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JULY 1991

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Mechanism of Neocarzinostatin Action: Role of DNA Microstructure in Determination of Chemistry of Bistranded Oxidative Damage

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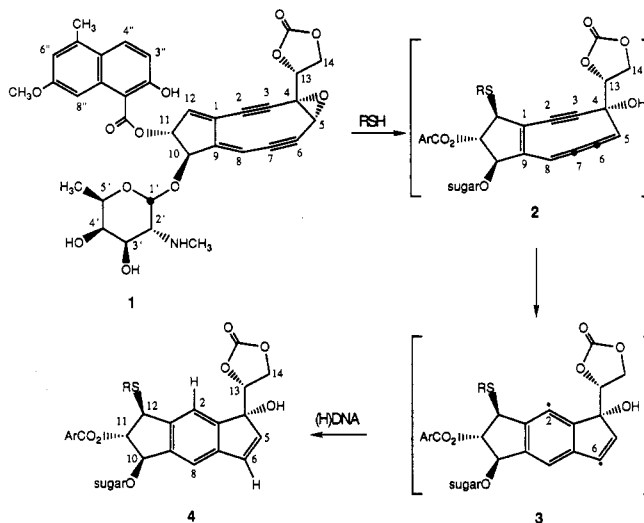
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Received March 6, 1991 (Revised Manuscript Received May 30, 1991)

DNA is the biological target of a number of antitumor antibiotics. DNA structure and function can be altered by either physical or covalent interactions between such agents and sites on the DNA that offer a particular kind of geometry and/or functionality.¹ Covalent complexes generally consist of adducts formed on the bases of the DNA; a smaller number of agents attack DNA deoxyribose, resulting in the formation of direct strand breaks and alkali-labile breaks at abasic sites. Among the latter are the bleomycin and bicyclic enediyne families of antitumor antibiotics. Bleomycin,^{1f} like another DNA-damaging agent, ionizing radiation, causes chemical changes in DNA deoxyribose by way of some form of reactive oxygen, drug-bound in the case of bleomycin and freely diffusible hydroxy free radicals in the case of ionizing radiation. By contrast, antibiotics containing the unprecedented bicyclic enediyne structures are themselves converted into diradical species that attack DNA deoxyribose, with dioxygen playing a secondary role in the expression of the oxidative damage.^{1e,2} These agents are proving to be useful probes of DNA microstructure. Furthermore, elucidation of the chemistry of DNA damage has uncovered novel mechanisms that have implications for their use as potent cancer

Irving H. Goldberg received his M.D. degree from Yale in 1953, after his B.S. from Trinity College in 1949. Following clinical training at the Columbia-Presbyterian Medical Center in New York, he earned his Ph.D. in 1960 under Professor F. Lipmann at the Rockefeller University. After an academic appointment at the University of Chicago, he moved in 1964 to the Harvard Medical School, where he is the Otto Kraymer Professor in the Department of Biological Chemistry and Molecular Pharmacology. At Harvard he has served as Chairman of the Division of Medical Sciences and Chairman of the Department of Pharmacology. For 30 years his research has focused on the molecular mechanisms of action of antitumor antibiotics that alter DNA and ribosome structure and function. His work with actinomycin D in the early 1960s provided the first data showing that such agents act by forming specific complexes with DNA.

Scheme I
Proposed Mechanism of NCS-Chrom Activation and Action



chemotherapeutic agents and also has shed light on the chemistry of nitroaromatic radiation sensitizer action. In addition, an unusual mechanism of mutagenesis has been uncovered.

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Neocarzinostatin Structure and Activation

