Oxidative Strand Scission of Nucleic Acids: Routes Initiated by Hydrogen Abstraction from the Sugar Moiety

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I. Introduction

Oxidants with free-radical character are well-known instigators of DNA and RNA damage. The group includes certain activated antibiotics, metal complexes, redox-active metalloenzymes, oxygen metabolites, and molecules ionized by high-energy radiation. While the heterocyclic bases of nucleic acids are important sites of free radical-mediated alteration (reviewed by Burrows and Muller in this issue[1]), the sugar−phosphate backbone is also highly vulnerable to attack. Abstraction of a hydrogen atom from ribose or deoxyribose produces a carbon-based sugar radical that can rearrange, culminating in scission of the nucleic acid strand.

These lesions, if unrepaired, may contribute to mutagenesis, carcinogenesis, aging, inherited disease, and cell death. While oxidative strand scission is generally considered to be deleterious, synthetic nucleases have been developed in recent years that have been useful in the treatment of disease and in research as probes of macromolecular structure.[2−8] (While the term "nuclease" is commonly used in the literature, these molecules cleave nucleic acids by oxidative attack at the sugar moiety, whereas most enzymatic nucleases cleave DNA by hydrolytic mechanisms. Hydrolysis affects only the phosphodiester bond and does not result in sugar damage.) The number of chemical species capable of damaging nucleic acids is immense and covers a broad range of molecules; however, certain intermediates appear to be common to many of these systems. Thus, determination of one mechanistic pathway has helped to elucidate others. As scientists have begun to think about the rational design of new nucleic acid-cleaving molecules, it has become apparent that design must be preceded by a thorough understanding of cleavage mechanisms. In addition, mechanistic information has been useful in interpreting experiments where oxidative cleavage patterns are used to determine structures of biomolecules. For these reasons, it has been of great interest to try to understand the mechanistic pathways by which oxidative agents cleave DNA and RNA.

The study of radical-mediated nucleic acid cleavage is not a simple task. Both the oxidizing species and the sugar radicals they produce tend to be extremely short-lived. Furthermore, chemical similarities in the degradation products make them difficult to separate and identify. Researchers have been innovative in devising solutions to these challenges. Ab

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Wendy Pogozelski was born in Schenectady, NY, in 1966. She earned her B.S. in Chemistry from Chatham College, Pittsburgh (1988) and her Ph.D. from The Johns Hopkins University (1994) with Thomas D. Tullius studying mechanisms of hydroxyl radical-mediated DNA scission. She was an Office of Naval Research Postdoctoral fellow in radiation chemistry with Michael Xapsos and William Blakely at the U.S. Naval Research Lab and Armed Forces Radiobiology Research Institute. Since 1996 she has been Assistant Professor of Chemistry at the State University of New York at Geneseo. Her laboratory currently investigates radiation effects, protein–DNA cross-linking, and mitochondrial DNA damage in diseases related to oxidative stress.

Thomas D. Tullius was born in Fontana, CA (1952), received his B.S. in Chemistry from UCLA in 1973, and his Ph.D. from Stanford University in 1979 (with Keith O. Hodgson). He was an NIH postdoctoral fellow in Stephen J. Lippard's laboratory at Columbia University from 1979 to 1982, where he worked on the sequence specificity of Cisplatin binding to DNA. In 1982 he joined the Chemistry Department at Johns Hopkins University as Assistant Professor. He spent 15 years at Johns Hopkins, eventually having appointments as Professor of Chemistry, Biophysics, and Biology, and as codirector of the Institute for Biophysical Research on Macromolecular Assemblies. In 1997 he moved to the Department of Chemistry at Boston University as Professor and Chairman. He was a Searle Scholar, a Camille and Henry Dreyfus Teacher–Scholar, and a Fellow of the Alfred P. Sloan Foundation. He currently is a Fellow of the AAAS and recently was named the 1998 Herbert A. Sober lecturer of the American Society of Biochemistry and Molecular Biology. His main research interest is in the development of new chemical methods to study the structure of DNA and DNA-protein complexes in solution. His laboratory introduced the technique of hydroxyl radical footprinting for the study of nucleic acid structure.

initio calculations have been developed and extensive studies with nucleic acid models have been performed. (For reviews, see Colson and Sevilla[9] and Beckwith et al.[10]) Additional insight has come from detailed product analysis, kinetic studies, NMR analysis of short oligonucleotides, low-temperature spectroscopy, EPR, ENDOR, Monte Carlo simulations, and the generation of specific deoxyribosyl radicals. (For examples, see ref 11−16.)

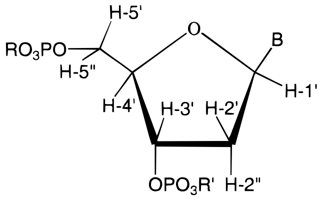


Figure 1. The seven deoxyribose hydrogen atoms.

An especially rich source of information has been the detailed analysis of the products of nucleic acid cleavage. Initially, gas chromatography coupled with mass spectrometry was used for product analysis but lately this approach has been replaced by electrophoretic methods. While electrophoresis has long been known as a means of separating oligonucleotides of different lengths for sequencing purposes, chemists have further exploited the high-resolution capabilities of gel electrophoresis to resolve DNA fragments that are identical in length (having the same number of nucleotides) but different in the chemical identity of the terminating functional group. By combining autoradiography with high-resolution polyacrylamide gel electrophoresis (PAGE), researchers have been able to visualize and quantify scission products with high sensitivity. Other laboratories have further extended the usefulness of this technique by following fates of incorporated isotopes. The introduction of isotopically labeled bases and sugars has provided great insight into mechanisms of nucleic acid damage.

Many excellent reviews have appeared over the years addressing oxidative DNA damage. (See Stubbe and Kozarich,[17] Hutchinson,[18] von Sonntag,[19] Breen and Murphy,[20] and Pratviel et al.[21]) While other authors have focused on the elucidation of molecular rearrangements after hydrogen abstraction from deoxyribose, here we emphasize attempts to identify the hydrogen abstraction event that initiates the cleavage process. At least one degradation pathway associated with each abstraction event is also described. While the vast majority of the literature focuses on DNA chemistry, we include RNA where appropriate. Last, we summarize the common features of these mechanisms and show how certain products may be used as diagnostic markers for investigating pathways of nucleic acid-cleaving agents whose chemistry is yet unknown.

II. Theoretical Considerations of Radical-Mediated Hydrogen Abstraction

A deoxyribose residue in the backbone of a DNA strand (Figure 1) has seven hydrogen atoms attached to carbon which, in principle, are available for abstraction by an oxidizing agent or free radical. These atoms are generally designated H-5′, H-5″, H-4′, H-3′, H-2′, H-2″, and H-1′. Ribose in RNA has six such hydrogen atoms, since one hydrogen at the 2′-carbon has been replaced by an alcohol. The 5′- and 2′-positions each contain two hydrogens and are prochiral. Some confusion exists with regard to the nomenclature at these positions. IUPAC has designated these atoms as H-5′, H-5″, H-2′, and H-2″.

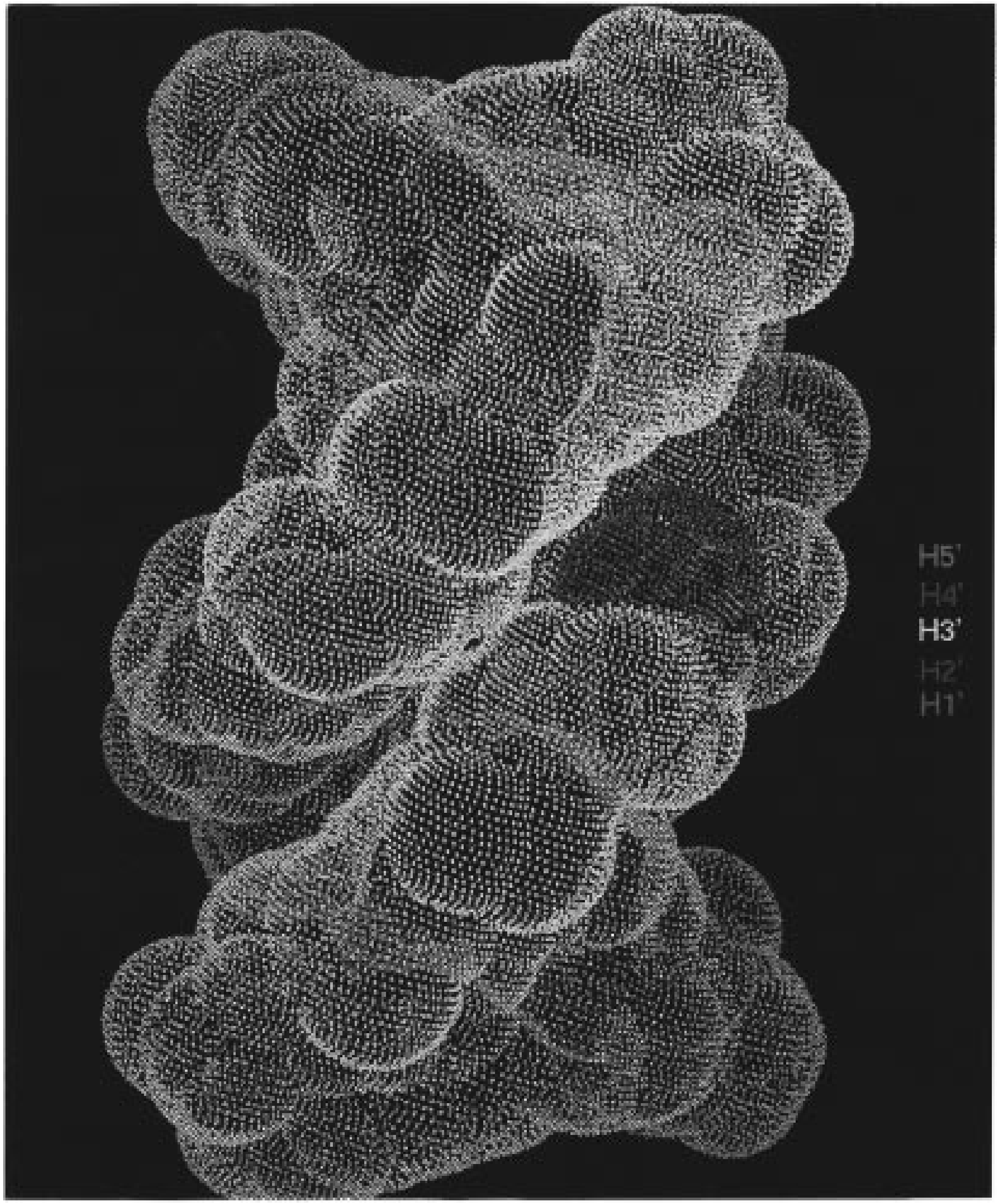


Figure 2. Illustration of solvent accessibility in a model B-form DNA duplex. A Conolly surface was calculated for a DNA decamer using the program Quanta (Molecular Simulations). The surfaces corresponding to the hydrogen atoms of one deoxyribose were colored as indicated.

