion should also bind to the open complex. The validity of this suggestion has been demonstrated by two experiments. First, (2,9-dimethyl-$OP)_2Cu^+$ blocks the nicking of the open complex by $(OP)_2Cu^+$ of the open complex (Figure 11). Secondly, (2,9-dimethyl-$OP)_2Cu^+$ is an effective inhibitor of transcription. Moreover, both processes exhibits a similar concentration dependence on the complex.

In addition to providing novel support for the postulated mechanism of action of the nuclease activity of 1,10-phenanthroline, these experiments have led to the discovery of a novel new class of transcription inhibitors. The finding that (2,9-dimethyl-$OP)_2Cu^+$ is an effective inhibitor of transcription may contribute to the cytotoxicity of this relatively inert coordination complex to eucaryotic and procaryotic cells.[62-65] It should be possible to generate even more effective inhibitors of transcription since there is a great potential for additional variation. For example, since we have shown that 5-phenyl-1,10-phenanthroline–cuprous ion reacts more efficiently with the open complex formed with lac UV-5 than $(OP)_2Cu^+$,[66] redox stable analogs of this complex should prove to be better inhibitors of transcription than (2,9-dimethyl-$OP)_2Cu^+$.

The reaction of the $(OP)_2Cu^+$ with protein–DNA complexes is proving to be a very fertile area of investigation. In one interesting study, 5-phenyl-1,-10-phenanthroline–copper was able to detect bending induced in DNA by the transcriptional activator Mer R.[67] Of perhaps greater significance is that both

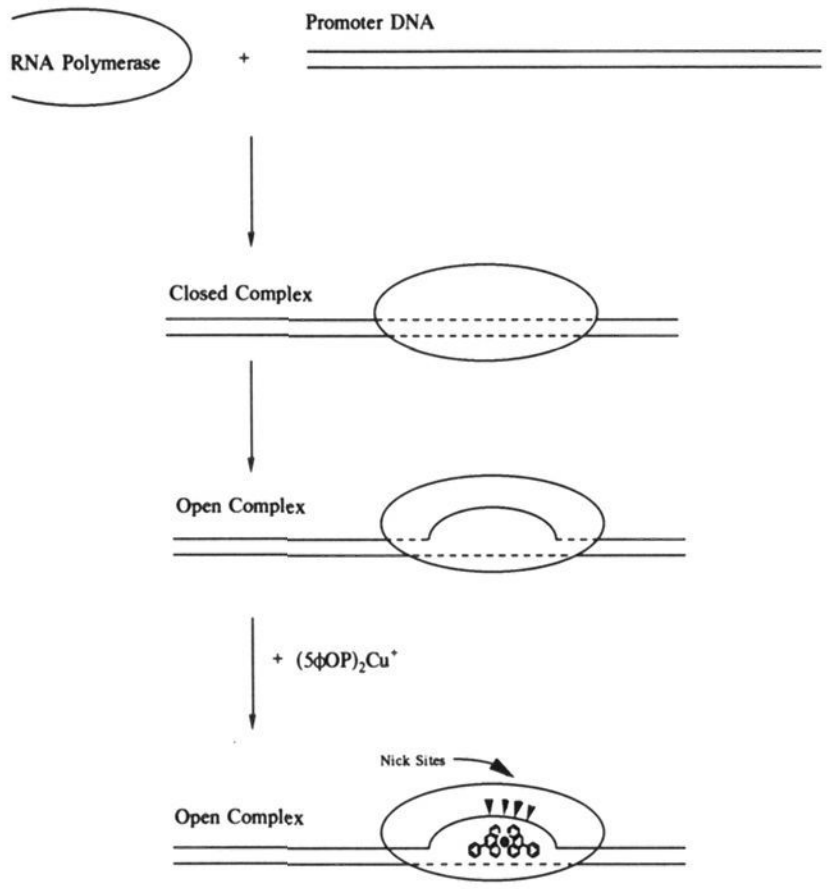


Figure 10. Model for the scission of transcription start sites of an open-complex by $(OP)_2Cu^+$ and its derivatives. Formation of the transcription bubble creates a binding site for $(5\phi OP)2Cu^+$ in this case.

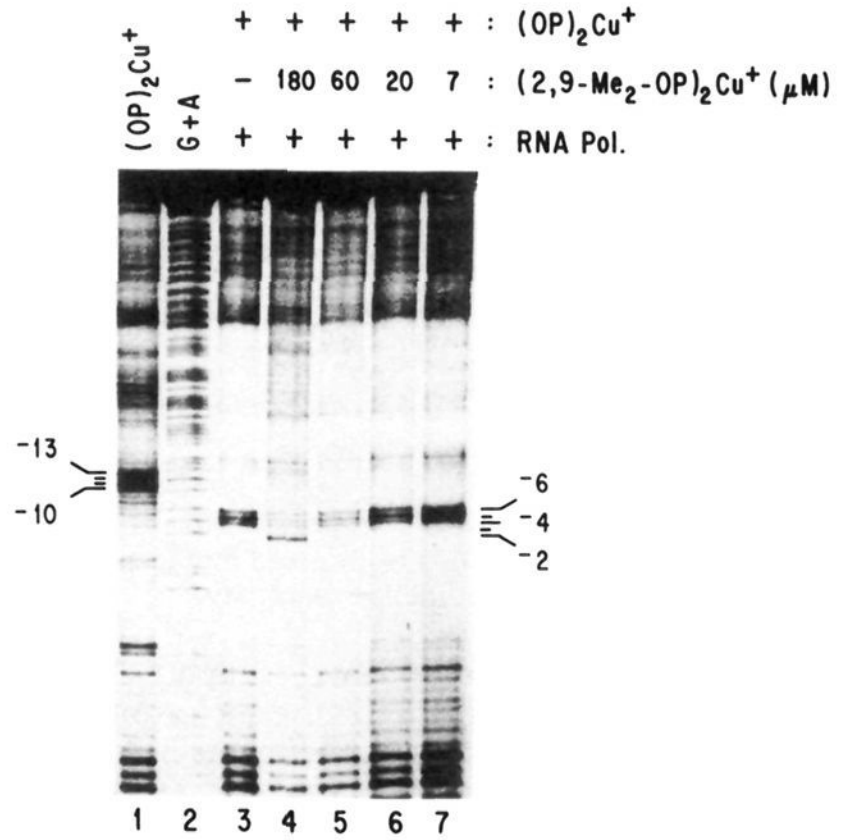


Figure 11. Protection of $(OP)_2Cu^+$ hyperrreactive bands by (2,9-dimethyl-OP)$_2Cu^+$. The open-complex composed of lac UV-5 and *E. coli* RNA polymerase is cleaved by $(OP)_2Cu^+$ in the presence of the indicated concentrations of (2,9-dimethyl-OP)$_2Cu^+$. The redox-stable coordination complex binds to the transcription start site and protect the template strand from the cleavage reaction in addition to inhibiting the enzyme.

eucaryotic transcriptional units and replication initiation sites are also hyperreactive to $(OP)_2Cu^+$.[68,69]

III. Octahedral Complexes of 1,10-Phenanthroline

Other metals form complexes with 1,10-phenanthroline and its derivatives which bind to, and react with, DNA and RNA in informative ways although they do

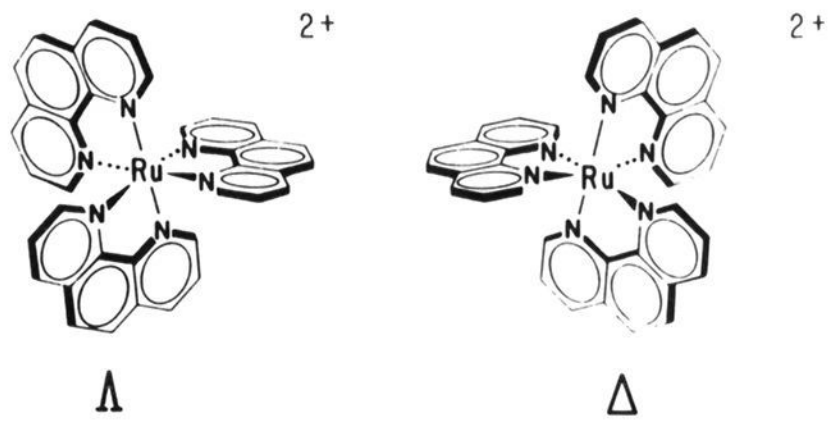


Figure 12. Structure of Λ (left) and Δ (right) isomers of 3:1 1,10-phenanthroline–ruthenium.

not cleave DNA in the presence of only thiol and oxygen like the copper complex of 1,10-phenanthrolines. However, there are significant differences in the interaction of the "*tetrahedral*" copper complexes and the "*octahedral*" complexes generated with ruthenium, rhodium, iron, zinc, and cobalt. The tetrahedral cuprous complex binds primarily to the minor groove to DNA while the octahedral complexes tend to bind to the major groove.[70] This distinction does not appear to be rigid. Although the octahedral complexes are at least 10 Å in diameter and too large to interact snugly in the minor groove, recent 2D NMR studies indicate that $(OP)_3Ru^{2+}$ primarily binds in this domain.[71]

Intercalation contributes to the interaction of the octahedral complexes with DNA but as discussed above does not seem to be essential in the interaction of $(OP)_2Cu^+$ with DNA. Coulombic and electrostatic interactions will modulate the interaction of both types of coordination complexes which will in turn be influenced by the sequence-dependent variability of DNA structure.[70] One difficulty inherent in the use of any of these relatively large hydrophobic cations as probes for DNA structure is their potential to alter the DNA structure upon binding. Intercalating agents, like ethidium bromide, for example, can induce the transition of DNA from the Z to B form.