The first of the bicyclic enediyne antibiotics to be discovered was neocarzinostatin (NCS).³ This agent was initially identified as a simple protein ($M_r = 11\,000$) antitumor antibiotic with the ability to inhibit DNA synthesis and induce the degradation of DNA in cells.⁴ It was later shown that DNA strand breakage was a primary effect and that this reaction took place in vitro, provided that a cofactor, such as thiol, was included.⁵ It was, however, not until almost 15 years after its discovery that it was appreciated that the biological activity of NCS was not due to the protein but rather to a previously unrecognized tightly, but noncovalently, bound ($K_D \sim 10^{-10}$ M), labile nonprotein chromophore (NCS-Chrom).⁶ The apoprotein contains a hydrophobic cleft where NCS-Chrom is believed to reside and is protected from degradation.⁷ Elucidation of the structure of NCS-Chrom resulted from the efforts of several laboratories.⁸ The structure (1) shown in Scheme I, based on the proposal of Edo et al.^{8f} with the stereochemical assignments of Myers et al.,⁹ has received support from biosynthetic studies¹⁰ and chemical synthesis.¹¹ At the time, the structure of NCS-Chrom was unique for a natural (and synthetic) product in possessing a bicyclic ring system containing two acetylenic bonds in the nine-membered ring. The major species (form A) consists of three subunits: a 5-methyl-7-methoxynaphthoate, a 2,6-dideoxy-2-(methylamino)galactose moiety, and an interconnecting C₁₂ subunit bearing a cyclic carbonate and an epoxide. The C₁₂ subunit consists of a novel, highly strained bicyclic [7.3.0]dodecadienediyne system.

Early studies on the mechanism of action of NCS-Chrom, prior to elucidation of the enediyne character of its structure, suggested that the active form of the drug was a radical species.^{12,13} It was found that

thiol-activated (or borohydride-activated) NCS-Chrom abstracted ³H into the drug from C-5' of deoxyribose of thymidylate residues in DNA.¹³ These and other experiments, to be described later, led to the proposal that the nucleophilic addition of thiol to NCS-Chrom converted the drug into a diradical species responsible for hydrogen atom abstraction from the DNA sugar.^{13b} Elucidation of the NCS-Chrom structure and analogy with the Bergman-type aromatization¹⁴ of esperamicin/calicheamicin¹⁵ led Myers,¹⁶ in assigning the structure of the chromophore-thiol adduct from the ¹H NMR and MS data of Hensens et al.,^{8c} to propose the mechanism for NCS-Chrom activation shown in Scheme I. In this mechanism nucleophilic attack by thiol at C-12 and epoxide ring opening generate a cumulene intermediate (2),¹⁷ which cyclizes to form the indacene diradical with radical centers at C-2 and C-6 (3). The bicyclic dienediyne core and a leaving group at C-5 (epoxide or chloro group)¹⁸ are required for diyl formation. Presumably, the cyclic nature of the enediyne structure facilitates aromatization at low temperatures. Reaction with thiol involves β -face attack at C-12, trans to the naphthoate at C-11.¹⁹ The diradical then abstracts hydrogen either from DNA to form the reduced chromophore (4) or from some other source to form the same product. Support for this formulation comes from experiments showing that in the absence of DNA deuterium is incorporated from borodeuteride in deuteriated solvent into C-12, C-6, and C-2 of NCS-Chrom, whereas in its presence deuterium is incorporated only into C-12 and hydrogen into C-6 and C-2.²⁰ These results indicate that DNA is the source of hydrogen atom donation to the two radical centers at C-6 and C-2 of NCS-Chrom.

Although thiol stimulates DNA damage by greater than 1000-fold, NCS-Chrom does cleave DNA in the absence of thiol, and this reaction is favored at more acidic pH's;²¹ possibly acid-induced opening of the epoxide is the initiating event. Recently, evidence has been obtained for another mechanism of NCS-Chrom aromatization. It was found that the aerobic treatment of NCS-Chrom with a very low concentration of thiol (methyl thioglycolate, 1.5×10^{-2} mM) in methanolic acetic acid leads to the formation (10% yield) of the indacene 12-oxo derivative.²² It was proposed that the ketone derivative is produced by hydroperoxy radical (formed by reaction of dioxygen and thiol) attack at C-12 of NCS-Chrom. In the process of hydroperoxide homolysis of the radical intermediate, there is an in-

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tramolecular shift of the benzylic hydrogen at C-12 to C-2.