However, some textbooks and computer programs use the nomenclature H5′1, H5′2, H2′1, and H2′2.[22] Biochemists, borrowing from carbohydrate chemistry, often designate the 2′-hydrogens as H-2′α (below the plane of the sugar) and H-2′β (above the sugar plane) while organic chemists tend to prefer the terms *pro-S*-H and *pro-R*-H. According to structures obtained by X-ray crystallography of oligonucleotides, the 5′-, 4′-, and 1′-positions of B-form duplex DNA are accessible from the minor groove, while the 3′- and one 2′-position are accessible from the major groove. One 5′-hydrogen atom points directly into the minor groove; the other points away from the backbone toward solvent.[23] As Figure 2 illustrates, the minor groove 5′- and 4′-hydrogen atoms are the most accessible in a typical B-form duplex.[24]

A key question in elucidating mechanisms of nucleic acid degradation is which of these hydrogen atoms is abstracted in the formation of a strand break. One approach has been to perform quantum mechanical calculations to elucidate the energetics governing the abstraction of a deoxyribose hydrogen mediated by a nonselective oxidant.[9] Osman and coworkers calculated the energy of hydrogen abstraction initiated by the hydroxyl radical (•OH) for positions 1′, 2′, 3′, and 4′ of the model sugar 2-deoxy-D-ribose and found that the theoretical probability of abstraction correlated with C−H bond strength.[25] They also determined that abstraction of H-1′, H-3′, or H-4′ required similar amounts of energy, presumably because the resulting sugar radical was stabilized by partial π-bond formation with the lone pair of electrons on the adjacent oxygen atom. Abstraction of the 2′- and 2″-hydrogens, on the other hand, required more energy because these positions were one bond removed from oxygen.[26]

Colson and Sevilla performed calculations on model sugars such as deoxyribose diphosphate and showed that abstraction of H-1′ was energetically favorable in these molecules, while abstraction of H-2′ was least favorable.[9] When calculations were performed on a deoxyribose sugar that was part of a double-stranded helix, solvent accessibility became a critical factor. In a study performed on double-stranded B-form DNA, the accessibility of H-1′ to a solvent-borne oxidant was reduced to virtually zero and showed little probability of abstraction. The 4′- and 5′-hydrogen atoms were significantly more exposed to solvent, and, therefore were much more likely to be abstracted.[26]

Similarly, using Monte Carlo simulation, Spotheim-Maurizot and co-workers calculated probabilities of hydroxyl radical-induced hydrogen abstraction from an 80 base pair duplex and found that abstraction was most probable from the 4′- and 5′-positions. Interestingly, these researchers also reported that accessibility of the 4′-hydrogen and one 5′-hydrogen (H-5″) were related to the width of the minor groove.[16]

According to these calculations, then, the propensity of a particular deoxyribose hydrogen in duplex DNA to be abstracted by a given oxidizing free radical is likely to be governed by proximity to oxygen (and hence, carbon−hydrogen bond strength) and the accessibility of the hydrogen atom to the oxidizing species.

III. Spectroscopic Examination of Hydrogen Abstraction Events

Identification of hydrogen abstraction events by techniques such as EPR has proved somewhat elusive due to the transient nature of the carbon-based sugar radicals, similarities in signals, and possible broadening of signals due to multiple sugar radical conformations.[27] Researchers have tried to overcome these limitations through low-temperature studies, ENDOR, simulations, and the use of model compounds and spin traps.[27−31] Most studies have utilized models of ribose or deoxyribose and are reviewed elsewhere. (See Colson and Sevilla,[9,28] Beckwith et al.,[10] and Close[27]). One interesting result comes from Close and co-workers, who irradiated single crystals of 2′-deoxyguanosine 5′-monophosphate at low temperature and, with the use of ENDOR spectroscopy, detected radicals at all five carbon atoms.[32] The radicals were not observed in irradiated duplex DNA.[27] Using spin trapping, ESR, and HPLC, Kuwubara et al. irradiated the model sugar thymidine 5′-monophosphate and detected C-1′, C-4′, and C-5′ radicals.[33] These researchers then extended treatment to the single-stranded oligonucleotides $(dC)_{10}$ and $(dT)_{10}$. To determine which DNA radicals led to strand breaks, the trapped radicals were subjected to denaturing gel electrophoresis. According to these results, only 4′-radical species were found to be precursors to immediate strand breaks.[33] These results confirm theoretical predictions that radicals can abstract hydrogen atoms from all positions of deoxyribose itself but also show that within a duplex, abstraction from certain positions may be more highly favored. While the double-helical structure of DNA likely contributes to such differences, these studies also suggest that hydrogen abstraction from certain positions may not lead to immediate strand breaks, but rather may make the DNA molecule more susceptible to strand breakage by other reagents or conditions.

IV. Chemical Studies Involving a Nonspecific Oxidant

A. Fenton-Generated Hydroxyl Radical ($^{\bullet}$OH)

How are theoretical predictions borne out by experiment? The question of which deoxyribose hydrogens are preferentially abstracted in the formation of strand breaks was investigated experimentally for the hydroxyl radical ($^{\bullet}$OH). This nonspecific, diffusible species was generated by the Fenton−Udenfriend reaction, in which $[Fe(EDTA)]^{2-}$ is oxidized by hydrogen peroxide:[34−36]

$$[Fe(EDTA)]^{2-} + H_2O_2 \rightarrow [Fe(EDTA)]^{1-} + {}^{\bullet}OH + OH^- \quad (1)$$

Unlike most of the other DNA-cutting systems reviewed here, the Fenton-generated oxidant is produced in bulk solution. The negatively charged $[Fe(EDTA)]^{2-}$ complex is electrostatically repelled by polyanionic nucleic acids; the hydroxyl radical, by contrast, has no charge and is diffusible. When generated in this manner, the highly reactive hydroxyl radical does not show specificity for cleavage at a particular nucleotide (A, C, G, or T) in B-DNA.[37,38] This characteristic distinguishes the Fenton oxidant from other DNA-cutting reagents that bind to DNA. For example, bleomycin·Fe(II) binds DNA and generates an oxidizing species in close proximity to minor groove hydrogen atoms. As a consequence, the drug shows sequence specificity and more specific chemistry than is observed with the Fenton-generated hydroxyl radical.[39,40] While some researchers have argued against formation of $^{\bullet}$OH by the Fenton reaction,[41−44] results from our laboratory and others are consistent with production of a small, neutral oxidant[45,46] that yields strand break patterns identical to those generated by ionizing radiation,[47,48] which is known to produce the hydroxyl radical.[49]

To determine how this diffusible and highly reactive species reacts at deoxyribose to cleave DNA, we selectively incorporated deuterium at each individual deoxyribose position and measured kinetic isotope effects on the production of strand break products. Results showed that the hydroxyl radical is able to abstract hydrogen from each deoxyribose carbon of B-form DNA, but the 5′- and 4′-positions are most important. Preference for individual hydrogens was found in the order H-5′ > H-4′ > H-2′ ≈ H-3′ > H-1′.[50] (The stereochemical preference of abstraction at positions 2′ and 5′ is not well-established.) This order of reactivity correlates well with the accessibility to

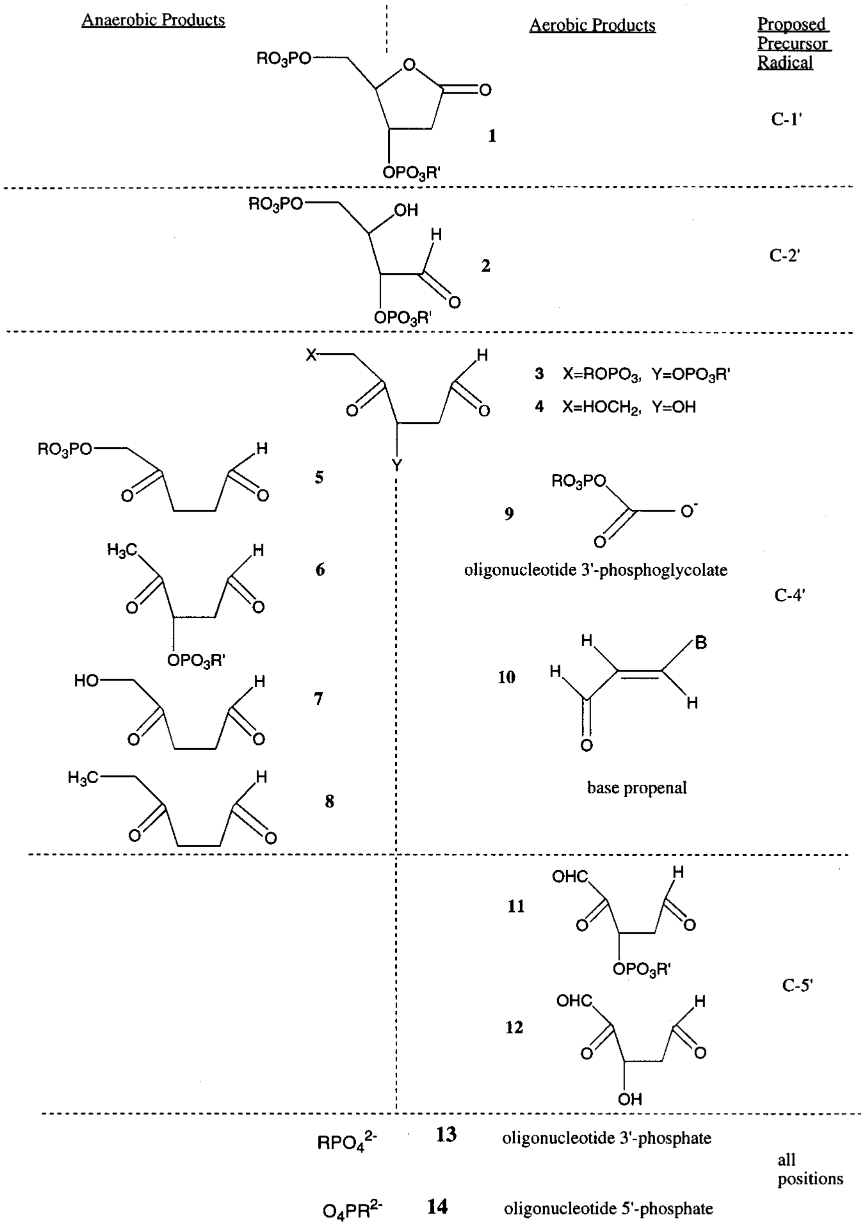


Figure 3. Products of gamma radiolysis-mediated DNA scission as identified mainly through derivatization and GC/MS analysis. DNA fragments in the center are identified under both aerobic and anaerobic conditions. (Figure adapted from von Sonntag[19] and Stubbe and Kozarich.[17])

solvent of individual hydrogen atoms in the sequences studied.[50,51] These results confirm that the accessibility of a hydrogen atom is critical for abstraction, even with a nonspecific, diffusible oxidant.

B. Radiation-Produced Hydroxyl Radical ($^{\bullet}$OH)

Ionizing radiation is another means of generating a nonspecific oxidant. A high-energy photon or particle induces the homolysis of a water molecule, forming the hydroxyl radical which initiates strand breaks in DNA and RNA.[49] The earliest investigations of nucleic acid scission pathways were in fact performed by radiation researchers (reviewed by von Sonntag).[19] Schulte-Frohlinde, von Sonntag, and their co-workers irradiated model systems such as aqueous poly(U) under aerobic and anaerobic conditions. Following derivitization and detection by GC/MS techniques, researchers found a wide spectrum of ketone sugars and DNA fragments containing ketone sugars, as well as DNA fragments terminated with phosphate groups (Figure 3). Mechanisms to account for these products are discussed in section V. While not every abstraction event resulted in an immediate strand break, the observation of a ketone group at each position was thought to reflect hydrogen atom abstraction at that position.[19,52,53]

Somewhat different results were obtained from the laboratories of Hecht and Haseltine.[54,55] In these studies, radiolabeled duplex DNA was used as a radiation target and products were separated by high-resolution gel electrophoresis. A simpler pattern of products was observed than had been seen with poly(U) by GC/MS. The cutting pattern on the gel showed only one or two products at each nucleotide position. When the DNA was radiolabeled with

^{32}P at the 5′-end, products **9** (DNA fragments terminated at the 3′-end with phosphoglycolate) and **13** (DNA fragments terminated at the 3′-end with phosphate) were seen. DNA radiolabeled at the 3′-end showed product fragments terminated at the 5′-end with phosphate (**14**). Terminal groups were identified by treating the products with various enzymes and observing the electrophoretic behavior relative to known standards. For example, products thought to contain a phosphate group in the 5′-position (**14**) were treated with polynucleotide kinase. This enzyme converts phosphate groups to hydroxyl groups;[56] hence, a fragment with a 5′-phosphate terminus, when treated with the enzyme, is converted to a fragment with a 5′-hydroxyl terminus. Oligonucleotides with hydroxyl termini can be identified by their slower electrophoretic mobility relative to phosphate-terminated strands.[54,55] The products observed with these methods were identical to those produced by the Fenton-generated hydroxyl radical.[47]

These two sets of radiolysis results may reflect different radiation conditions, differences in the sensitivities of the detection methods, and/or differences in postirradiation treatment. However, another explanation for the discrepancy in the number of products observed in the different studies is simply the fact that cleavage chemistry is influenced by duplex structure. Many of the early radiation studies were performed on single-stranded nucleic acids such as poly(U). Duplex DNA or RNA would have fewer sites accessible for hydrogen abstraction and this difference could result in fewer products being generated.