A. Enantiomeric Specificity

Nevertheless, octahedral complexes are capable of one level of discrimination not available to tetrahedral complexes. The octahedral complexes exist as enantiomers.[70,72–74] It is therefore possible to explore the binding properties of the Λ and Δ enantiomers using a variety of physical and spectroscopic techniques (e.g. electrophoresis, fluorescence, and circular and linear dichroism)[75] (Figure 12). Interesting results have been obtained with the two enantiomers of the ruthenium complex of 4,7-diphenyl-1,10-phenanthroline (DIP).[72,70] The Λ isomer binds readily to B-DNA. One of its DIPs intercalates and the two remaining DIPs can be accommodated into the major groove. In contrast, the intercalation of one DIP of the delta isomer into B-DNA leads to a steric clash between the remaining two DIPs and the DNA strands. The binding of the Δ isomer to B-DNA is therefore disfavored. In contrast, both enantiomers bind to Z-DNA with comparable affinities because the shallow major groove can accommodate either. The Δ isomer is therefore specific for Z-DNA although both isomers bind to this left-handed form of DNA of as yet undetermined biological significance.

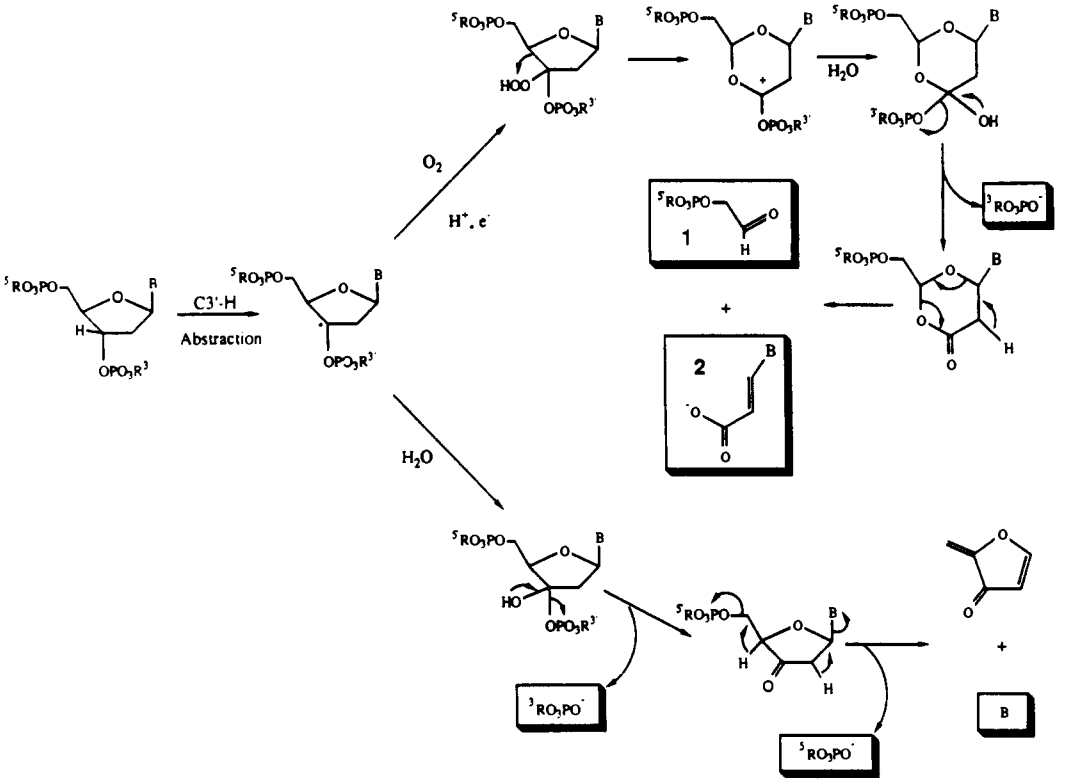


Figure 13. Postulated mechanism of scission by abstraction of a H-atom from the C-3 of the deoxyribose moiety.[78]

B. Scission Chemistry

1. Base-Directed Reactivity

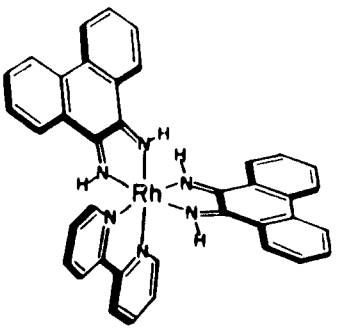
The relative stability with respect to oxidation of the octahedral complexes has allowed extensive studies of the binding of these ligands to DNA. It is also possible to convert these complexes into scission reagents by irradiation since they can either serve as photosensitizers for the formation of singlet oxygen or catalyze DNA cleavage by hydrogen atom abstraction.[76,77] In contrast to the copper-dependent scission reaction, the cleavage chemistry of the octahedral complexes does not require a geometric change during the reaction.

The ruthenium complexes act by generating singlet oxygen and will cleave at a site proximal to the binding site of the coordination complex. Unlike the chemical nuclease activity $(OP)_2Cu^+$, the scission chemistry involves oxidative attack on the bases rather than on the deoxyribose. The order of reactivity is dGMP > dTMP > dCMP = dAMP.[76] Oxidation of the bases does not necessarily lead to cleavage of the phosphodiester backbone. In order to generate a sequencing ladder the oxidized DNA must be treated with 10% v/v piperidine at 90 °C for 30 min to accomplish strand scission as in the Maxam–Gilbert sequencing reactions. Given the nucleotide-dependent variability in the chemistry, cleavage reactions, which proceed via singlet oxygen and react with the bases, may not be the best way to probe the sequence-dependent variability of the binding of the coordination complex of B-DNA. Nevertheless, it has been possible to use this chemistry to demonstrate the preference of the Λ enantiomer tris(tetramethylphenanthroline)ruthenium(II) for A-form DNA by showing the preferential cleavage of polynucleotides which adopt this structure.[76]

2. Ribose-Directed Reactivity

The rhodium complexes of 1,10-phenanthroline and its analogs cleave DNA and RNA in a light-dependent hydrogen atom abstraction reaction.[78] In the case of DNA, tritium labeling studies have indicated that the C-3H is the site of initial attack by the nucleic acid-bound reagent (Figure 13). This mechanism of cleavage does not involve any diffusible intermediates. In contrast to the cleavage mechanism involving singlet oxygen, the reaction is ribose directed and should therefore be nucleotide independent like the reactions observed with ferrous-EDTA and $(OP)_2Cu^+$.

Altering the ligands to the rhodium can generate complexes with a variety of specificities. For example, the complex bis(phenanthrenequinone diimine)bipyridylrhodium(III) ($Rh(phi)_2(bpy)^{3+}$) is sequence neutral



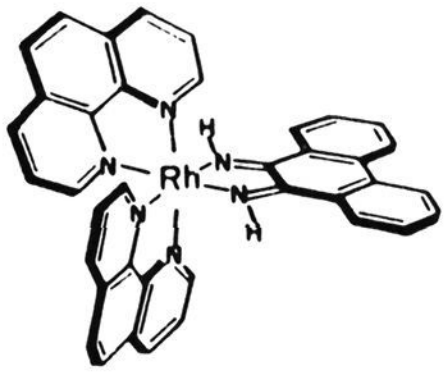
$Rh(phi)_2(bpy)^{3+}$

in its reaction with DNA.[77] Although its reactions with DNa may not provide insight into the structural variation of nucleic acids, it has advantages as a

footprinting reagent. Its light dependence and insensitivity to divalent cations, EDTA, reducing agents, or glycerol should make it reliable in the study of the protein–DNA interactions. *In vivo* footprinting may be yet another important application of this reagent since it does not require any cofactor.