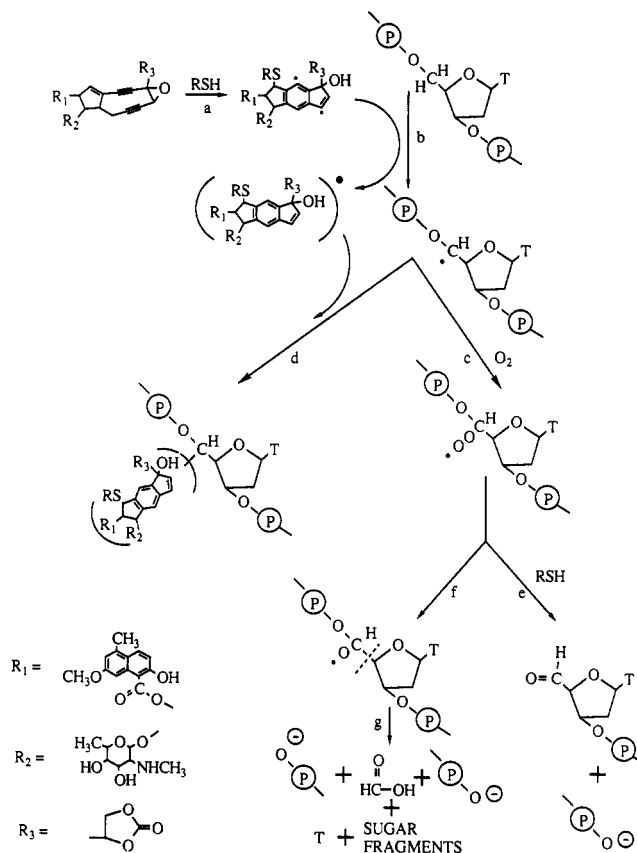
The source of the hydrogen atoms abstracted by the activated drug into C-2 and C-6 in the absence of DNA appears to be complex and raises some interesting issues. Earlier experiments at low ratios of thiol to drug (2–5 equiv) failed to reveal incorporation of exchangeable deuterium from solvent into the chromophore.^{8a,c,20} In contrast, with greatly increased thiol (methyl thioglycolate) concentrations (300 equiv), significant (>80%) deuterium was incorporated at C-2 and C-6, in addition to the formation of a 6,12-dithiol adduct.⁹ In a subsequent study the amount of deuterium incorporated from solvent was shown to vary with the ratio of thiol to drug and with the source of the carbon-associated hydrogen.¹⁷ Since in the earlier experiments^{8a,c,20} all possible sources of hydrogen atom donation were eliminated except for the nonexchangeable hydrogens of the thiol, it was suggested that one of the carbon-bound hydrogens of glutathione (either that α to the sulfur or one on an NH_2 -substituted carbon) might be the source of the hydrogen incorporated into the chromophore.²⁰ The transfer of a carbon-bound hydrogen atom from the adducted thiol or from the C-12 position to the radical center at C-2 could account for the predominance of single-strand (SS) over double-strand (DS) lesions, since under these conditions a bifunctional molecule would be converted into a monofunctional one. This could also explain why C-5' attack by C-6, not C-2, is the main mechanism of SS break formation.

Interaction with DNA

NCS-Chrom binds to DNA in a two-step process.²³ The first involves external binding, and the second, intercalation of the chromophore between adjacent DNA base pairs. NCS-Chrom binds duplex DNA with $K_D \sim 10^{-6}$ M, with an overall preference for DNA rich in T and A residues.^{6c} The physical structure of DNA is altered by drug intercalation so that the helix unwinds by 21° and the molecule is lengthened by 3.3 Å for each chromophore molecule bound.²⁴ Electric dichroism measurements show that the naphthoate portion of the chromophore is oriented approximately parallel to the DNA bases, in accord with its being the intercalating moiety.²⁴ The chromophore binds in the minor groove of B-DNA, as bulky moieties in the major groove of DNA fail to interfere with NCS-Chrom binding, whereas minor groove specific agents, such as the antibiotics netropsin and distamycin, block drug binding.²⁵ These studies led to the proposal of a model for NCS-Chrom-DNA complex formation in which the active portion (enediyne) of NCS-Chrom is positioned in the minor groove of DNA by intercalation of the naphthoate moiety and electrostatic interaction of the positively charged amino sugar moiety with the negatively charged sugar phosphate backbone of the DNA.²⁶

The initial work on *in vitro* DNA scission showed that NCS-Chrom produced primarily SS breaks and there was *base* but little clear-cut *sequence* specificity.²⁷

Scheme II
Proposed Mechanism of NCS-Chrom-Induced Damage at C-5' of Deoxyribose of Thymidylate in DNA