V. Proposed Mechanisms of DNA Degradation

Many nucleic acid-cleaving molecules, particularly those that bind to nucleic acids, can be quite specific in the pathway by which they form strand breaks. Mechanisms have been proposed for DNA degradation reactions beginning with hydrogen abstraction from each deoxyribose position. While many of the intermediates in these reactions remain to be elucidated, research has progressed to the point that products can be accounted for rationally. In the following sections we describe a representative set of DNA-cleaving pathways, organized by initial hydrogen abstraction event, and show how pathways can be deduced by observation of various unique marker products.

A. Abstraction of H-1′

1. General Considerations

In a B-DNA helix, H-1′ is buried in the minor groove and is thought to be relatively inaccessible to solvent.[24,26,57] Calculations performed by Miaskiewicz and Osman predicted a low probability of abstraction by the hydroxyl radical at this position in a B-form duplex.[26] Likewise, deuterium kinetic isotope effect experiments for the reaction of the hydroxyl radical (generated by Fenton chemistry) with duplex DNA indicated low reactivity at this position.[50] Hence, the importance of H-1′ as a reactive site appears to be limited primarily to minor groove-binding molecules in which the oxidant is generated within the groove and oriented toward this hydrogen.

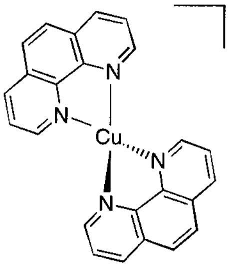


Figure 4. Bis(1,10-phenanthroline)copper(I).

2. Bis(1,10-phenanthroline)copper(I)

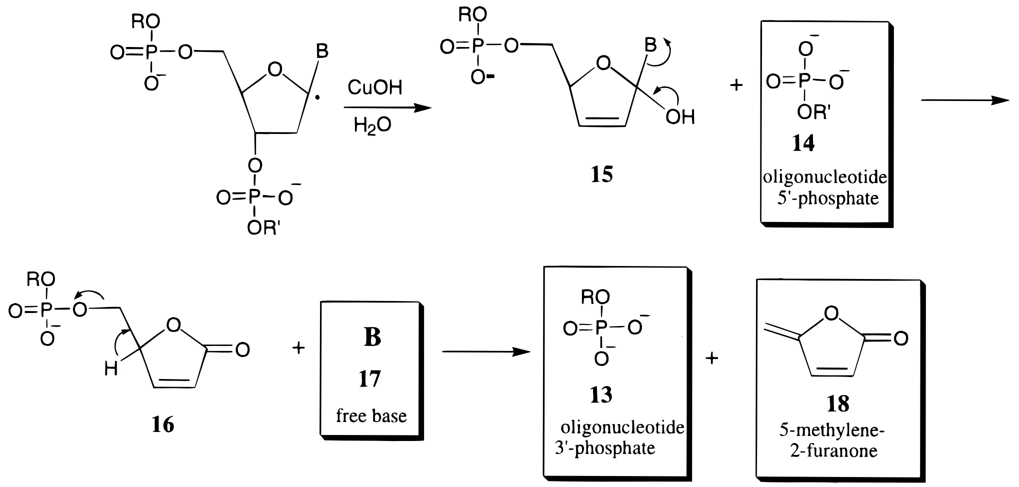
One of the best-characterized DNA cleavage reactions resulting from H-1′ abstraction is that promoted by bis(1,10-phenanthroline)copper(I) (abbreviated Cu(OP)$_2$). This "artificial nuclease", discovered by Sigman and co-workers[58] and shown in Figure 4, binds to DNA in the minor groove in a sequence-dependent manner[59] and degrades double-stranded DNA in the presence of hydrogen peroxide.[60] Researchers disagree as to the identity of the oxidizing species; it may be free hydroxyl radical,[61,62] but more likely is a copper-bound oxidant such as $[CuO]^+$, $[CuOH]^{2+}$, or CuO_2H.[63,64]

A mechanism proposed[65] for DNA cleavage promoted by Cu(OP)$_2$ is shown in Scheme 1. Scission products observed include free base (**17**), equal amounts of DNA fragments with 3′-phosphate (**13**) and 5′-phosphate (**14**) termini, and the monomeric product 5-methylene-2-furanone (MEF) (**18**).[59,66,67] Oxygen-labeling studies have shown that the carbonyl oxygen of 5-methylene-2-furanone (**18**) is derived from solvent.[65] To accommodate this observation, the initial 1′-deoxyribosyl radical is proposed to be converted to a carbocation, probably by copper-mediated oxidation. Nucleophilic attack by water at C-1′ produces intermediate **15**, which eventually decomposes to yield a fragment of DNA bearing a 3′-phosphate terminus (**13**) and the furanone (**18**). A key feature of this pathway is that a strand break is observed at room temperature; heat and alkali treatment are not necessary for strand cleavage. Another interesting observation is that, unlike most other cleavage pathways, the sugar ring is not opened in this pathway.

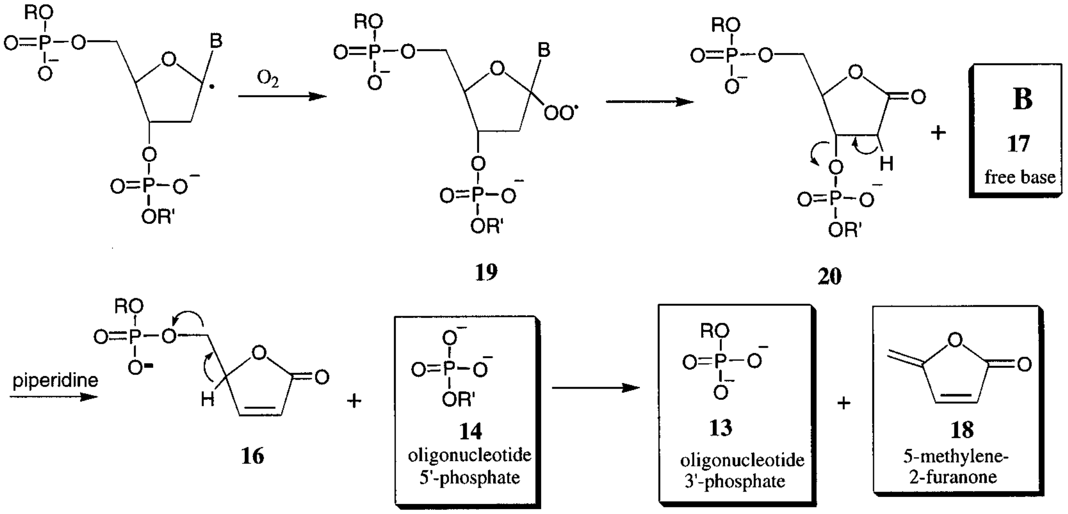
Cu(OP)$_2$ is also reported to cleave DNA by a minor pathway that begins with abstraction of H-4′. As the 4′-hydrogen atom is highly accessible from the minor groove, such a pathway is reasonable. This mechanism is discussed in section V.D.

The cleavage chemistry of non-B-form nucleic acids also was studied to determine how changes in structure alter modes of degradation. Cu(OP)$_2$ was found to cleave A-DNA less efficiently than B-DNA, presumably because of fewer favorable contacts between the complex and the widened minor groove of the A-form double helix.[68] The reduced cleavage of A-DNA was not attributed to altered accessibilities of the 1′- and 4′-hydrogen atoms. These positions are also very accessible in RNA. When Cu(OP)$_2$ was

Scheme 1. Proposed H-1′-Abstraction Pathway for Oxidative DNA Strand Scission by Bis(1,10-phenanthroline)copper(I) (adapted from Meijler et al.[65])



Scheme 2. Proposed H-1′-Abstraction Pathway for DNA Strand Scission Mediated by Neocarzinostatin (adapted from Goldberg[71])



allowed to react with Z-DNA, a conformation in which the DNA has little or no minor groove, the Z-form was not cleaved at all.[69,70] While Cu(OP)$_2$ cleaves double-stranded DNA more efficiently than single-stranded DNA, the reverse was found to be true of RNA. Single-stranded loops of RNA were cleaved more efficiently than duplex regions.[68]

3. Neocarzinostatin

Another class of DNA-cleaving agents believed to react at the 1′-deoxyribose position are the enediyne antibiotics. These natural products are characterized by the presence of an enediyne unit that can form a diradical. The group includes neocarzinostatin[71,72] (abbreviated NCS), calicheamicin,[73,74] dynemicin A,[75,76] esperamicin A$_1$,[77] C1027,[78] and maduropeptin.[79] The best-characterized of these molecules is neocarzinostatin. The nonprotein chromophore, shown in Figure 5, contains a 1,5-diyne-3-ene as part of a bicyclo[7.3.1] ring system. It binds preferentially to A/T-rich sequences[80] and, when activated by thiol, undergoes Michael addition of the thiolate to the α,β-unsaturated bridgehead ketone. Rearrangement[81] and subsequent aromatization of the enediyne produces a diradical that can span the minor groove and react with both strands of a DNA duplex, producing a double-strand break.[71] Deuterium kinetic isotope effect experiments have shown that the drug abstracts a 1′-hydrogen from one of the DNA or RNA strands.[82−84] Different chemistry occurs on the complementary strand. (See section V.D.4).

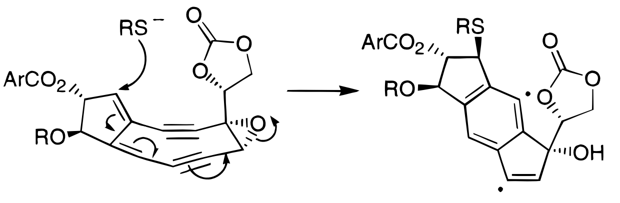


Figure 5. Activation of the neocarzinostatin chromophore to form the diradical species.

The mechanism proposed for strand cleavage by NCS is somewhat different from that proposed for Cu(OP)$_2$. The basis of this difference is that Cu(OP)$_2$ treatment of DNA gives rise to an immediate strand break, while the initial lesion generated from H-1′ abstraction by neocarzinostatin requires base treatment to produce a strand break. As Scheme 2 illustrates, the 1′-deoxyribosyl radical generated by NCS is proposed to react with molecular oxygen to form peroxyl radical **19**. Reduction of the peroxyl radical and loss of water yields the 2′-deoxyribonolactone **20** and free base.[82] Thus, an abasic site is generated which, upon treatment with hot piperidine, leads to strand scission.[65,84] Alternatively, the 1′-radical could be quenched by a thiol, generating an abasic site that also leads to strand breaks on treatment with alkali.[85] The products produced by these mechanisms are identical.