C. Scission of RNA

In contrast to $Rh(phi)_2(bpy)^{3+}$, bis(phenanthrolinephenanthrenequinone diimine)rhodium^{+3} ($Rh(phen)_2phi^{3+}$) and tris(4,7-diphenyl-1,10-phenanthroline)-rhodium^{3+} ($Rh(DIP)_3$) show specific scission patterns. For example, the latter complex can recognize G–U mismatches in double-stranded regions of RNA[79,80] and also nicks DNA near cruciform structures.[81] The former complex nicks RNAs at a range of tertiary structures including G–U mismatches, bulges containing more than one unpaired nucleotide, and stem-loops. The mechanisms of cleavage of RNA by $(OP)_2Cu^+$ and $Rh(phen)_2phi^{3+}$ are not known although extrapolating from their reaction with DNA the former should abstract the C-1 H and the latter the C-3 H.



$Rh(phen)_2phi^{3+}$

Yeast $tRNA^{phe}$ has been used as a substrate for both $(OP)_2Cu^+$ and $Rh(phen)_2phi^{3+}$. The preferred sites of attack are compared in Figure 14 along with the cleavage sites of methidium propyl–EDTA which primarily cleaves in stem structures since it is an intercalating agent. Given the disparity in their cleavage specificities, $(OP)_2Cu^+$ and $Rh(phen)_2phi^{3+}$ must bind to RNA differently. The scission of other RNAs will be required before the specificity preference of these coordination complexes can be fully summarized.[82]

IV. Nucleolytic Reactions of Porphyrins

Since porphyrins and metalloporphyrins are hydrophobic cations, they also have affinity for DNA and RNA. The tethering of ligands with independent affinity for DNA such as a deoxyribooligonucleotides or intercalating agents enhances this DNA binding affinity.[83] In the absence of metal ions, porphyrins can serve as photosensitizers in the scission of DNA presumably via the intermediacy of singlet oxygen. This metal ion-independent reactivity has generated interest because porphyrins accumulate in tumor cells which then become vulnerable to irradiation.[84]

The mode of binding of porphyrins to DNA is a function of its coordination state. Unliganded, the porphyrins are intercalating agents. In the presence of metal ions which bear axial substituents, they are minor groove binders. The modes of binding have distinct spectroscopic signatures. Intercalators have negatively induced circular dichroism bands in the Soret region

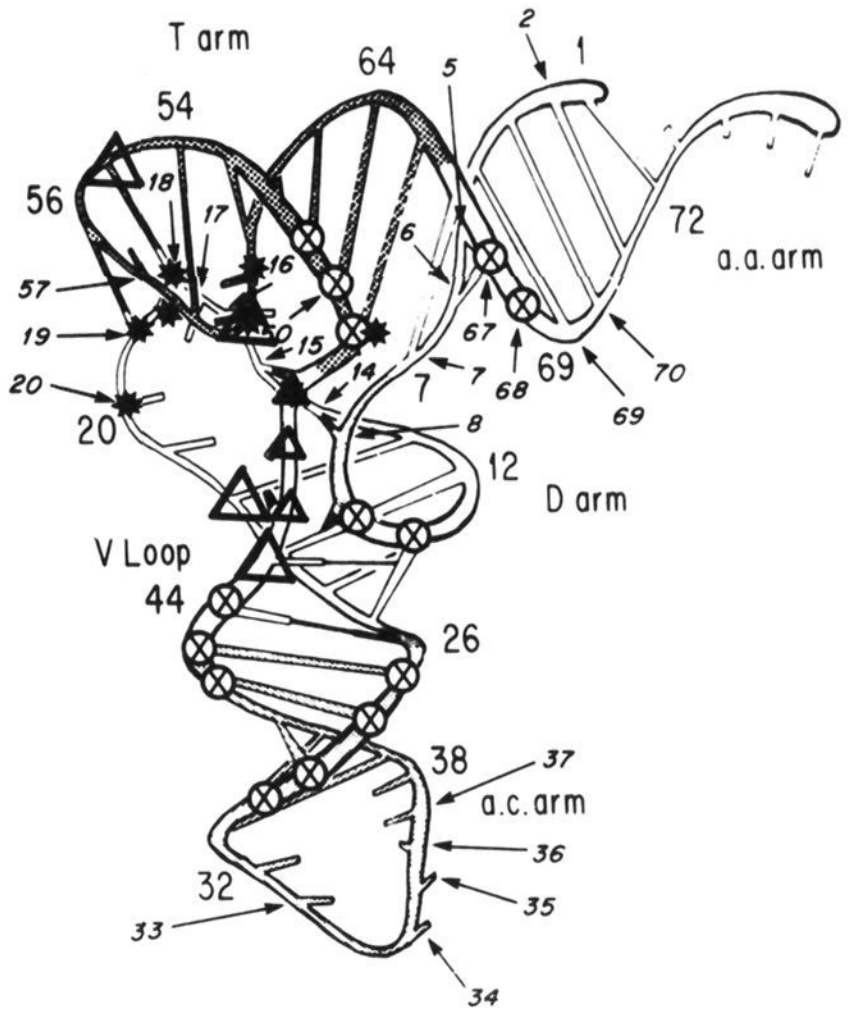


Figure 14. Comparison of sites of scission of $tRNA^{phe}$ by $(OP)_2Cu^+$ (→), MPE (⊗), and $Rh(phen)_2phi^{3+}$ (△) and sites protected from hydroxyl radicals generated by ferrous–EDTA in the presence of Mg^{2+}.

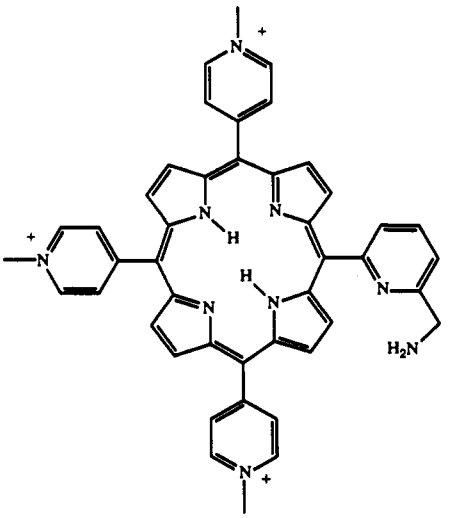
while groove binders have positively induced CD bands.[84] The structure of functional groups on the periphery also has an influence on the mode of binding.

The scission chemistry of metalloporphyrins coordinated to metal ions (e.g. Co^{3+}, Fe^{+3}, and Mn^{+3}) which retain their axial ligands has been studied. For the redox-active metal ions, Co^{3+}, Fe^{+3}, and Mn^{+3}, scission is observed in the presence of either ascorbic acid, superoxide, or iodosobenzene in A–T, but not G–C rich regions. A possible explanation for this observation is that the porphyrin is a minor-groove ligand whose interaction with DNA is inhibited by the 2-NH_2 of guanine. Analysis of the reaction products indicates the formation of phosphomonoester termini but no thiobarbituric acid positive base–propenals. As would be expected for a ribose-directed oxidative activity, scission is observed at all four nucleotides.[85]

Extensive studies of the cleavage of DNA by a cationic manganese porphyrin, [*meso*-[tetrakis(4-*N*-methylpyridiniumyl)porphyrinato]manganese(III)], in association with potassium monopersulfate, have been carried out. This reaction system is efficient at cleaving supercoiled DNA at concentrations of porphyrin as low as 0.5 nM. In comparing the structure of the products generated by the manganese porphyrin with those generated by DNases I and II, it was demonstrated that the cleavage reaction leads to both 5′- and 3′-phosphomonoesters in analogy to the product formed by $(OP)_2Cu^+$ (Figure 2). Even closer correspondence to $(OP)_2Cu^+$ chemistry is provided by the isolation of 5-methylenefuranone as a product of oxidation of the deoxyribose moiety.[86,87]

A novel approach in developing porphyrins as site-specific scission reagents has been the synthesis of 6′-[(aminomethyl)pyridyl]porphyrin. In this derivative,

the 6-aminopyridine is a strong ligand for the coordination of transition metals. The nonmetalated porphyrin intercalates into B-DNA as indicated by spectroscopic measures. Robust nicking of covalently closed circular DNA is observed in the presence of copper ion. However, efficient cleavage is not observed with Zn^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Pb^{2+}, Mn^{2+}, Fe^{3+}, or Cd^{2+}. The copper-dependent reaction is inhibited by catalase suggesting that the detailed course of the scission may be very similar to that $(OP)_2Cu^+$.[88]



porphyrin derivative capable of targeting scission by copper ion coordinated by a pendant aminopyridine ligand[88]

V. Ferrous–EDTA

A. Chemistry

Redox-active metals can catalyze the reduction of hydrogen peroxide, generating hydroxide anion and the highly reactive hydroxyl radical

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + {}^{\bullet}OH + OH^- \quad (1)$$