About 75% of the breaks were at T residues ($T \gg A \gg C > G$). The break at a T residue consisted of a DNA fragment with 3'-phosphate and 5'-thymidine 5'-aldehyde ends.²⁸ This lesion resulted from the selective abstraction of a hydrogen atom by the activated drug from C-5' of thymidylate in DNA (Scheme II, reaction b).¹³ Using $^{18}\text{O}_2$, it was found that the oxygen of the 5'-aldehyde is derived entirely from dioxygen, not H_2O .²⁹ This result is compatible with reaction c, in which dioxygen adds to the carbon-centered radical at C-5' to form a peroxy radical species that, following reduction by thiol, results in the formation of nucleoside 5'-aldehyde and strand break (reaction e). This mechanism accounts for more than 80% of the breaks generated by NCS-Chrom as the result of 5' chemistry. It is consistent with kinetic studies³⁰ showing that the initial reaction of the chromophore with a single molecule of thiol (reaction a) occurs in the absence of dioxygen and that drug activation is rapidly followed by the uptake of 1 mol of O_2 /mol of chromophore and the subsequent utilization of at least an additional sulfhydryl group, which occurs only in the presence of dioxygen.

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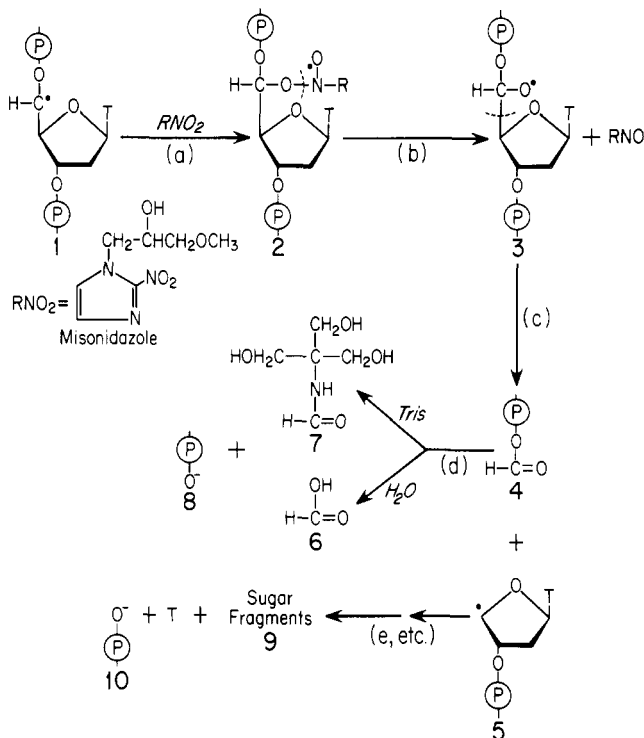
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Scheme III
Proposed Mechanism of Involvement of Misonidazole
(RNO₂) in NCS-Chrom-Induced Damage at C-5'



Fewer than 20% of the breaks are actually single-nucleoside gaps with phosphate at each end.^{28b} The mechanism of generation of this lesion requires further clarification, although the observed products are compatible with reactions f and g (Scheme II), in which the peroxy radical species converts to an oxy radical, which undergoes β -fragmentation with cleavage between C-4' and C-5' to generate at 3'-(formyl phosphate)-ended fragment.³¹ It is not obvious, however, how the peroxy radical intermediate converts to the oxy radical species. Although simple peroxy radicals can dimerize to form tetraoxide intermediates that undergo Russell fragmentation to lose dioxygen and produce alkoxy radicals,³² steric constraints in the case of DNA make this scenario problematic. The 3'-(formyl phosphate)-ended DNA fragment, an energy-rich formyl donor, either spontaneously hydrolyzes to form formate and a 3'-phosphate-ended fragment (reaction g) or donates its formyl moiety to an available nucleophile.^{31,33} The four-carbon product derived from the deoxyribose has recently been identified.³⁴ Kawabata et al.³⁴ have proposed that a Criegee-type rearrangement of a hydroperoxide intermediate at C-5' results in the cleavage between C-4' and C-5'. It is difficult, however, to invoke a Criegee-type mechanism in the absence of metal involvement or acidic conditions,^{1f} unless the methyl-amino group of the chromophore sugar serves as a proton donor.

The oxy radical mechanism discussed above is analogous to that shown likely to occur when the ni-

troimidazole compound and radiation sensitizer, misonidazole, substitutes for dioxygen in NCS-Chrom-induced DNA damage (Scheme III).³¹ Single-nucleoside gaps with phosphate moieties at each end are the main lesions under these conditions.³⁵ Despite the difference in the relative distribution of the final damage products, abstraction of a 5'-hydrogen atom by the thiol-activated drug to form a carbon-centered radical on C-5' of deoxyribose is a common initial step in both the dioxygen- and misonidazole-dependent reactions. With misonidazole the formation of formate or its derivative (following transfer to available nucleophiles) is the major reaction, and the nitroaromatic compound appears to undergo reduction of the nitro group to the nitroso level.³¹ This reaction is dependent on the presence of DNA, indicating the involvement of a nascent form of DNA damage in the process and ruling out a direct action of activated NCS-Chrom on the misonidazole to generate a species that reacts with the DNA.