While double-stranded RNA has not been found to be a good substrate for neocarzinostatin,[86] DNA·RNA hybrids have been shown to be cleaved by the drug. A substantial kinetic isotope effect was observed when the 1′-position of the RNA strand of a hybrid was deuterated.[84] The RNA strand was shown to be cleaved exclusively by an H-1′ pathway while the complementary DNA strand reacted via an H-5′ abstraction pathway. When the RNA was replaced with DNA, the analogous DNA strand also showed 5′-H chemistry.[84] As DNA·RNA hybrids are presumed to have an A-form structure, the observation of 1′-H abstraction on the RNA strand may indicate increased accessibility of this position due to the wider minor groove.

4. Other Examples of Chemistry at the C-1′ Position

The antitumor antibiotic bleomycin, discussed in section V.D.3 and reviewed by Burger in this issue,[87] has been proposed to cleave RNA by a pathway beginning with abstraction of H-1′,[88] but DNA·RNA hybrids did not exhibit H-1′ chemistry when cleaved by bleomycin·Fe(II).[89−91] In fact, no bleomycin-induced damage was observed in the RNA strand.[92] However, when self-complementary DNA oligonucleotides containing a single *ribo*- or *ara*-nucleoside were used as substrates for activated bleomycin, the products showed evidence of H-1′ abstraction at the altered nucleoside.[93]

B. Abstraction of H-2′

1. Gamma Radiolysis of Poly(U)

To date, the 2′-position of deoxyribose is not a commonly invoked abstraction site in DNA, presumably because of the low accessibility or low reactivity of these hydrogens. An aldehyde sugar thought to arise from H-2′ abstraction was identified from gamma ray-treated poly(U) by GC/MS.[94] The 2′-OH in the single-stranded poly(U), which replaces the 2′-*pro-R*-H in DNA, may have facilitated hydrogen abstraction from this position. Quantum mechanical calculations suggest that in short single-stranded oligonucleotides, ionizing radiation can induce a conformational change that shortens the distance between the base and the sugar, enabling a base radical to abstract a 1′- or 2′-hydrogen from the sugar.[14,95] Uracil, furthermore, is known to form radicals that are powerful hydrogen abstractors and may be able to abstract hydrogen atoms from adjacent sugars as well. An erythrose-containing site thought to arise from H-2′ abstraction also was reported as a product of gamma radiolysis of calf thymus DNA,[96,97] and the hydroxyl radical as generated by the Fenton reaction showed a small kinetic isotope effect on the production of phosphate-terminated DNA strands when the 2′-position of double-stranded DNA was dideuterated.[50] These results suggest that abstraction of a 2′-hydrogen atom may contribute to strand scission even in B-form DNA. However, the contribution of this cleavage pathway appears to be minor.

2. Photolysis of Oligonucleotides Containing Halogenated Uracil

Certain chemical modifications of DNA appear to enhance the contribution of a 2′-H mechanism. Recent results suggest that when an oligonucleotide containing a halogenated uracil at one site is cleaved by photooxidation, products can be accounted for by a mechanism involving H-2′ abstraction.[13,98−100] In support of this mechanism, Sugiyama et al. reported a kinetic isotope effect in erythrose-containing DNA products when DNA containing a halogenated uracil moiety was specifically labeled at the 2′-position with deuterium and photooxidized.[100] A mechanism proposed by these researchers is shown in Scheme 3. The halogenated uracil is believed to abstract a 2′-H from the deoxyribose moiety of the adjacent adenosine (in the 5′-direction). Oxygen reacts with the 2′-deoxyribosyl radical to form a peroxide (**21**) that may undergo homolytic or heterolytic cleavage to form the erythrose-containing site (**22**). Upon treatment with alkali, **22** undergoes a retroaldol reaction that cleaves the DNA, forming fragments with phosphoglycaldehyde termini (**24** and **25**).[99] These fragments have been detected and quantified by HPLC.[84]

As mentioned previously, the 2′-position is prochiral. Sugiyama et al. investigated the stereochemistry of abstraction from this position, again using DNA that contained halogenated uracil and incorporating deuterium into the 2′-position of the adjacent nucleotide. The two stereoisomers were designated 2′α-D (or 2″-D) and 2′β-D (or 2′-D). There was a substantial kinetic isotope effect on the yield of the erythrose-containing product **22** for the 2′α-D substrate, perhaps for the simple reason that the α-position (below the plane of the sugar) is closer to the uracilyl radical of the adjacent nucleotide, facilitating electron transfer from the base to the adjacent sugar.[100]

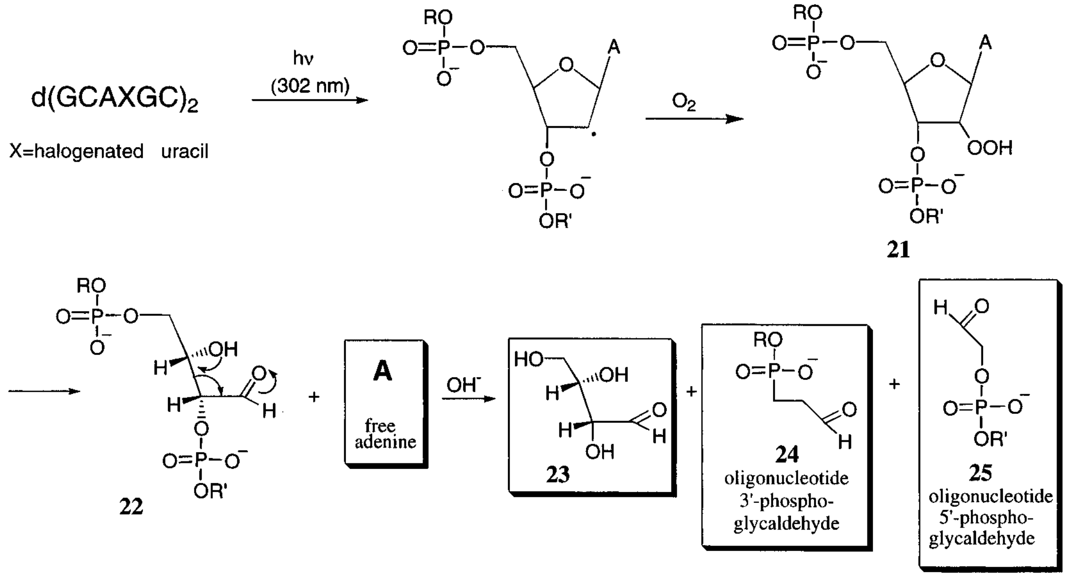
Collectively, these results suggest that abstraction of H-2′ does not contribute greatly to most pathways of DNA strand scission, but abstraction of a 2′-H may be facilitated in RNA, in processes involving excited states, or by the presence of adjacent halogenated bases.

C. Abstraction of H-3′

1. General Considerations

The 3′-hydrogen, located in the major groove, appears to be involved in few pathways investigated thus far. This infrequency may reflect the fact that the majority of known oxidative cleavage agents bind in the minor groove rather than the major groove. However, deuterium kinetic isotope effect measurements for the Fenton-generated hydroxyl radical

Scheme 3. Proposed H-2′-Abstraction Pathway for Photolysis-induced DNA Strand Scission at a Nucleotide Containing a Halogenated Uracil (adapted from Sugiyama et al.[99])



suggested that even with a very nonselective, diffusible oxidizing species, abstraction of hydrogen from the 3′-carbon of deoxyribose is a minor contributor to strand break formation in B-form duplex DNA. Therefore, the scarcity of known 3′-based mechanisms may be due to limited accessibility or to reduced stability of a 3′-deoxyribosyl radical. Colson and Sevilla have suggested that the reactivity of H-3′ may be affected by sugar pucker.[28]

2. Photoactive Rhodium(III) Complexes

One class of metal complexes has been established to cleave DNA through abstraction of the 3′-hydrogen. Rhodium(III) coordinated by well-known intercalating ligands is able to bind to duplex DNA and generate strand breaks in the presence of light. It is interesting to note that these complexes also slightly unwind DNA. This group of photoreactive agents includes [Rh(phen)$_2$phi]$^{3+}$, [Rh(phi)$_2$bpy]$^{3+}$ (Figure 6),[101] and both enantiomers of [Rh(en)$_2$-phi]$^{3+}$ [102] (where en = ethylenediamine, phen = 1,-10-phenanthroline, bpy = 2,2′-bipyridyl, and phi = 9,10-phenanthrenequinone diimine). The rhodium complex is believed to form a phi cation radical via photoinduced ligand-to-metal charge transfer. In the metal–DNA complex, the cation radical is proposed to be pointed toward H-3′. Chemical modification, HPLC analysis, and gel electrophoresis studies revealed the products of this reaction to be, as shown in Schemes 4 and 5, 3′- and 5′-phosphate-terminated DNA strands (**13** and **14**, respectively), a DNA strand terminating in a 3′-phosphoglycaldehyde group (**24**), free base, and base propenoic acid (**33**).[101] Oxygen was found to be necessary for formation of the 3′-phosphoglycaldehyde-terminated fragment and base propenoic acid, but not for formation of free base or the 3′-phosphate-terminated product. The partitioning between anaerobic and aerobic pathways best correlated with how the shape of the rhodium complex was expected to limit access of oxygen to the cleavage site.[101] Abstraction of H-3′ was proposed to account for the products, which, interestingly, had been predicted earlier.[17] The anaerobic pathway (Scheme 4) involves oxidation of the radical, followed by solvolysis to yield an alcohol (**26**) that then undergoes β-elimination of the phosphate and release of free base. This mechanism predicts the production of an unopened sugar lactone derivative (**28**). In the oxygen-dependent mechanism (Scheme 5), molecular oxygen reacts with the 3′-radical to give the hydroperoxide radical. Subsequent rearrangement of **29** results in insertion of an oxygen atom into the deoxyribose ring, forming intermediate **30**. Decomposition yields base propenoic acid (**33**) and DNA fragments with 5′-phosphate (**14**) or 3′-phosphoglycaldehyde (**24**) termini.[101] The product containing phosphoglycaldehyde is identifiable by its reduced electrophoretic mobility relative to the fragments that contain only phosphate termini.