If the reaction is to cycle, the $M^{(n+1)+}$ produced must be reduced again by a one-electron donor. A particularly efficient reductant is superoxide anion (O_2^-):

$$M^{(n+1)+} + O_2^- \rightarrow M^{n+} + O_2 \quad (2)$$

The overall reaction summarized in eq 3 has been termed Fenton Chemistry and may play a central role in oxygen toxicity.[90] Iron and, to a lesser extent, copper, are primarily responsible for carrying out the reaction *in vivo*.[91]

$$O_2^- + H_2O_2 \rightarrow O_2 + OH^- + {}^{\bullet}OH \quad (3)$$

Ferrous–EDTA is an efficient mediator of Fenton chemistry. It is readily reduced by superoxide and ascorbic acid and efficiently oxidized by hydrogen peroxide. Moreover, its compact structure restricts its reactivity with the hydroxyl radicals that it generates. Hydroxyl radicals generated by γ irradiation or chemically by ferrous–EDTA cleave DNA efficiently by reaction mechanism(s) which have not yet been fully elucidated.[92,29] With either radiatively or chemically generated hydroxyl radicals, the 5′-termini are phosphomonoesters and the 3′-termini are 3′-phosphomonoesters or 5′-phosphomonoesters. The former products presumably are generated by C-1 hydrogen abstraction while the latter are generated by 4-H hydrogen abstraction from the deoxyribose. Although free bases can be detected, the bases themselves can also react.[93]

Reaction with the bases does not in itself lead to cleavage of the DNA, but can result in cleavage after subsequent chemical treatment. Aruoma *et al.*[94] observed two major base damage products, 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 8-hydroxyguanine, when DNA was treated with hypoxanthine/xanthine oxidase/Fe^{3+}–EDTA. Hydroxyl radical was confirmed as the causative agent because •OH radical scavengers such as dimethyl sulfoxide and mannitol inhibited base damage. Furthermore, superoxide dismutase, catalase, and desferrioxamine also inhibited base damage.

Prigodich and Martin[95] studied the reaction of hydroxyl radical, generated with Fe^{2+}–EDTA, with single-stranded oligodeoxyribonucleotides. They observed damage to the DNA could not be visualized by electrophoresis until piperidine treatment, implying the hydroxyl radicals react extensively with the bases of the DNA as well as to the deoxyriboses. Although the hydroxyl radical is believed to be the primary (and perhaps only) species involved, the possible involvement of higher valent iron–oxo species Fe(IV) and Fe(V) may also play a role.[96–101] Strong evidence for Fe(V) in the absence porphyrin has been provided in the "Gif" hydroxylation systems.[102]

B. Applications

1. Footprinting

Tullius and Dombroski[30] have used the •OH, generated by Fe^{2+}–EDTA reduction of hydrogen peroxide,[103] to footprint DNA–protein interactions. Ascorbate can be used as a reductant in the Fe^{2+}-catalyzed Fenton reaction, although at millimolar concentrations, ascorbate is also known to act as an •OH scavenger.[104] The reactions are typically quenched by the addition of thiourea. Thiourea reacts with H_2O_2 and alkoxy radicals[105,106] and can also chelate metal ions necessary for the Fenton reaction to proceed. Because the Fe^{2+}–EDTA complex used is negatively charged, there should be no electrostatic association of the complex with DNA. This is an important feature of this reagent because it limits the interaction of the complex with the protein–DNA complex with two important implications. First, the conformation of the DNA would not be altered by binding Fe^{2+}; and second, the actual probe (hydroxyl radical) is small. Because of the diffusible nature and small size of the •OH and the resulting sequence independence of the cleavage, high resolution footprints are obtained. In an initial report, hydroxyl radical footprinting demonstrated that the bacteriophage lambda repressor and Cro proteins bind to only one side of the recognition helix. Only a few nucleotides on each strand were protected from hydroxyl radical, and these were all on the same side of the DNA helix.[30] These results provided more detail than a DNase I footprint and complemented existing information from ethylation interference experiments. A limiting feature of this reagent is that glycerol, commonly present in many protein storage buffers, is an efficient •OH

scavenger. Dilution of the glycerol concentration to less than 0.5% is necessary for this footprinting technique to be successful.

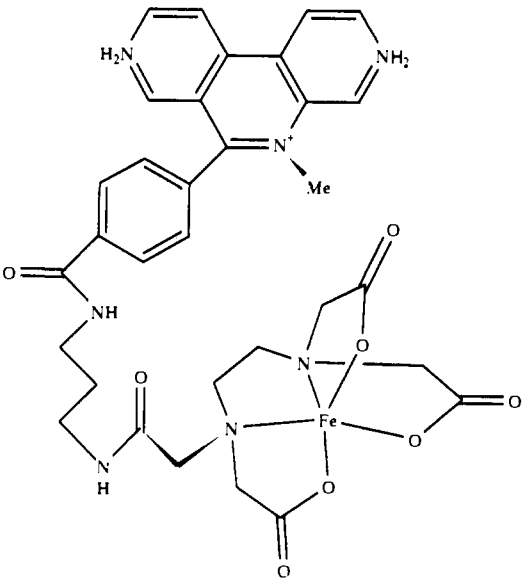
2. Analysis of Secondary Structures

Burkhoff and Tullius[107] used the hydroxyl radical, generated by ferrous–EDTA, to study the structure of adenine tracts (A tracts). These repeat sequences are observed in kinetoplast DNA and produce a small bend in a DNA helix. The efficiency with which the hydroxyl radical cleaved the A tract decreased in the 5′ to 3′ direction. This result was in striking contrast to the usual pattern of cleavage generated with hydroxyl radicals in which every base is cleaved to the same extent. The cutting pattern of the complementary strand was offset by one or two base pairs as would be expected for chemistry occurring in the minor groove. The authors concluded that the decreased cutting was indicative of the width of the minor groove decreasing smoothly in the 5′ to 3′ direction in an A tract. Furthermore, at elevated temperatures, where the anomalous electrophoretic behavior and presumably the bending of the A tracts disappears, a uniform cutting pattern is observed with the hydroxyl radical.

Muller *et al.*[108] have used hydroxyl radicals generated by Fe^{2+}–EDTA to footprint the interactions of T7 RNA polymerase with its promoter. They observed two protected regions on each strand which are located on the same side of the DNA helix and concluded that the enzyme interacts with the DNA from this side. Latham and Cech[109] have shown that Fe^{2+}–EDTA can be used to probe the structure of the *Tetrahymena* ribozyme. They observed regions of cleavage alternating with those of protection throughout the entire RNA in the presence of magnesium ion. The catalytic core of the ribozyme displayed significantly diminished cleavage. They concluded that the ribozyme folds into a tertiary structure with an interior and exterior analogous to protein enzymes. The equivalent reactivity of Fe^{2+}–EDTA toward single- and double-stranded nucleic acids contrasts with the reactivity of 1,10-phenanthroline–Cu(I). The latter preferentially cleaves duplex DNA[23] and single-stranded regions of RNA.[54] The sites of reaction of ferrous–EDTA with $tRNA^{phe}$ in the presence of Mg^{2+} are compared to those of $(OP)_2Cu^+$ in Figure 14.

3. Covalent Linkage to Intercalating Agents

Hertzberg and Dervan[38] have exploited the concept of using a DNA-binding ligand to deliver a metal ion to a site in a DNA helix by linking the DNA intercalator methidium to Fe(II)–EDTA via a propyl group (4). They found that while Fe(II)–EDTA at a concentration greater than 0.1 mM is required to convert supercoiled (form I) pBR322 to its nicked (form II) state, only 1 μM MPE–Fe(II) is required for the same reaction. If dithiothreitol was used as a reducing agent to regenerate the Fe(II) from Fe(III), only 10 nM MPE–Fe(II) was required to cleave the plasmid. The reaction is inhibited by superoxide dismutase probably because superoxide is essential for the efficient one electron reduction of Fe^{3+} to Fe^{2+}. Superoxide dismutase does not inhibit the chemical nuclease activity of $(OP)_2Cu^+$ unless superoxide provides the only source of reducing equivalents for the reduction of cupric ion to cuprous ion.[110]



methidium propyl–EDTA

Ni(II) and Zn(II), which form stable complexes with EDTA (the log K for binding Ni(II) and Zn(II) to EDTA is 18.62 and 16.5, respectively) block cleavage by competing with iron for the coordination site of MPE.[38]

Kean *et al.*[111] used the intercalative and cleaving ability of MPE–Fe(II) to detect intercalator sites in various RNA molecules. They found that the reagent bound to three or four locations in $tRNA^{Phe}$ with an affinity of 10^5–10^6 M^{-1} at pH 7.6 and 37 °C. The sites of scission by MPE–Fe(II) are compared to those of other chemical nucleases in Figure 14. Although MPE will generally cut in double helical regions, unanticipated scission preferences will be observed because, like $(OP)_2Cu^+$, it is a hydrophobic cation and is likely to bind at structural discontinuities. For example, both reagents react at the branch points of model Holliday junctions.[112] The intercalating agent propidium inhibits the scission by both reagents at the branch point.