The above results suggest a mechanism (Scheme III)³¹ in which the carbon-centered radical at C-5' (1), generated by hydrogen atom abstraction by activated NCS-Chrom, reacts with the nitro group of misonidazole (RNO₂) (reaction a) to form a nitroxide radical adduct intermediate (2). Precedence for the formation of such intermediates comes from studies on the addition of carbon-centered radicals to the oxygen of the nitro group of tetranitromethane and nitrobenzenes.³⁶ The ease of formation of such adducts depends on the one-electron redox potential of the nitro compound,³⁶ in agreement with the results showing a similar relationship for NCS-induced DNA damage.³⁵ The adduct can undergo a fragmentation reaction³⁷ (reaction b) to form an oxy radical (3) and the nitroso reduction product of misonidazole, a two-electron process. Oxy radicals undergo β -fragmentation reactions,³² resulting in cleavage between C-5' and C-4' (reaction c) to form 3'-(formyl phosphate)-ended DNA (4) and other fragments (9) from the remaining four carbons of deoxyribose (5). Formyl group transfer from the labile (formyl phosphate)-ended DNA (reaction d) results in the formation of a gap with phosphates at both ends (8 and 10). Strong support for this novel mechanism, involving cleavage of the oxygen-nitrogen bond, comes from ¹⁸O studies in which the carbonyl oxygen of the formate was shown to come exclusively from the nitro group oxygen of misonidazole.³⁸

Under anaerobic conditions there is little, if any, DNA strand breakage; instead the radical at C-5' of deoxyribose interacts with the bound chromophore to form a covalent drug-DNA adduct (Scheme II, reaction d).³⁹ Adducts on deoxyribose of DNA had not been observed before; clearly, in order to form such a product, one of the carbons of deoxyribose must first be "activated". By mapping of the adducts as exonuclease termination sites in defined-sequence DNA, it has been

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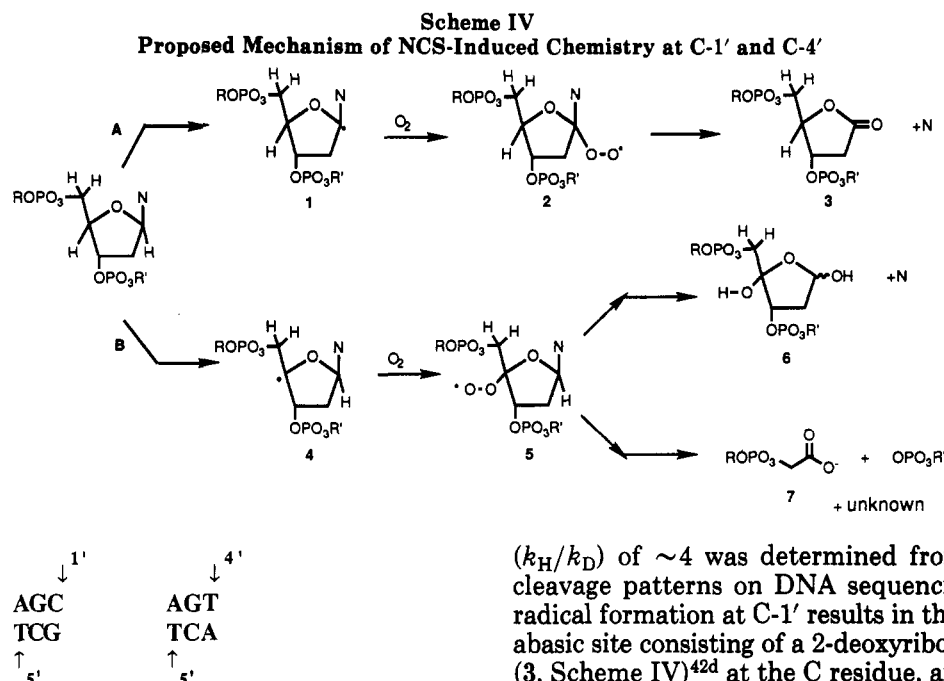


Figure 1. Chemistries of sequence-specific bistranded lesions.

shown that stable adducts occur with the same base specificity as strand-break formation.⁴⁰ It appears that the carbon-centered radical on deoxyribose is a precursor of both strand breaks and adducts, with dioxygen and DNA-bound drug competing for addition to the sugar.