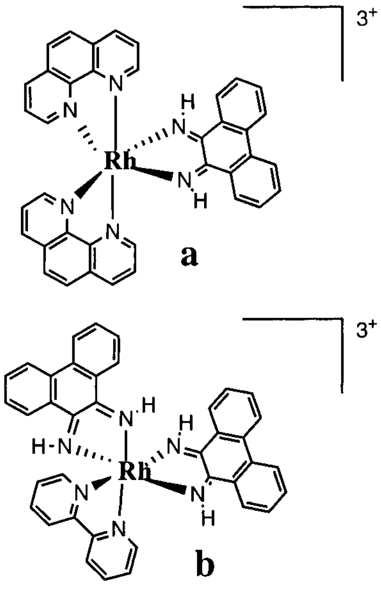
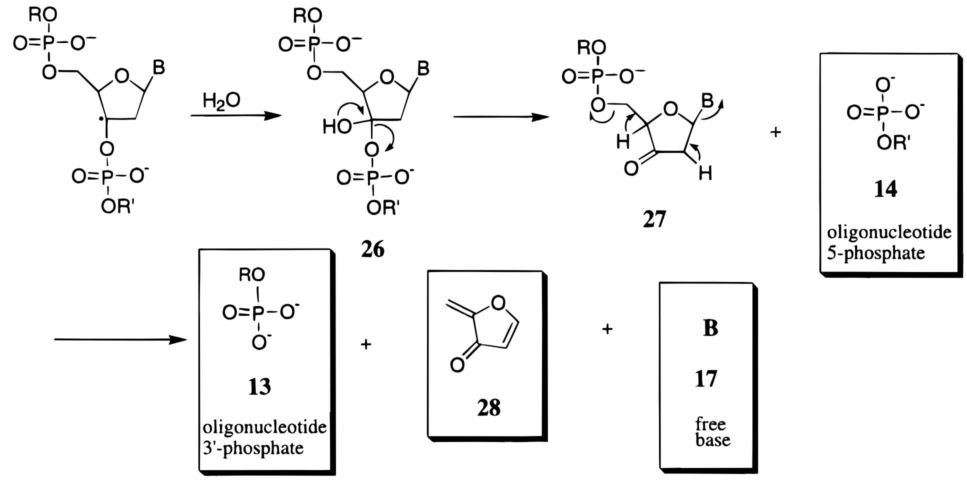
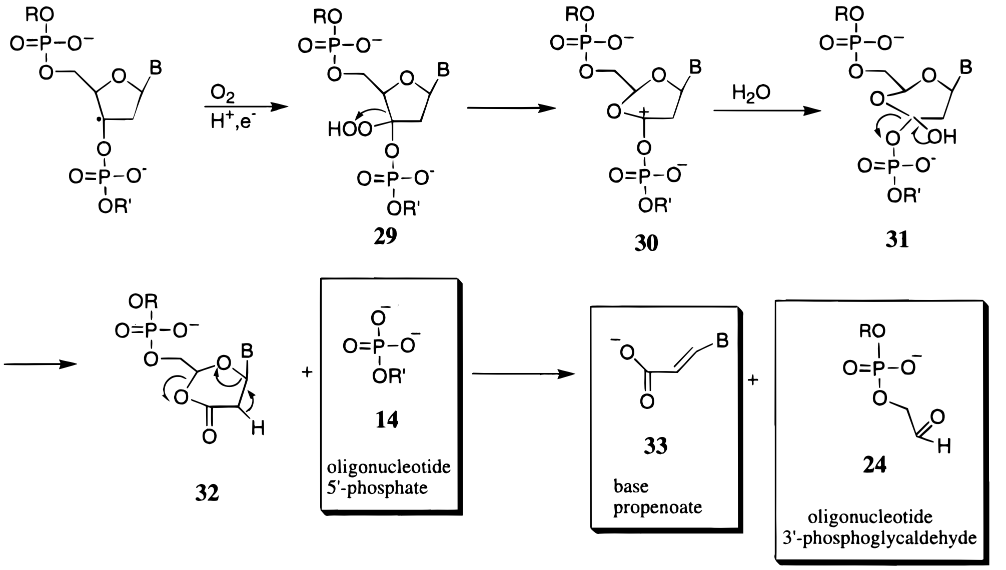


Figure 6. [Rh(phen)$_2$(phi)]$^{3+}$ (a) and [Rh(phi)$_2$(bpy)]$^{3+}$ (b).

Scheme 4. Proposed H-3′-Abstraction Pathway for DNA Strand Scission Mediated by $[Rh(phen)_2(phi)]^{3+}$ or $[Rh(phi)_2bpy]^{3+}$ under Anaerobic Conditions (adapted from Sitlani et al.[101])



Scheme 5. Proposed H-3′-Abstraction Pathway for DNA Strand Scission Mediated by $[Rh(phen)_2(phi)]^{3+}$ or $[Rh(phi)_2bpy]^{3+}$ under Aerobic Conditions (adapted from Sitlani et al.[101])



D. Abstraction of H-4′

1. General Considerations

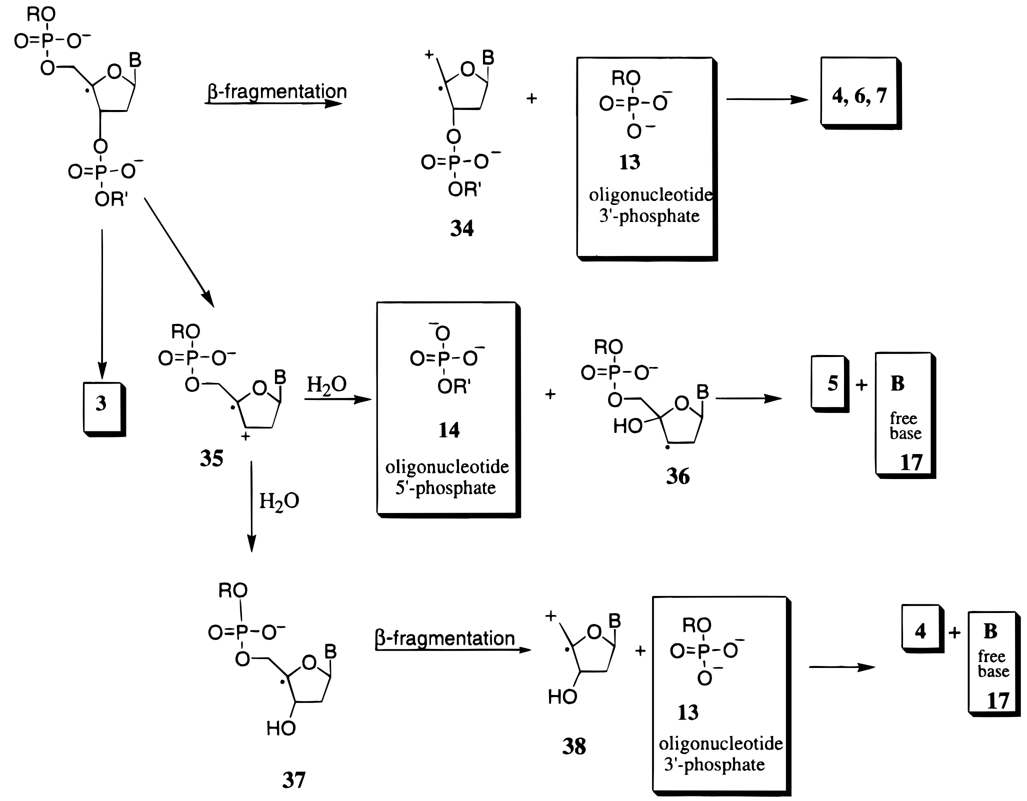
Strand scission resulting from abstraction of H-4′ has been observed in many systems. This deoxyribose position is believed to be very accessible in B-form DNA;[26,57] therefore it is not surprising that many DNA-cutting molecules appear to react at this site. DNA damage initiated from 4′-hydrogen abstraction has been proposed for ionizing radiation,[19] methidiumpropyl(EDTA)·Fe(II),[17,103] Fenton-generated hydroxyl radicals,[104] and the drugs bleomycin,[17,90,105] calicheamicin,[106] neocarzinostatin,[71,107] elsamicin A,[108] and C1027.[76] A pathway involving H-4′ may also be of minor importance in DNA scission mediated by $Cu(OP)_2$.[109]

2. Gamma Radiolysis

A mechanism beginning with 4′-hydrogen abstraction was first proposed for gamma ray-induced DNA strand breaks to account for the production of scission fragments with phosphate termini and the ketone sugars shown in Figure 3. As Scheme 6 shows, Schulte-Frohlinde, von Sonntag, and co-workers proposed an oxygen-independent pathway based on model chemistry with alkyl phosphates and ribose 5′-phosphate. An alkyl radical, when adjacent to a phosphate ester, undergoes hydrolysis followed by β-elimination of phosphate. Hence, in the mechanism proposed for DNA a C-4′ radical is formed followed by β-elimination of one or both phosphates. In the process, a radical cation intermediate (**34** or **35**) is generated, to which water is added and a proton is released.[110,111] The resulting radical is believed to be stabilized by the lone pair of electrons on the ring oxygen.

Phosphate elimination and formation of radical cations were also observed in the model studies of Giese.[112] The proposed pathway predicts that the final sugar products (Figure 3) will depend on whether water adds to the cation or to the carbon-centered radical. Recent results from studies involving phosphate esters from the Giese and Crich groups[113,114] also raise the possibility of phosphate migration.

Scheme 6. Proposed H-4′-Abstraction Pathway for DNA Strand Scission by Gamma Radiolysis under Anaerobic Conditions (adapted from von Sonntag et al.[19] and Schulte-Frohlinde[53])



However, long segments of DNA may be significantly less likely to rearrange in this fashion.

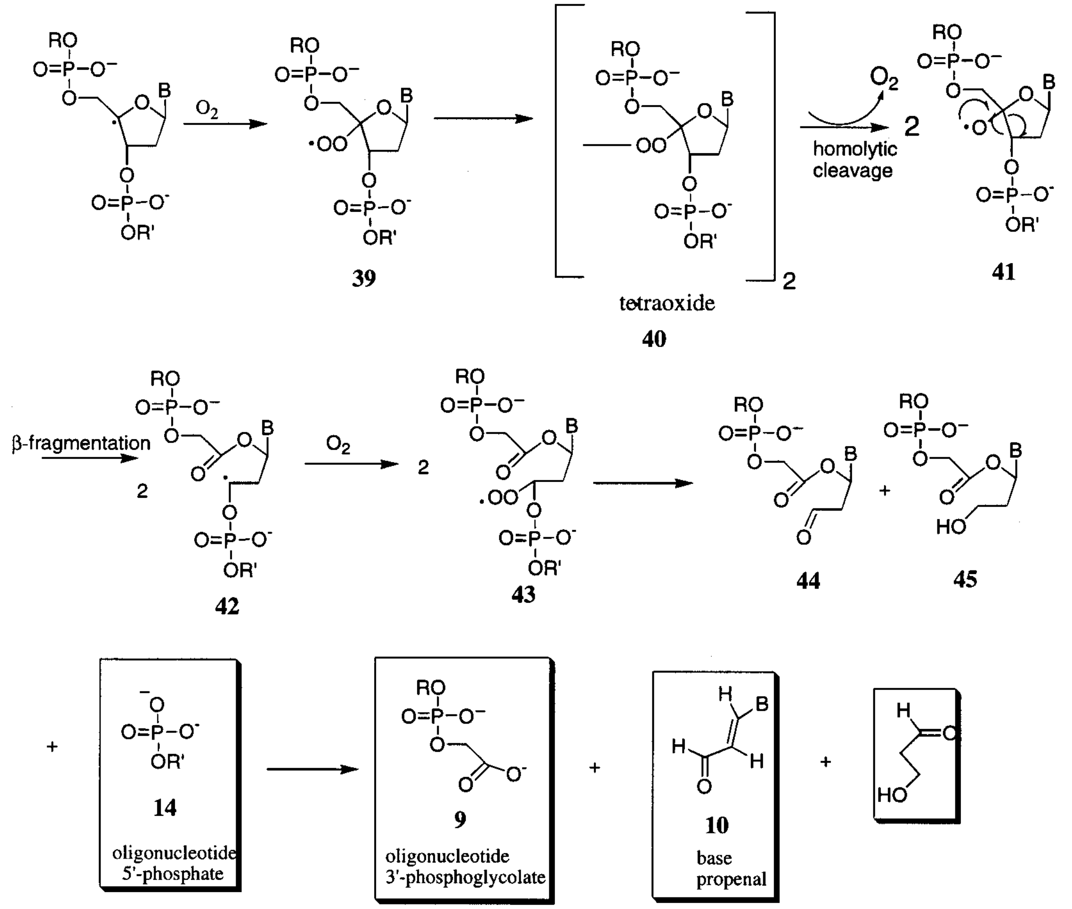
A different mechanism was proposed for gamma radiolysis to account for products produced under aerobic conditions (Scheme 7). In this pathway oxygen reacts at the diffusion-controlled rate with the 4′-radical center to form peroxyl radical **39**. In a Russell-type mechanism, two such peroxyl radicals combine to form a tetraoxide (**40**).[115,116] Heterolytic or homolytic cleavage of the tetraoxide results in phosphate- or phosphoglycolate-terminated products.[96,117,118] While tetraoxide formation has been demonstrated in small molecules, dimerization of two polyanionic duplexes appears problematic due to steric and electronic barriers. Such species may be somewhat more likely to form in cases of high-energy deposition such as that provided by high-LET or densely ionizing radiation. Additional details regarding proposed radiation-induced cleavage pathways can be found in the reviews of von Sonntag,[19] Hutchinson,[18] and Breen and Murphy.[20]