VI. Uranyl Acetate

A. Chemistry

Buchardt and colleagues[113] have reported that photochemically activated uranyl salts cleave DNA and can be used as a footprinting reagent to study protein–DNA interactions. Photofootprinting with uranyl acetate has the advantage that the DNA cleavage can be initiated without the addition of any extraneous activator. Photofootprinting could be especially useful for intracellular applications but a possible difficulty with uranyl acetate is that millimolar concentrations are necessary for useful cleavage. An important feature of the chemistry is that cleavage is not inhibited by radical scavengers dithiothreitol (at 1 mM) or 2% glycerol. This observation suggests that the reaction does not proceed via a diffusible hydroxyl radical. These findings are consistent with previous studies of the photosensitized reactivity of uranyl acetate which has identified two prominent pathways.[114] The first is an inner-sphere mechanism involving reaction of the central atom, U(VI), with equatorial ligands. The rate-determining step of such a reaction is usually the inner-

sphere electron transfer to U(VI). The second mechanism is a charge-transfer hydrogen abstraction mechanism leading to the reduction of the uranyl salt.

Possibly, the cationic uranyl ion binds to the phosphodiester backbone of DNA like other inorganic ions. The ability of uranyl salts at high concentrations to precipitate DNA and form complexes with inorganic phosphate supports this view. It is unlikely for steric reasons that B-form DNA can form the planar complexes observed with uranyl nitrate and acetate but it is possible that one phosphate of the backbone could directly coordinate the uranyl ion and a second anion could be contributed by the buffer.[115] Since the cleavage reaction is sequence neutral, binding to the phosphodiester backbone provides an excellent explanation for the sequence neutrality of the scission in the absence of any evidence for the production of a diffusible hydroxyl radical. In this view, the uranyl ion would oxidize the deoxyribose proximal to the phosphate of the DNA to which it is complexed.

B. Applications as a Footprinting Reagent

In their initial report, Nielsen *et al.*[116] have used uranyl acetate and uranyl nitrate to probe DNA–protein contacts in the λ phage repressor bound to its operator, O_{R1}. The induced photocleavage was sequence independent, and the resulting products had 3′- and 5′-phosphate ends, as determined by their comigration with products from a Maxam–Gilbert chemical sequencing reaction. The protection from uranyl cleavage afforded by the bound λ phage repressor was located at nearly identical sites as those seen with ferrous–EDTA footprinting.

Nielsen *et al.*[117] have also used uranyl photofootprinting to detect the width of the minor groove in bent kinetoplast DNA, in DNA containing strong binding sites for minor groove binders, and in an internal control region (ICR) of the 5S RNA gene. The unusual conformation of kinetoplast DNA was recognized by the uranyl ion, and the usual sequence neutrality was not observed. Instead, a modulation of the cleavage intensity was apparent. With uranyl ion, the strongest reactivity is at the 3′ end of the tract as opposed to strongest cleavage by Fe(II)–EDTA at the 5′ end.[107]

Recently, Sigman and colleagues[56] used light-activated uranyl acetate to probe the interactions between HeLa cell nuclear protein and HIV TAR RNA. The reagent was also found to cleave RNA in a primary and secondary structure-independent manner. The utility of this photochemical nuclease has thus been extended to provide a means for studying RNA–protein interactions in addition to DNA–protein interactions. Uranyl acetate is an important reagent because most nucleases which cleave RNA do so in a very specific manner. For example, RNase T1 cleaves only at guanines in single-stranded regions. RNase A cleaves only at pyrimidines in single-stranded regions, and α-sarcin cleaves only at purines but in both single- and double-stranded regions.

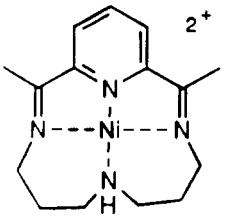
VII. Metal-Chelating Tripeptides

A. Glycyl-glycyl-L-histidine

This tripeptide comprises the Cu(II) transport site of albumin and binds to the metal ion with a stoichiometry of 1:1 in the pH range of 6.5 to 11.[118] The complex of GGH with Cu(II) has $\epsilon_{max} = 10^3$ at $\lambda_{max} = 525$ nm and a dissociation constant of 1.18×10^{-16} M at pH 7.53 and ionic strength 0.16 M. The dissociation constant of the albumin–Cu(II) complex is 6.61×10^{-17} M.[118]

Recently, Inoue and Kawanishi[119] have provided evidence that activated oxygen species are produced with the Ni(II) complex of GGH. Mack and Dervan[120] have exploited the oxidizing potential of metal-chelated GGH by attaching the tripeptide to the amino terminus of the DNA-binding domain of Hin recombinase. This chimeric protein is capable of binding to DNA at four sites consisting of 13 base pairs (termed hixL and secondary) and cleaving specifically at these sites in the presence of either copper, hydrogen peroxide, and ascorbate or nickel and monoperoxyphthalic acid.

The reactivity of nickel complexes unlinked to a targeting carrier is of interest. In the presence of $KHSO_5$ (oxone), $NiLi_1^+$ (5) oxidizes the N-7 of guanosine in single-stranded regions of DNA. This modification creates base sensitive scission sites which can be revealed by the treatment of the modified DNA with piperidine. This reaction is not observed with the nickel complex of gly-gly-his despite its reactivity when linked to the N-terminus of the hin recombinase peptide. It is also of interest to note that linked to hin recombinase, the chemistry appears to be ribose-directed although the cleavage is greatly enhanced by piperidine treatment prior to its application to a sequencing gel. The G-specific reaction of the free ligand provides a useful and rapid method for the detection of guanine not involved in Watson–Crick B-DNA structures.[121]



nickel complex which attacks guanosine in the presence of oxone[121]

B. Glycyl-L-histidyl-L-lysine

In 1980, Pickard *et al.*[122] found that a growth-modulating tripeptide, glycyl-histidyl-lysine, GHK, could form a complex with Cu(II). The association of this tripeptide for Cu(II) and the homology similarity between this complex and copper transport sites on albumin and α-fetoprotein, where the cupric ion is bound to a histidyl residue adjacent to a basic amino acid, suggested that GHK may act as a copper transport factor. A crystal structure of a GHK–Cu(II) complex revealed a polymeric structure where GHK forms a planar, tridentate bonding arrangement involving the N-terminal group of glycine, the nitrogen of the first amide bond, and the imino nitrogen of the imidazole ring (Figure 15). The fourth and fifth ligands are oxygens and bound to Cu(II) in an adjoining molecule forming a binuclear metal complex, analogous to crystalline copper(II) acetate.[123] The ε-terminal amino group of lysine is not bound to the copper.

Freedman *et al.*[124] later used optical, EPR, and electron spin–echo envelope spectroscopies to examine

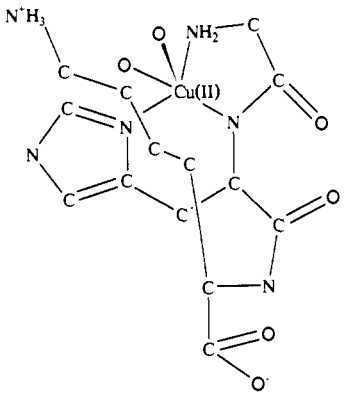


Figure 15. Crystal structure of GHL–Cu.

the GHK–Cu(II) complex *in solution*. Their findings were consistent with the crystal structure conclusions presented above except for the fact that the oxygen-bridged Cu(II) pairs do not exist in solution as they do in the solid state polymeric structure. They proposed that Cu(II) binds to GHK with a stoichiometry of 1:1.

Chikira *et al.*[125] used EPR to examine the orientation of Cu(II)–GHK complex binding to DNA. They observed that the square planar complex (formed by the tridentate liganding) binds parallel to the DNA fiber axis consistent with a minor groove interaction. With this assumption, they hypothesized that the tridentate complex binds to an oxygen in the phosphodiester backbone to give a square planar configuration. Finally, they determined the orientation of the complex bound to DNA to be dependent primarily on the glycyl-histidine part of the tripeptide. The contribution of the lysine (i.e., its positive charge) in determining orientation is minimal.

Recently, Bailly *et al.*[126] have created a sequence-specific nuclease which links the copper-chelating ability of GHK with the minor groove binding ability of netropsin (Net) and the intercalating ability of an acridine derivative (GA). In the presence of copper, hydrogen peroxide, and ascorbate site-specific cleavage of DNA was observed. The netropsin–acridine hybrid (NetGA) had an affinity constant of $9.1 \times 10^5\ M^{-1}$ while that of the derivatized hybrid (NetGA–GHK) had an affinity 20 times higher ($K_a = 2.2 \times 10^7\ M^{-1}$), revealing that the tripeptide contributed positively to DNA binding.