Sequence-Specific Bistranded DNA Damage

Whereas SS breaks are due mainly to 5' chemistry, DS lesions (DS break or abasic site with a closely opposed SS break) also involve other chemistries (1' or 4'). It is likely that a single molecule of the diradical species of NCS-Chrom reacts in a concerted fashion with appropriately situated deoxyribose moieties on both strands of the DNA so as to generate carbon-centered radicals at C-5' and C-1' or C-4'. The DS lesions are sequence-specific and more important than SS lesions in terms of mutagenicity and cytotoxicity. The SS breaks are rapidly repaired, whereas persistent DS breaks result in cell-killing.⁴¹

The first DS lesion to be identified involves 1' chemistry (Scheme IV, pathway A) at the C residue of the sequence AGCGCT and 5' chemistry at the T residue two nucleotides to the 3'-side on the complementary strand (Figure 1).⁴² This bistranded lesion has been shown to be responsible for GC to AT transitions in λ phage and *Escherichia coli*.⁴³ Direct evidence for hydrogen atom abstraction from C-1' comes from experiments in which deuterium replaced hydrogen at C-1' of the C residue.^{42e} A deuterium isotope selection

(k_H/k_D) of ~ 4 was determined from alkali-induced cleavage patterns on DNA sequencing gels. Peroxy radical formation at C-1' results in the formation of an abasic site consisting of a 2-deoxyribonolactone moiety (3, Scheme IV)^{42d} at the C residue, and that at C-5' of the T residue eventuates in a strand break with thymidine 5'-aldehyde formation (Scheme II, reaction e). Because every abasic site at the C residue is accompanied by a direct break at the T residue on the complementary strand,^{42c} it is likely that it occurs as part of a bistranded lesion resulting from the concerted action of the two radical centers at C-2 and C-6 of a single NCS-Chrom molecule. However, because there are more strand breaks at the T residue than abasic sites at the C residue,^{42b,44} it is clear that some strand breaks are not part of a bistranded lesion. Dioxygen (or misonidazole) is required in the formation of both the abasic site and the direct strand break.

The nature of the thiol used as the activator/reductant is critical in determining the extent of formation of the bistranded lesion; glutathione is superior to 2-mercaptoethanol or dithiothreitol.^{42a,b} As noted later in the discussion of DS breaks, the influence of the structure of the thiol in determining the extent of bistranded damage is a recurring theme. Since the size, shape, and charge of the activated drug will differ depending upon the particular thiol, these differences might be expected to result in change in binding to the DNA minor groove, as well as in the orientation of the diradical species for attack on the DNA deoxyribose.

The importance of DNA microstructure in the generation of the abasic site at the C residue is further indicated by the finding that substitution of an I residue, which lacks a 2-amino group on the base, for the G residue of AGC markedly reduces abasic site formation, whereas placement of an I residue opposite the C residue enhances (4–5-fold) abasic site formation in oligodeoxynucleotides.^{42b} Interestingly, the latter base replacement eliminates the deuterium isotope effect on abasic site formation at the C residue.^{42e} This is the expected result, if the enhanced reaction is due to an increase in the relative rate of hydrogen atom abstraction by the activated drug versus its dissociation from the DNA. Further, such a consideration may be

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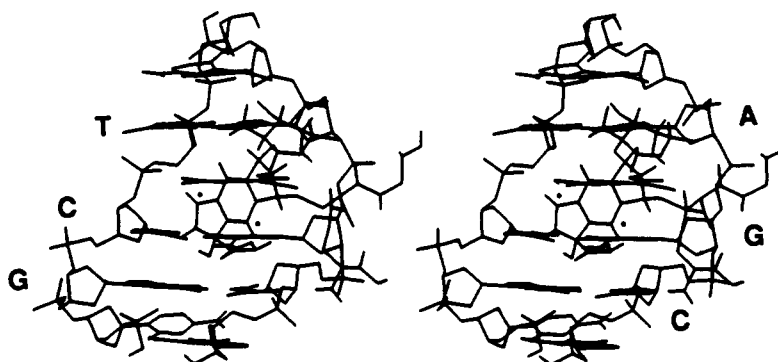