3. Fe(II)•Bleomycin

One of the best-characterized pathways of DNA cleavage from H-4′ abstraction is that mediated by the antitumor antibiotic bleomycin•Fe(II) (Figure 7). Bleomycin-mediated cleavage mechanisms are discussed in detail by Burger in this issue.[87] The drug binds to DNA in the minor groove and is activated either by molecular oxygen and two electrons, or by H_2O_2.[119,120] The actual identity of the oxidizing species in activated bleomycin has been an issue of debate. Electrospray mass spectrometry has provided evidence of a ferric hydroxide or a high-valent iron–oxo species such as $Fe^{V}{=}O \leftrightarrow Fe^{IV}{-}O^{\bullet}$, capable of abstracting a hydrogen atom from deoxyribose.[91,119] Several other reactive species such as the hydroxyl radical have also been proposed, but are less well supported in the literature.[121]

Researchers investigating bleomycin were first to experimentally identify hydrogen abstraction events in DNA. By incorporating tritium or deuterium into specific positions in deoxyribose sugars and measuring kinetic isotope effects, Kozarich and Stubbe and co-workers were able to show that abstraction of H-4′ was the rate-limiting step in DNA degradation mediated by the drug.[17,122−124] Extensive characterization of products by gel electrophoresis, enzymatic analysis, base treatment, sodium borohydride reduction, and chromatographic separation led the pathways proposed in Schemes 8 and 9. Products include free base, base propenal (**10**), DNA fragments with 3′-phosphate end groups (**13**), DNA fragments with 3′-phosphoglycolate end groups (**9**), and DNA fragments with 5′-phosphate termini (**14**). The existence of two base products and two types of 3′-termini suggested that partitioning of the 4′-deoxyribosyl radical occurs. This prediction was supported by experiments which showed that formation of the base propenal (**10**) and 3′-phosphoglycolate-terminated strands (**9**) could be suppressed when oxygen concentration was low.[39,125] In low amounts of oxygen (Scheme 8) the 4′-deoxyribosyl radical is oxidized, producing a 4′-hydroxyl species (**46**) and free base. *β*-Elimination yields a DNA strand with a 5′-phosphate terminus (**14**) and a 4′-keto-1′-aldehyde-terminated strand (**49**). This latter intermediate is alkali-labile; hence, in the presence of heat and a base such as piperidine, it

Scheme 7. Proposed H-4′-Abstraction Pathway for DNA Strand Scission by Gamma Radiolysis under Aerobic Conditions (adapted from Breen and Murphy[20])



decomposes, producing a DNA strand with a 3′-phosphate end (**13**).[39] Alternatively, a pathway has been proposed in which oxidation of the C-4′ radical (perhaps by an electron-transfer process) generates a C-4′ oxonium ion and releases free base. Nucleophilic attack of water at C-4′ produces the keto aldehyde **49**.[126] Phosphates are eliminated in the presence of alkali, generating DNA strands with phosphate termini.

Under neutral pH conditions and high amounts of oxygen, a different mechanism is proposed (Scheme 9). Molecular oxygen reacts at the carbon-centered radical to form a peroxyl radical (**39**) or peroxide (**51**). The intermediate may undergo a Criegee-like rearrangement.[17,127–129] While the occurrence of a Criegee rearrangement has been questioned due to a requirement for strong acid, the drug itself may be able to act as a Lewis acid and effect this rearrange-

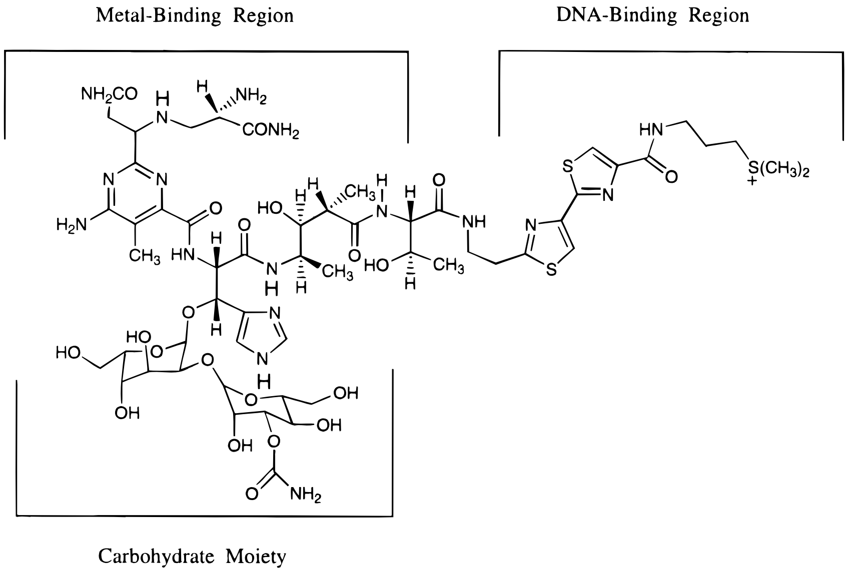
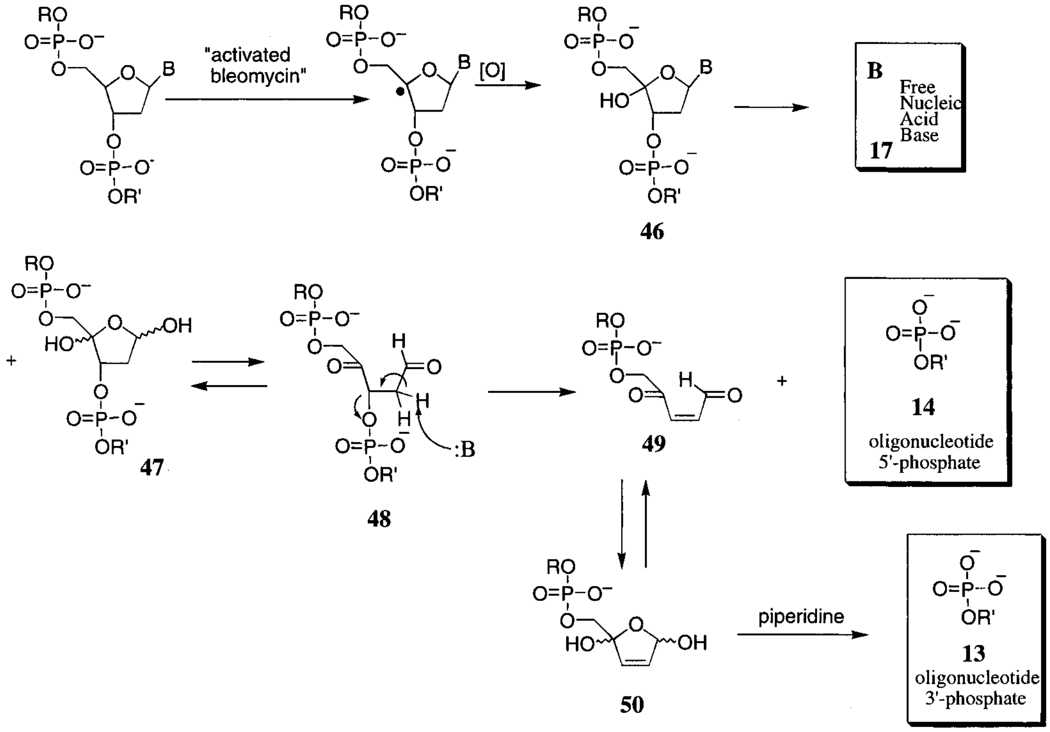
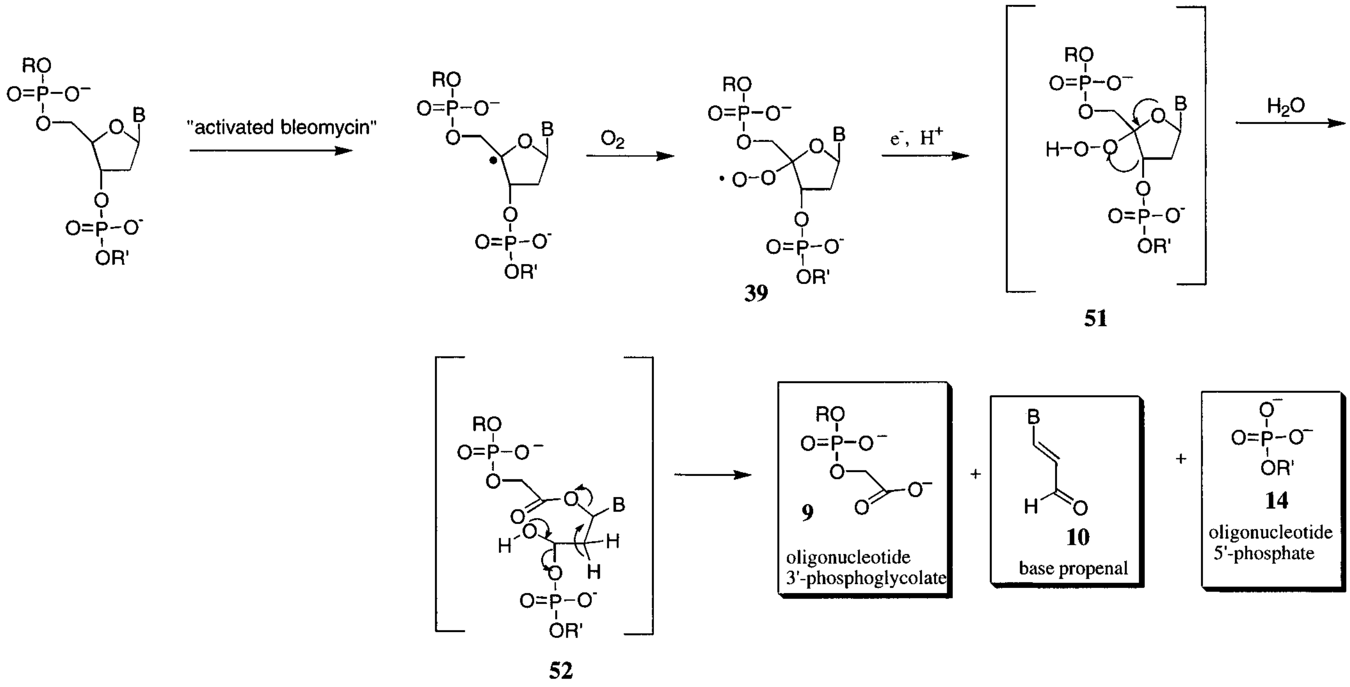


Figure 7. The antitumor antibiotic bleomycin.