VIII. Cleavage of DNA by Elimination Mechanisms

A. Reactions Catalyzed by L-Lysyl-L-tryptophanyl-L-lysine

In the early 1970s, Helene and colleagues[89,127] directed their research toward peptides containing an aromatic amino acid which could recognize nucleic acids via stacking interactions of their aromatic ring side chains with nucleic acid bases. They observed that lysyl-tryptophanyl-lysine (KWK) bound to DNA[128] and single-stranded polynucleotides according to the following two-step model:

$$\text{KWK} + \text{nucleic acid} \overset{K_1}{\leftrightarrow} \text{complex I} \overset{K_2}{\leftrightarrow} \text{complex II} \quad (4)$$

Both complexes involve electrostatic interactions between the lysyl residues and α-amino group of the tripeptide and the phosphodiester backbone of the nucleic acid. Moreover, the fluorescence quantum yield of complex I is identical with that of the free peptide. However, complex II involves a stacking of nucleic acid bases with the aromatic side chain of the tryptophan as determined by complete quenching of its fluorescence. Maurizot *et al.*[89] also determined that in a nucleic acid with both single- and double-stranded regions, this tripeptide will preferentially interact with the single-stranded region, as seen by a higher value of K_2 for denatured as compared to native DNA.

In 1981, the labs of Helene[129] and Laval[130] simultaneously and independently discovered that this tripeptide, KWK, could not only recognize DNA at an abasic site but also nick there as well. Helene and colleagues determined the value of K_2 (measuring the ratio of the concentrations of stacked to unstacked complexes) to be approximately 200.[131] This value was much higher than that for single-stranded DNA (4.9) and native DNA (0.3). They hypothesized that the recognition of an abasic site came via stacking interactions whereby the tryptophanyl residue occupies the vacant site left by removal of the nucleic acid base. This stacking brings the lysyl residues into close proximity of the abasic site, which exists as an equilibrium between the hemiacetal and ring-opened aldehydic tautomers, and increases the rate of strand scission at the aldehydic site.[132]

Laval and colleagues also found that this nicking is ionic strength-dependent. Although no nicking of depurinated DNA was observed at 100 mM NaCl, nicking did occur at lower salt concentrations. Presumably, at elevated salt concentrations, no interactions between phosphates and lysyl residues could occur, inducing a dissociation of the tripeptide from the nucleic acid as seen in eq 4. They also calculated that the scission of depurinated DNA had an activation energy of 21 ± 2 kcal/mol.

B. Stereochemistry

In 1990, Mazumder *et al.*[7] investigated the mechanistic and stereochemical course of the elimination reaction catalyzed by KWK. They found that the tripeptide catalyzes a β-elimination of the 3-phosphate group, leaving a 4(*R*)-hydroxy-2-pentenal esterified via its 5′-hydroxyl to the 3′-phosphate of the nucleotide 5′ to the abasic site. Furthermore, they found that this reaction was an *anti* β-elimination, with the 2′-proR hydrogen being abstracted by a general base, presumably the lysyl group of KWK, resulting in an alkenal which had a *trans* geometry about the double bond (Figure 16).

IX. Nucleophilic Displacement Reactions

A. RNase A Models

The remainder of this review provides a brief summary of recent developments in the scientifically challenging problem of designing chemical nucleases which hydrolyze phosphodiesters. While only very limited success has been achieved in terms of catalytic rates and turnover, efficient hydrolytic catalysts would

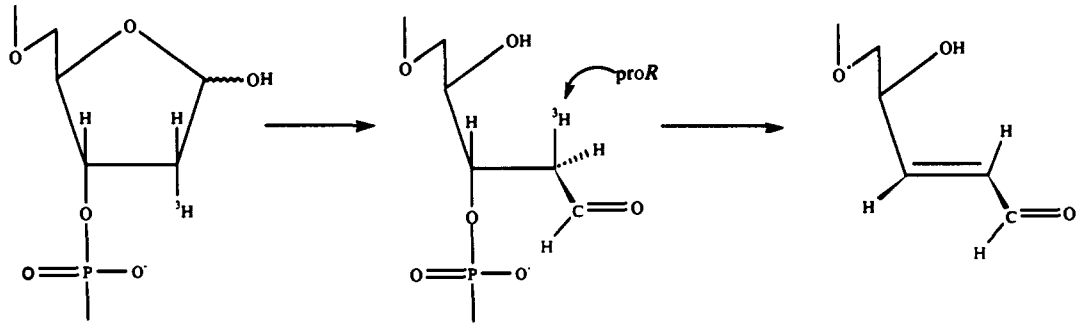


Figure 16. Elimination reaction at abasic sites catalyzed by KWK.

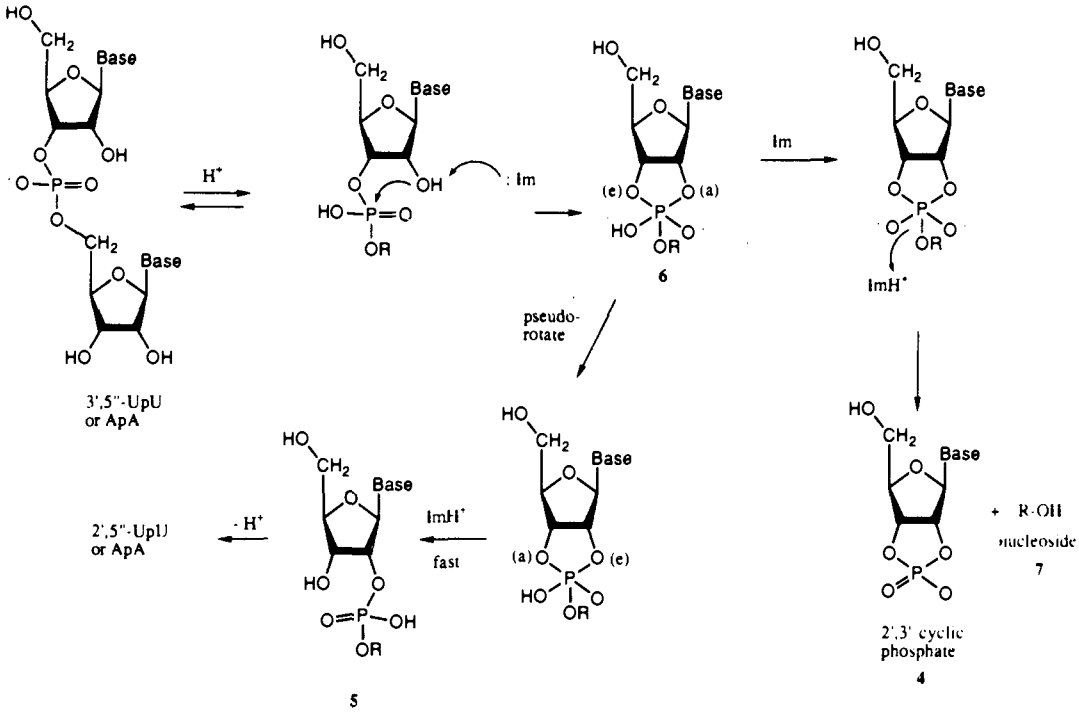


Figure 17. Proposed mechanism for ribonuclease A-catalyzed hydrolysis.[136]

represent a substantial improvement over the oxidative coordination complexes discussed previously in this review. Since the reaction mechanisms of bovine pancreatic ribonuclease, staphococcal nuclease, and bovine pancreatic deoxyribonuclease have been extensively studied and are well understood, they have provided important points of departure in the design of simple bioorganic systems capable of hydrolyzing phosphate esters and anhydrides.

RNase A in particular has played an important role in the history of enzymology. Besides being the first enzyme whose primary structure was determined, extensive chemical modification studies of its active site demonstrated that two histidine residues (12 and 119) participated directly in the reaction mechanism as acid/base catalysts both in the formation of 2',3'-cyclic phosphate intermediates and in their subsequent hydrolysis (Figure 17).[133,134] Both phases of this two-step reaction mechanism proceed by a simple SN_2 (P) reaction mechanism with inversion of configuration at the phosphorous.[8]

To mimic the mechanism of action of RNase A, β cyclodextrins have been derivatized with two imidazole groups.[135,136] β-Cyclodextrins are composed of seven glucose (A-G) units which are cyclized to define a hydrophobic binding site. To prepare the cyclodextrin model systems, imidazoles were linked to the A,B; A,C; and A,D glucose residues. The A,B bis-imidazole dextrin was the most efficient catalyst of *tert*-butylcatechol 1,2-cyclic phosphodiester. The pH rate profile was bell-shaped, indicative of bifunctional catalysis, and had a pH optimum of 6.2. The reaction yields the 2-phosphate as the exclusive product as would be anticipated by an in-line mechanism characteristic of the enzymatic reaction.[137] However, the rate of the reaction is substantially slower than RNase-catalyzed hydrolysis. The k_{cat}/K_m for the RNase A-catalyzed hydrolysis of CpC is 200 $s^{-1}/4 \times 10^{-3}$ M or 5×10^4 s^{-1} M^{-1} while that for the cyclodextrin is 1×10^{-3} $s^{-1}/5 \times 10^{-3}$ M or 0.20 s^{-1} M^{-1}. Both of these reactions exhibit simultaneous bifunctional catalysis, a property not shared by imidazole buffer model systems.[135,138] Possibly greater efficiency can be built in these systems by adding guanido groups on the edge of the cyclodextrin[139] but the ability of cyclodextrins to bind to RNAs and DNAs rather than mononucleotides has not been