Figure 2. Stereodrawings of optimized structure of reacted form of NCS-Chrom complexed with AGC-GCT-containing oligodeoxynucleotide. C-2 and C-6 of NCS-Chrom are labeled with dots. Glutathione is attached to C-12. Modified after Galat and Goldberg.⁴⁶

the basis for the significant variation in isotope effects observed at different sites in the DNA, in particular, for that involving chemistry at C-5', where the values vary from 1.0 to 2.6.^{42a,45}

Molecular model building based on energy minimization and molecular dynamics simulations has led to a proposal for the activated NCS-Chrom-AGC-GCT complex in which the radical center at C-6 abstracts a hydrogen atom from C-5' of the T residue (3.31 Å) and C-2 abstracts a hydrogen atom from C-1' of the C residue (4.10 Å) (Figure 2).⁴⁶ The naphthoate moiety of NCS-Chrom is intercalated between the A:T and G:C base pairs, and the positively charged amino sugar interacts with the negatively charged sugar phosphate backbone so as to place the indacene diradical in the DNA minor groove, projecting toward the 3'-end of the (+) strand. The intercalated naphthoate adds the equivalent of a base pair to the interaction site so that separation of the lesions on each strand by two rather than three (as with the nonintercalating calicheamicin)^{2a} base pairs represents the shortest distance across the DNA minor groove. The glutathione adduct at C-12 of the chromophore forces the radical center at C-2 deeper into the minor groove so as to facilitate attack at C-1' of the C residue, perhaps accounting for the increased formation of the abasic lesion with this thiol. To corroborate the model and to clarify further the underlying mechanism, the C-5' position of the T residue was labeled with deuterium, and its incorporation into the spent NCS-Chrom was analyzed by ¹H NMR.⁴⁴ These studies showed that the deuterium was incorporated only into C-6 of the chromophore, as predicted. Of the two prochiral hydrogen atoms at C-5' of deoxyribose, it is expected that H_β, the one projecting into the minor groove, is the one abstracted by the drug.

The finding that direct cleavage at the T residue exceeds abasic site formation at the C residue, however, raises the possibility that deuterium abstraction from C-5' of the T residue by the radical center C-6 is in part due to a different mode of drug-DNA binding that leads only to direct SS breaks. The number of SS breaks that are not part of a bistranded lesion decreases dramatically when an I residue is placed opposite the C of AGC (S. M. Meschwitz and I. H. Goldberg, un-

published data), consistent with the increase in abasic lesions at the C residue that are part of a bistranded lesion. The SS break lesion could be due either to a different binding mode or to the same mode that leads to the bistranded lesion but with abasic site formation being less efficient for either chemical or geometric reasons. If a different energetically allowable binding mode is involved in SS break formation, such as intercalation at the G:C:C:G step with the diradical core extending toward the 5'-end of the (+) strand,⁴⁶ it is still necessary that C-6 of the activated drug attack C-5' of the T residue, because there is no detectable deuterium incorporation at C-2.⁴⁴ In this case the diradical core would have to be rotated so that it is almost perpendicular to the helix axis, resulting in a distance of 3.47 Å between C-6 of the drug and C-5' of the T residue.

Until recently there was considerable uncertainty as to the mechanism involved in the formation of DS breaks. It had been found that in mammalian cells the ratio of SS to DS breaks was on the order of 5:1,⁴¹ but when DNA strand cleavage was characterized in vitro, the ratio was 30:1 to 50:1.⁴⁷ The latter value gave rise to the conjecture that DS breaks result from the random placement of SS breaks at closely opposed sites and that DS breakage as a discrete event occurred rarely, if at all. The paradox existing between the in vivo and in vitro data was resolved once it was appreciated that the difference in ratios of SS:DS breaks might be related to the fact that glutathione is the thiol in vivo and 2-mercaptoethanol was used in vitro. In fact, when glutathione was used in vitro, the SS:DS ratio was found to be 6.1.^{47b} Further, the number of DS breaks increased 7-fold. Analysis of a number of NCS-treated restriction fragments for DS cleavage sites revealed that DS breaks were sequence-specific and that most of the lesions occurred at the T residue of a GT step.