Scheme 8. Proposed H-4′-Abstraction Pathway for DNA Strand Scission Mediated by Activated Bleomycin·Fe(II) under Anaerobic Conditions (adapted from Worth et al.[39] and Hecht[90])



Scheme 9. Proposed H-4′-Abstraction Pathway for DNA Strand Scission Mediated by Activated Bleomycin·Fe(II) under Aerobic Conditions (adapted from Worth et al.[39] and Hecht[90])

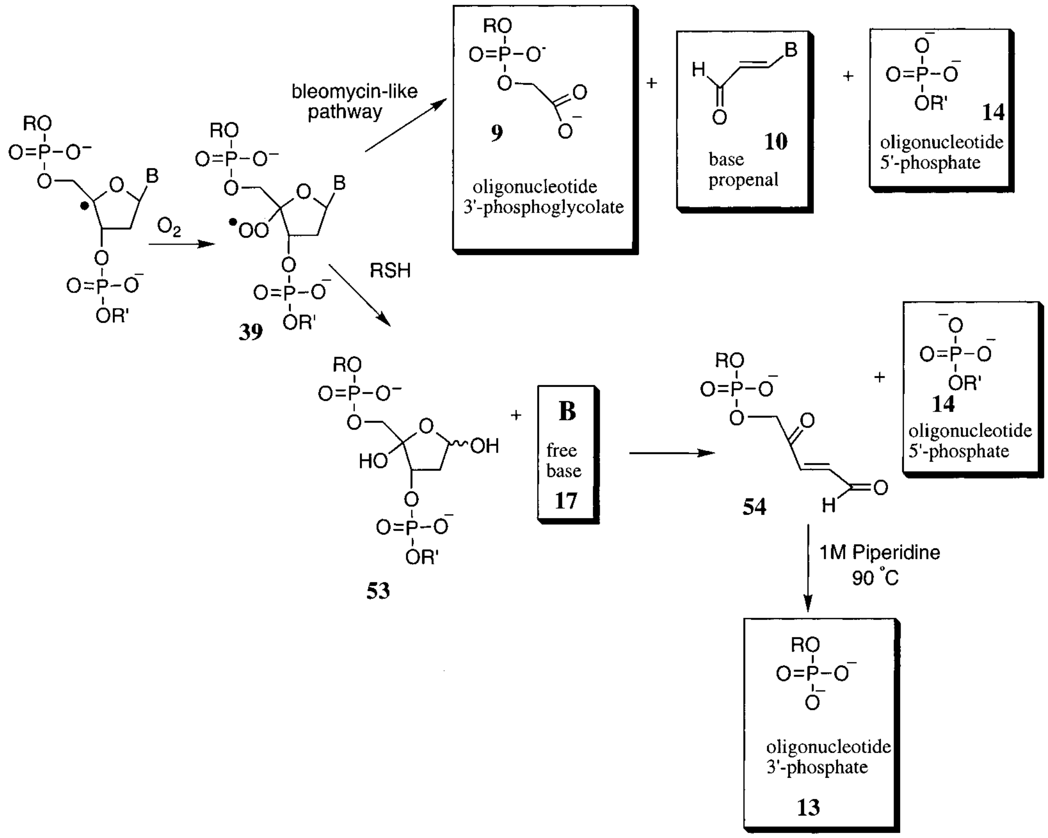


ment,[130] producing DNA fragments with 3′-phosphoglycolate termini (**9**), base propenal (**10**) and DNA fragments with 5′-phosphate termini (**14**). The base propenal is unique to 4′ chemistry and can be identified by a colorimetric reaction with thiobarbituric acid.[105] The phosphoglycolate-terminated strand, also a marker of oxidation at the 4′-position, can be identified by its faster mobility, relative to a phosphate-terminated strand, in an electrophoresis gel.[40,122,131]

4. Neocarzinostatin

In DNA cleavage promoted by the enediyne drug neocarzinostatin, abstraction of 4′-H on one strand produces a 4′-deoxyribosyl radical that subsequently partitions into two pathways. As Scheme 10 shows, the 4′-radical can react with oxygen as proposed for bleomycin (see Scheme 9), resulting in formation of a 3′-phosphoglycolate-terminated DNA fragment (**9**).[132−134] Presumably base propenal is also produced

Scheme 10. Proposed H-4′-Abstraction Pathway for DNA Strand Scission Mediated by Neocarzinostatin in the Presence and Absence of Thiol (adapted from Frank et al.[107])



but it has not been well-characterized in this system. This pathway appears to be favored when the thiol used to activate neocarzinostatin is negatively charged or large, such as glutathione, with low affinity for DNA. It is thought that an intermediate of neocarzinostatin-mediated strand scission is sensitive to reducing agents and so the phosphoglycolate-forming pathway results from an intermediate that has not been reduced by thiol.[135]

The alternate pathway is also oxygen-dependent, but accounts for products observed in the presence of thiols that are neutral, small, and have high reducing power, such as β-mercaptoethanol.[135] The hydroperoxide is reduced to the alkali-labile hemiacetal **53** that can subsequently decompose to nucleotides with 3′- or 5′-phosphate termini (**13** and **14**, respectively).[132] Partitioning along this pathway results in increased yields of phosphate-terminated fragments and decreased yields of phosphoglycolate-terminated product.[107]

E. Abstraction of H-5′

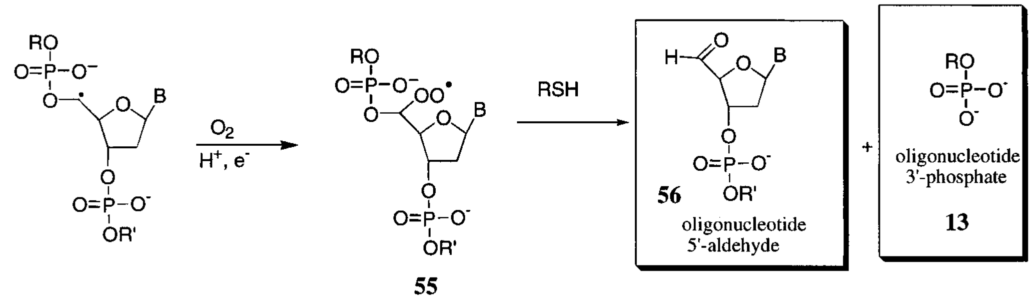
1. General Considerations

The two hydrogen atoms attached to the 5′-carbon also are highly accessible in a B-DNA helix.[28,57] When the sum of the accessible surface areas of both 5′ hydrogens is considered, solvent accessibility is even greater. While both atoms are accessible from the minor groove, it should be noted that one atom points away from the groove toward solvent. Pathways involving abstraction of hydrogen from the 5′-position have been proposed for DNA scission mediated by the enediyne antibiotics,[82,107,133,134] Fenton-generated hydroxyl radicals,[50] gamma radiolysis,[19,118,136] cationic metal porphyrins,[137] and the perhydroxyl radical, •OOH.[138]

2. Neocarzinostatin

In the mechanistic pathway proposed for neocarzinostatin (shown in Scheme 11), a 5′-deoxyribosyl radical reacts with oxygen to form a 5′-peroxyl radical (**55**). Presumably the peroxyl radical is reduced by thiol, and subsequent chemistry yields a DNA strand terminated with a 5′-aldehyde group (**56**) and a DNA strand terminated with a 3′-phosphate group (**13**).[33] The aldehyde product is unique to agents that oxidize the C-5′ position and can serve as a marker for this pathway. This product has been characterized by HPLC and gel electrophoresis.[134] When run on an electrophoresis gel, the aldehyde-terminated DNA strand migrates anomalously slowly, approximately one or two nucleotides slower than the corresponding phosphate-terminated fragment of the same overall length. The aldehyde sugar can be removed by alkali or reduced to an alcohol by sodium borohydride, altering the electrophoretic mobility of the product.[133] It should be noted that in the pathway mediated by cationic porphyrins (discussed below), the 5′-aldehyde sugar is proposed to undergo further degradation.[137] It has been suggested that thiols inhibit this degradation in the neocarzinostatin system.[21] Another mechanism (not shown) proposes reduction of the peroxyl radical to a hydroperoxide that undergoes a Criegee-type rearrangement,[127] producing DNA frag-

Scheme 11. Proposed H-5′-Abstraction Pathway for DNA Strand Scission Mediated by Neocarzinostatin in the Presence of Thiol (adapted from Frank et al.[107])



ments with 3′-phosphate termini and 5′-phosphate termini.[139,140]

3. Cationic Metal Porphyrins

A different type of degraded sugar is observed as a product of 5′ chemistry mediated by cationic metal porphyrins. *meso*-Tetrakis(*N*-methylpyridinium-4-yl)porphyrinatomanganese(III) pentaacetate (Mn-TMPyP, Figure 8), in the presence of an oxygen atom donor such as $KHSO_5$, creates strand breaks in DNA.[137] The complex can abstract either a 1′-H or a 5′-H atom, depending on sequence. The 5′ mechanism appears predominant in poly(dA)•poly(dT) sequences, but less important in mixed-sequence DNA.[137] The proposed pathway of oxidation is shown in Scheme 12. A unique product produced by this pathway is an oxidized sugar bearing an aldehyde, known as furfural (FUR, **59**). The C-5′ radical is hydroxylated by the activated metal porphyrin, either in one step by the activated complex itself, or in two steps via electron transfer, formation of a carbocation, and reaction of the carbocation with water. The hydroxylated intermediate **57** degrades spontaneously, since the hydroxyl group is adjacent to a phosphate, releasing DNA strands terminated with a 3′-phosphate group and DNA strands terminated with a 5′-aldehyde, as observed with strand scission mediated by NCS. However, upon heating, two β-elimination reactions occur. The first reaction eliminates phosphate from the α,β-unsaturated aldehyde, releasing 5′-phosphate-terminated DNA (**14**). The second elimination releases free base and results in the formation of the oxidized sugar FUR (**59**).[137]

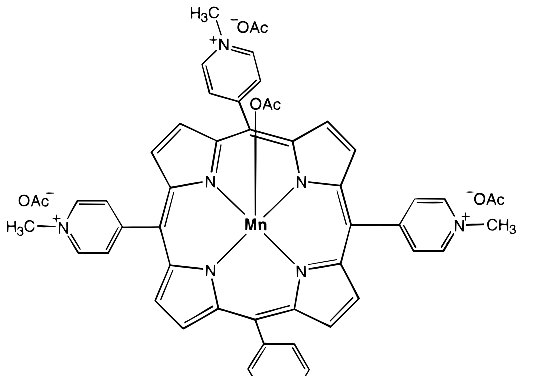


Figure 8. The cationic porphyrin *meso*-tetrakis(*N*-methylpyridinium-4-yl)porpyrinatomanganese(III) pentaacetate (Mn-TMPyP).

Pratviel et al. suggested that FUR was not observed as a product of NCS-mediated DNA cleavage because of the presence of thiols needed to activate the drug.[21]

4. The Stereochemistry of Hydrogen Abstraction by Calicheamicin

The 5′-position of deoxyribose (or ribose) is especially interesting because it is prochiral. Little is known, however, about the stereochemistry of hydrogen abstraction from the 5′-carbon. One report comes from Townsend and co-workers who investigated the cleavage of DNA by the enediyne drug calicheamicin. Using stereospecifically 5′-monodeuterated DNA substrates, they found that the drug abstracted the 5′-*pro-S*-hydrogen nearly exclusively.[106] Since the two 5′-hydrogen atoms are expected to have similar abstraction energies and calicheamicin is known to bind DNA in the minor groove, the preference for the 5′-*pro-S*-hydrogen is probably governed by the structure of the calicheamicin–DNA complex. Stereochemical preferences remain largely unexplored in other systems.