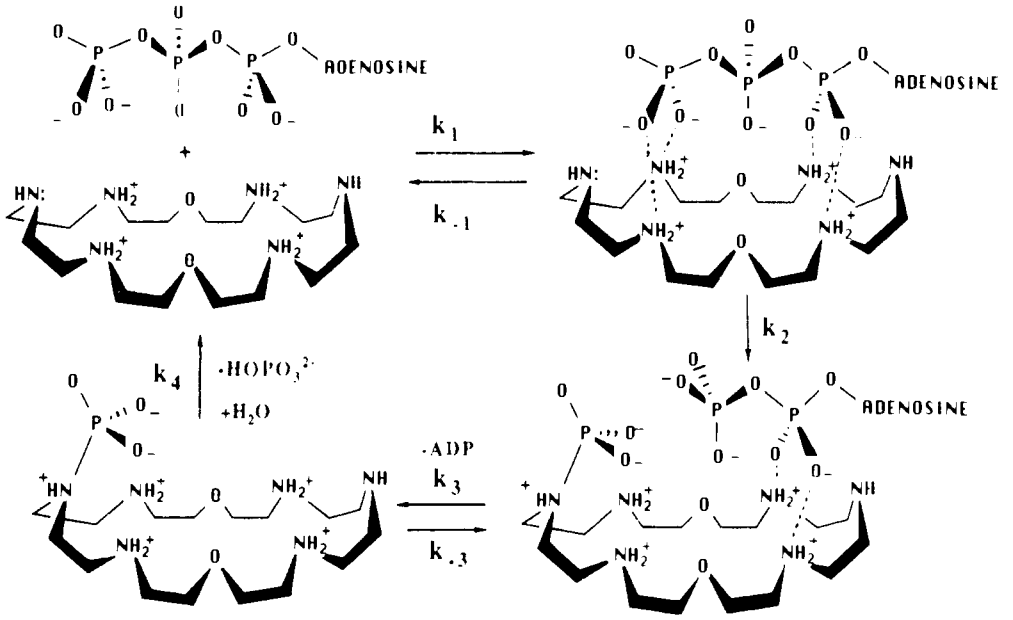
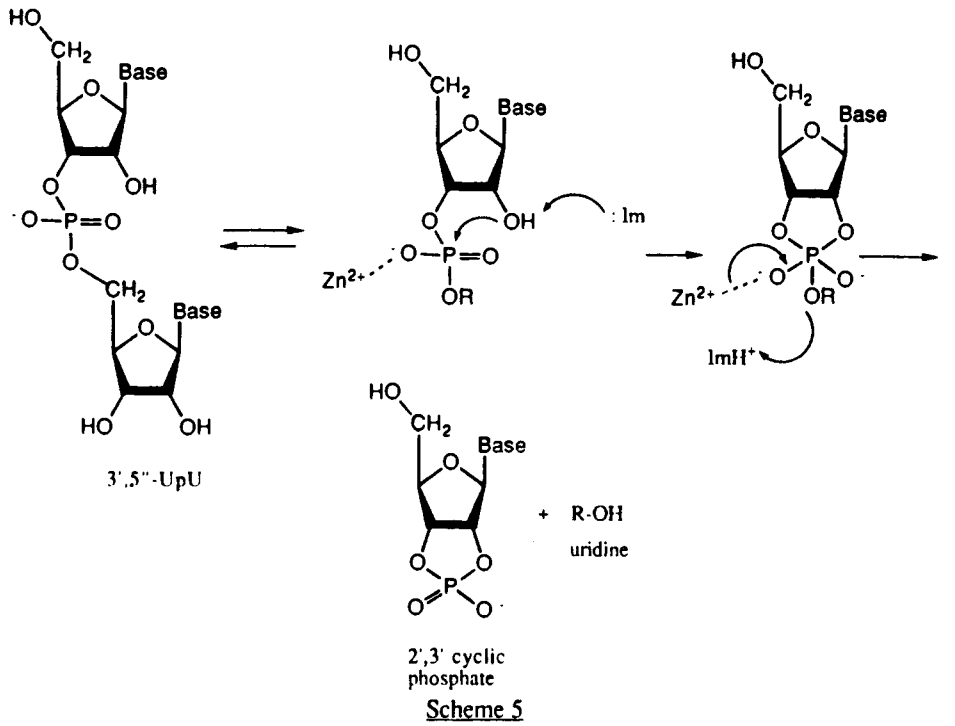


Figure 18. Mechanism of ATP hydrolysis catalyzed by a macrocycle.[156]



Scheme 5

Figure 19. Mechanism of the zinc ion-catalyzed hydrolysis of 3′,5′-UpU in an imidazole buffer.[135]

demonstrated.[140] However, the ATP hydrolysis catalyzed by macrocycle I indicates that it should be possible to engineer high-affinity binding sites into these molecules[139] (Figure 18).

B. Metal Ion-Dependent Phosphoryl Transfer

Redox-inactive metal ions are essential cofactors for the overwhelming majority of enzymes which catalyze phosphoryl transfer reactions. This requirement is not surprising in view of the well-known ability of metal ions to enhance the rate of nonenzymic reactions involving phosphoryl groups. A comprehensive review of this extensive literature is not within the scope of the review especially in light of the availability of excellent recent reviews on this subject.[141] However, it is possible to assert that two general roles are possible for a metal ion catalyzing nucleophilic displacement reaction. First, it could serve as a Lewis acid catalyst and enhance the electrophilicity of a reaction center. An example of this mode of catalysis is provided by the zinc ion-catalyzed hydrolysis of 4-*tert*-butylcatechol-1,2-cyclic phosphate in an imidazole buffer[135] (Figure 19). Secondly, it could serve as a specific base catalyst. In this mode, the substrate coordinates the metal ion and can be attacked by a metal bound nucleophile (e.g. hydroxide ion or hydroxymethyl). An example of this mode of catalysis is the zinc ion-catalyzed phosphorylation of 1,10-phenanthroline-2-carbinol by ATP[142] (Figure 20). This reaction proceeds through a mixed complex of 1 mol of zinc ion, 1 mol of 1,10-phenan-

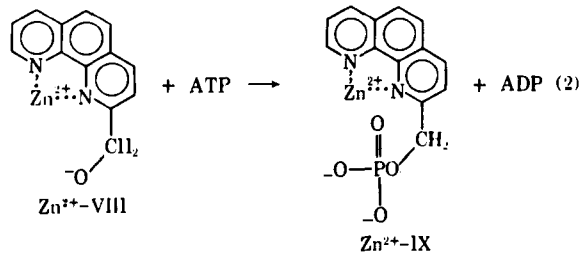


Figure 20. Zinc-catalyzed phosphorylation of 1,10-phenanthroline-2-carbinol by ATP.

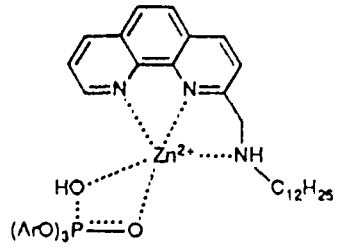


Figure 21. Hydrolysis of a phosphotriester by a 1,10-phenanthroline–zinc ion complex.[143]

throline-2-carbinol, and 1 mol of ATP. In a mixed micellar system, the zinc ion-catalyzed hydrolysis of diphenyl *p*-nitrophenyl phosphate by *N*-dodecyl-2-(aminomethyl)-1,10-phenanthroline also has been demonstrated[143] (Figure 21).