VI. Determining Mechanistic Pathways by Product Formation

Researchers have used a variety of techniques to elucidate cleavage mechanisms, including gel electrophoresis coupled with chemical and enzymatic modification, chromatographic techniques, EPR, ENDOR, NMR, isotopic labeling of oxygen, isotopic labeling of sugars, and synthesis of potential intermediates. Thanks to these efforts, it can now be relatively simple to deduce which hydrogen atom of the deoxyribose is abstracted by a cleavage agent, on the basis of the observation of a particular product. Some of the diagnostic cleavage products are shown in Tables 1 and 2. Table 1 summarizes the products of the chemical systems discussed in the previous section. Table 2 lists unique products that can be used as markers. An example is the 3′-phosphoglycolate-terminated strand (**9**). First identified by Grollman and co-workers as a product of DNA cleavage mediated by activated bleomycin under aerobic conditions (Scheme 9),[105] this terminus is uniquely formed by a mechanism that begins with abstraction of the 4′-hydrogen.[50,71,122,123] Strands terminated by a 3′-phosphoglycolate group are easily detected when radiolabeled DNA products are separated by high-resolution ($\geq$20% polyacrylamide) denaturing gel electrophoresis. This product has slightly greater gel mobility compared to the corresponding 3′-phosphate terminated strand of the same length

Scheme 12. Proposed H-5′ Abstraction Pathway for DNA Strand Scission Mediated by the Cationic Manganese Porphyrin *meso*-Tetrakis(*N*-methylpyridinium-4-yl)porpyrinatomanganese(III) Pentaacetate (Mn-TMPyP) (adapted from Pratviel et al.[137])

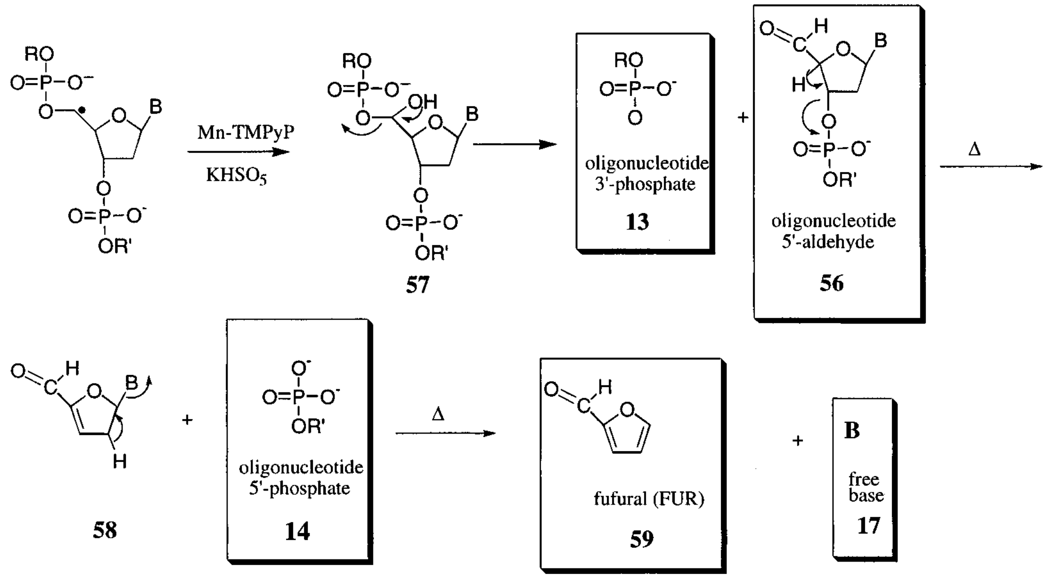


Table 1. DNA Cleavage Products

	Fenton •OH	gamma radiolysis	BLM•Fe(II)	$Cu(OP)_2$	NCS	Ru complexes	cationic metal porphyrins
free base	+	+	+	+	+	+	+
base propenal	+	+	+	+	+[a]	−	−
base propenoic acid	−	−	−	−	−	+	−
nucleotide 5′-phosphate	+	+	+	+	+	+	+
nucleotide 5′-aldehyde	+	−	−	−	+	−	+
nucleotide 3′-phosphoglycaldehyde	−	+	−	−	−	+	−
nucleotide 3′-phosphoglycolate	+	+	+	+	+	−	−
nucleotide 3′-phosphate	+	+	+	+	+	+	+
5-methylene-2-furanone	−	−	−	+	+	−	−
furfural	−	−	−	−	−	−	+
ketone sugars	−	+	−	−	−	−	−

[a] Presumed, but not characterized.

(that is, the same number of nucleotides).[39,47] Consequently the two products often appear as a closely spaced doublet in the image of the gel. Since phosphate-terminated fragments comigrate with products of Maxam−Gilbert sequencing reactions,[54,55,131] the faster-moving phosphoglycolate-terminated product is easy to identify.

Produced in a 1:1 ratio with the 3′-phosphoglycolate-terminated fragment is the product known as base propenal (**10**) (Scheme 9). This product contains a purine or pyrimidine base attached to a propenal moiety. It also has been shown to be a unique product of 4′-hydrogen abstraction, and can be isolated by HPLC or TLC and identified by a colorimetric assay using 2-thiobarbituric acid.[105]

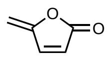
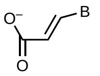
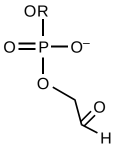
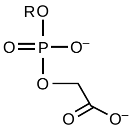
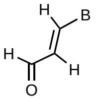
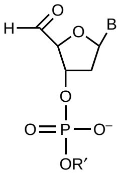
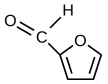
Another unique product is 5-methylene-2-furanone (MEF) (**18**), formed by abstraction of 1′-H. This lactone is a well-known product of $Cu(OP)_2$-mediated DNA cleavage and can be isolated and identified by HPLC,[59] although some researchers have found it to be sensitive to heat.[141]

Base propenoic acid (**33**) is a proposed product of H-3′ abstraction.[101] Produced concurrently with base propenoic acid is a strand that ends with a 3′-phosphoglycaldehyde (**24**). This glycaldehyde-terminated strand has reduced electrophoretic mobility relative to the corresponding phosphate-terminated strand and therefore can be detected by high-resolution gel electrophoresis.[101] Glycaldehydes have also been detected as products of 2′-H abstraction in the photolysis or radiolysis of modified oligonucleotides. However, since the appearance of this product occurs after treatment with alkali, **24** can still be used as a marker of 3′ chemistry that occurs under neutral conditions.

A DNA strand terminated with a 5′-aldehyde (**56**) is a unique product of a cleavage pathway beginning with H-5′ abstraction. On an electrophoresis gel, this product migrates one to two nucleotides more slowly than the corresponding phosphate-terminated strand of the same length.[133] The aldehyde can be reduced to an alcohol by sodium borohydride treatment. The alcohol runs just slightly behind the phosphate-terminated strand of the same length and comigrates with alkaline phosphatase-treated DNA. Alternatively, the aldehyde-bearing sugar can be removed by treatment with alkali, leaving a DNA strand that bears a 5′-phosphate terminus and comigrates with products of Maxam−Gilbert sequencing reactions.[104]

Another unique product of 5′ chemistry is furfural (FUR, **59**), detected as a product of DNA cleavage mediated by the cationic porphyrin Mn-TMPyP[137]

Table 2. Unique DNA Scission Products

marker product		position of hydrogen abstraction	how detected
 **18**	5-methylene-2-furanone	C-1′	HPLC
 **33**	base propenoate	C-3′	HPLC
 **24**	oligonucleotide 3′-phosphoglycaldehyde	C-3′	PAGE
 **9**	oligonucleotide 3′-phosphoglycolate	C-4′	PAGE
 **10**	base propenal	C-4′	reaction with thiobarbituric acid
 **56**	nucleotide 5′-aldehyde	C-5′	PAGE
 **59**	fufural (FUR)	C-5′	HPLC

and believed to be formed in the absence of reducing agents such as thiols.[21] Like MEF (**18**), this oxidized sugar is easily detected by HPLC but may also be sensitive to heat.[141]

VII. Summary

Pathways for strand scission involving abstraction of each of the deoxyribose hydrogens in B-DNA (except for the 2′-hydrogens) have been elucidated. Although all seven hydrogen atoms of deoxyribose are believed to be individually reactive toward free radicals, not all have equal probability of being abstracted from duplex nucleic acids. The pathway of cleavage is dependent on the helical structure of the nucleic acid and the orientation of the oxidant relative to the sugar. Molecules that bind to the minor groove of DNA as well as nonselective radical species appear to show the most oxidation at the 5′- and 4′-positions of deoxyribose in B-form DNA. These positions are the most accessible to solvent, are adjacent to an oxygen atom, and can be accessed from the minor groove. The single major groove-binding molecule discussed here cleaves DNA by pathways involving the 3′-hydrogen. This atom can be accessed through the major groove of B-DNA.

All of the DNA-cleaving molecules described initiate at least one pathway that results in production of DNA strands with phosphate termini. These products are easy to detect and measure because of their ubiquity, but their presence does not indicate much about the chemistry of their formation. More incisive as a marker of certain pathways is the detection of certain modified sugars at the site of a strand break.

Another important point is that not all oxidation events lead to immediate strand cleavage. Several pathways result in formation of metastable sugars or sites that are susceptible to base-catalyzed hydrolysis. Examples are abasic deoxyribose residues and situations in which a phosphate group is adjacent to (or in the β-position relative to) an alcohol. In these situations a strand break occurs after treatment with heat and high pH.

Once a deoxyribosyl radical is formed, it can partition along pathways dependent on external oxidants or reductants, oxygen, thiol, or pH. Dioxygen will react with a carbon-centered radical at diffusion-limited rates.[142] Peroxidation of carbon radicals is extremely important, for such radicals may facilitate dimerization of sugars or may commit a hydrogen abstraction event to a pathway that ultimately results in strand cleavage, resulting in a sensitizing effect. In the absence of oxygen, processes in which the sugar radical abstracts a hydrogen atom from another species can compete with strand cleavage. The effect of oxygen may be especially important when DNA damage in cells is considered. The cell nucleus is known to be poorly oxygenated. Mitochondrial DNA, however, located within an environment in which oxygen is actively metabolized, might be more susceptible to the effects of oxygen and suffer damage mediated by aerobic pathways. Cancer cells are often poorly oxygenated,[143] particularly within the dense interior of tumors, and this fact may explain why these cells are sometimes more resistant to oxidative DNA cleavage compared to normal cells. Anaerobic pathways of damage might predominate under these conditions.

Thiol, if present, may add hydrogen to a sugar radical and repair the original damage. Alternatively, thiol can reduce certain intermediates of a scission process, resulting in partitioning between pathways. The concentration of reducing thiols, such as glutathione, is known to vary from cell to cell and is altered in certain disease states.[144] Thiols, then, may have significant influence on reaction pathways after hydrogen atom abstraction.

Such observations have been put to use in the development of synthetic nucleases for specific purposes. By attaching a DNA-cleaving complex to an oligonucleotide or other DNA-binding molecule, researchers have been able to target specific sequences of DNA for cleavage. (For examples, see works by Sigman, Dervan, and others.[145−149]) Mechanistic information has also been immensely useful in interpreting not only ligand−DNA footprints, but experiments in which DNA cleavage is used to probe biomolecular structures. Strand scission reactions have been used in the study of structures such as bent DNA, four-stranded DNA junctions, RNA, and DNA•RNA hybrids as well as numerous protein-DNA and drug−DNA complexes. (For representative works, see refs 4, 5, 89, and 150−152.) Knowledge of cleavage mechanisms can greatly facilitate the interpretation and extend the amount of information available from these experiments.[101]

The elucidation of degradation mechanisms and the ability to use reaction products as diagnostic tools have greatly advanced our understanding of nucleic acid-cleaving molecules. Knowledge of the atom at which a small molecule attacks DNA or RNA and the pathway by which the damage occurs may, in some cases, be known simply by analysis of reaction products. The many efforts put forth to elucidate these pathways will undoubtedly be useful as researchers design new synthetic nucleases to target genes and fine-tune the chemistry of currently used antibiotics and chemotherapeutic agents.

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