A series of studies have demonstrated that exchange stable cobalt(III) ligand systems can catalyze the hydrolysis of phosphate esters. Using ^{18}O labeling and ^{31}P NMR, Jones *et al.* have demonstrated that a coordinated H_2O has a sufficiently low pK_a (approximately 8) to generate a metal ion-associated OH^- for intracomplex attack on the simultaneously coordinated phosphodiester to yield a pentacoordinate intermediate that subsequently hydrolyzes.[144] These reactions have first-order rate constants of 10^{-3} s^{-1} or lower and therefore are not efficient enough to operate on a biochemically useful time scale. Although the diaquatetraazacobalt(III) complexes afford some of the highest observed rate enhancements, they are poisoned by their tight coordination to the resultant phosphomonoester thus suffering from product inhibition and diminished efficacy as catalysts.[145,146] It has also been shown that the shape and size of the tetraamino ligand can effect the catalytic properties of such complexes. In addition, the metal ion itself can effect the rate of catalysis as seen by a 500-fold decrease in catalytic rate upon substitution of Ir(III) for Co(III).[147] This decrease was rationalized by noting that increasing the size of the metal lengthens the metal–O bond distance and conversely decreases the O–metal–O bond angle (Figure 22).[147] This results in greater strain in the four-membered ring intermediate. By disfavoring the formation of this intermediate, the catalytic activity is diminished.[147] Coordination complexes of copper and zinc also catalyze phosphate mono-, di-, and triester hydrolysis with generally lower rate enhancements.[148–150]

One example of a coordination complex capable of hydrolyzing phosphate esters is the 1:1 2,2′-bipyridine–copper complex. At pH 6.40, the second-order rate constant for the hydrolysis of diethyl 4-nitrophenyl phosphate to diethyl phosphate and nitrophenol is 0.1 M^{-1} s^{-1}. The reaction exhibited a dependence on an

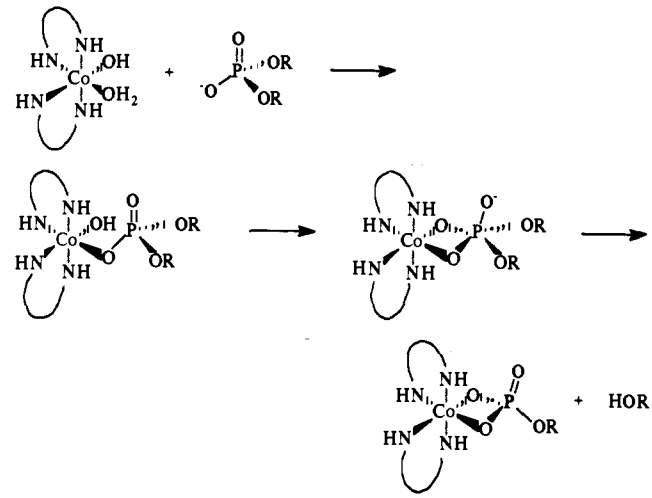


Figure 22. Cobalt-catalyzed hydrolysis of a phosphotriester.

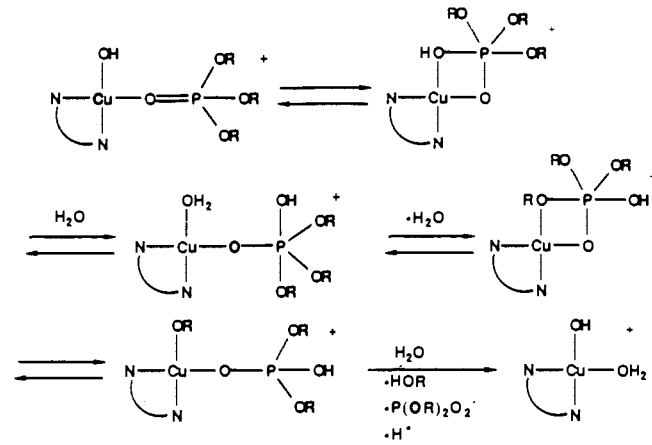
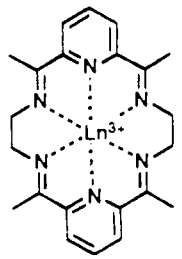


Figure 23. Metal ion-catalyzed hydrolysis of a phosphodiester catalyzed by 1,10-phenanthroline–copper.[148]

ionizable group of 6.9 which is approximately that expected for the first pK_a of Cu(bpy$(OH_2)^+$. The reaction mechanism proposed for this hydrolytic reaction is summarized in Figure 23. One unusual feature of this system is that about 20% of the hydrolytic product is ethanol and 4-nitrophenyl ethyl phosphate anion. A possible explanation for the substantial hydrolysis of ethyl phosphate relative to the nitrophenyl phosphate despite the 8 pK_a unit difference of the two leaving groups is that the copper ion coordinates to the leaving group and diminishes the sensitivity to leaving group tendencies.[148,151]

Although these simple catalytic systems demonstrate the principle of metal ion-catalyzed phosphodiester bond scission, they have not addressed directly the scission of the phosphodiester bonds of nucleic acids. Two interesting systems have appeared which catalyze the cleavage of the phosphodiester bonds of RNA. The ligand system (6) with lanthanide(III) metal ion catalyzes the scission of the dinucleotide adenyl-3′,5′-uridine 3′-monophosphate (ApUp) and oligomers of adenylic acid. The second order rate constant for the scission of ApU is approximately 9.7 M^{-1} s^{-1} compared to 5×10^4 M^{-1} s^{-1} for RNase A.[6] The primary reaction products are adenosine cyclic 2′,3′-monophosphate and 3′-uridine monophosphate. The metal–ligand complex therefore catalyzes a transesterification reaction and is specific for the scission of the phosphodiester backbone of an oligoribonucleotide but not a deoxyribonucleotide.

Since transesterification is observed, a hydroxide ion within the coordination sphere of the metal ion cannot possibly serve a nucleophilic role.

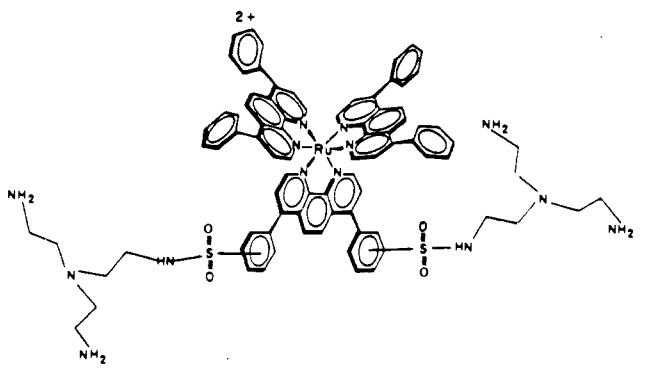


lanthanide complex which catalyzes hydrolysis of ribooligonucleotides[6]

C. RNA and DNA Scission

The scission of the phosphodiester backbone of RNA has also been achieved with $Cu(bpy)^{2+}$ and $Cu(trpy)^{2+}$.[4,5] As in the case of the lanthanide complexes, scission occurs by a transesterification reaction and therefore is specific for RNA. The rate constant is approximately 10^{-2} M^{-1} s^{-1} for $Cu(bpy)^{2+}$. No reaction is observed with DNA and the products are distinguishable from those generated by the oxidative scission of poly A by $(OP)_2Cu^+$. In an effort to generate targeted hydrolytic catalysts, $Cu(bpy)^{2+}$ was linked to deoxyuridines and thymidine residue on the 5-position of the base as well as the 5′ and 3′ terminus of the mononucleotide. Poly A was used as the substrate. Contrary to expectation, the free coordination complex was more efficient than the tethered complexes in the scission reaction by a factor of about 2–3.

The sole example of the scission of DNA in a metal ion-catalyzed hydrolysis reaction involved a ruthenium octahedral complex. This complex, like other octahedral complexes, binds to the major groove.[152] In the presence of 7 μM complex and 160 μM Zn(II), Cd(II), or Pb(II) ions, 30% scission of supercoiled DNA is observed after a 5–7-h incubation. Approximately 40% of the cleaved strands can be religated with T4 DNA ligase.[153] The ability to religate the strands is the primary evidence for inferring hydrolysis. If the DNA had been oxidatively cleaved by the addition of copper, none of the DNA could be religated even though the DNA had undergone extensive scission. From the data provided, a second-order rate constant of 7 M^{-1} s^{-1} can be estimated. This chemistry, at its present level of development, has not been applied to the analysis of DNA structure–function relationships.



coordination complex capable of catalyzing DNA hydrolysis in presence of added zinc ion[153]

X. Concluding Remarks

A variety of coordination complexes are now available which can cleave DNA and RNA via oxidative or free radical pathways. Many of these reagents have already provided useful footprinting reagents *in vitro*. The utility of these reagents in intracellular applications has not been established. This would appear to be a promising direction for future research. The chemistry must be refined so that the reagents are membrane permeable and will not be inhibited by normal cellular defense mechanisms against toxic xenobiotics. Possibly a useful intermediate step would be the development of methodology that would be applicable for concentrated cell lysates. However, future work with nucleolytic agents should be coupled with the development of improved methods of visualizing the products of the scission chemistry at specific genetic loci. Valuable progress in this area has already been made using polymerase chain reaction-based techniques.[154,155]

The understanding of the chemistry of the cleavage of many of the reagents discussed in this review is far from complete. A more thorough understanding of the covalent chemistry of the reagents that have already been described might be more generally useful than the report of yet another coordination complex which cleaves DNA by an unknown reaction mechanism. A precise understanding of both the DNA binding properties and the chemical reactivity of any scission reagent is essential for potential applications as a footprinting reagent or as a chemical probe of nucleic acid conformation. While many oxidative scission reagents have been described, no robust chemical reaction has yet been discovered which cleaves the phosphodiester bond of RNA or DNA by a hydrolytic mechanism at a rate that would make it useful to study nucleic acid structure–function relationships or to incorporate it into the design of a synthetic restriction endonuclease.

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