Analysis of the DS breaks showed predominantly a two base pair stagger to the cleavage site and showed that sequences, in particular AGT·ACT, containing a GT step are especially good DS cleavage sites.^{47b} The break at the T residue of AGT involved mainly (75%) 4' chemistry (Scheme IV, pathway B) with the formation of a 3'-glycolate-ended DNA fragment (7), whereas at the T residue of ACT at least 80% of the breaks were due to nucleoside 5'-aldehyde formation, the result of

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oxidative attack at C-5' of deoxyribose (Figure 1). The involvement of different chemistries at the staggered sites of the DS break is analogous to the bistranded lesions found at AGC-GCT and indicates that each radical center on NCS-Chrom reacts with C-4' of AGT or C-5' of ACT to produce DS breakage. In fact, it has recently been found that C-6 of the chromophore selectively abstracts deuterium from C-5' of ACT (S. M. Meschwitz and I. H. Goldberg, unpublished data). The location of these sites in the minor groove of B-DNA makes them accessible for attack by the bifunctional chromophore.

In contrast to DS break sites, analysis of SS breaks generated at the T residue of AGT revealed a near absence of 3'-glycolate-ended fragments.^{47b} This result is consistent with a model for SS cleavage in which a slightly different orientation of the bound drug attacks monofunctionally at C-5' of this T residue, rather than at nearby C-4'. This subtle difference in binding geometry might depend on the nature of the activating thiol. It is also possible that SS and DS breaks are due to two different binding modes, perhaps involving different sites of intercalation. Finally, it should be noted that partitioning of the 4' chemistry (Scheme IV) at the T residue of the AGT also leads to formation of the abasic 4'-hydroxylation product (6); this lesion, accompanied by the break at the T residue on the opposite strand, generates a bistranded lesion akin to that at AGC-GCT.

Evidence that NCS-Chrom can attack C-4' of T residues in DNA (Scheme IV, pathway B) first came from studies by Saito et al.⁴⁸ showing the formation of the 4'-hydroxylation abasic product (6), but very little, if any, 3'-phosphoglycolate (7) (<3%) with the self-complementary hexamer CGTACG. DNA restriction fragments and synthetic oligonucleotides containing the sequence TGT were used to obtain evidence for the partitioning of a 4'-peroxy radical intermediate (5) between 6 and significant amounts of 7 at the T residue of the GT step.⁴⁵ This partitioning is dependent on the thiol used as activator/reductant. Acidic thiols produce more glycolate product, and 4-hydroxythiophenol, the thiol used by Saito et al.,⁴⁸ produces little, if any, glycolate product and results in very few DS breaks (P. C. Dedon, Z.-w. Jiang, and I. H. Goldberg, unpublished data). The involvement of 4' chemistry was confirmed by the demonstration of a deuterium isotope effect ($k_H/k_D \sim 4$) at C-4' for the formation of both 6 and 7, suggesting a common precursor for both products.^{45b} Further, the partitioning between abstraction of either a 4'- or 5'-hydrogen was found to be modulated by deuteration at either position, indicating a shuttling between the two closely situated attack sites based on isotope selection effects. The steps involved in the partitioning of the 4'-peroxy radical intermediate remain to be elucidated, since, unlike with bleomycin,^{1f} dioxygen (or misonizadole) is required in the formation of the 4'-hydroxylation product, as well as the phosphoglycolate-ended fragment. Recent preliminary experiments (L. S. Kappen and I. H. Goldberg) show that, as in the case of bleomycin, the product designated "unknown" in Scheme IV is base propenal.

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Concluding Remarks

NCS-Chrom is endowed with unprecedented structural properties that enable it to aromatize to a diradical under mild conditions (0 °C) in aqueous solution in the presence of levels (2-5 mM) of the cellular thiol, glutathione, that exist in mammalian cells. In fact, the mechanism of its action in cells appears to be the same as that in vitro, as depletion of cellular glutathione results in lower toxicity,⁴⁹ mutagenesis,⁴⁹ and DNA strand breakage.⁵⁰ Also, the products of DNA deoxyribose damage and the pattern of strand breakage appear to be similar.⁵⁰

The ability of NCS-Chrom to act as a sequence-specific bistrand-reactive agent derives from its DNA binding moieties and the bifunctional nature of its active species. Elucidation of the role played by the adducted thiol in complex formation with DNA will require separation of the activator from the reductant properties of the nucleophile. The precise chemistry carried out on each strand from the DNA minor groove, however, is determined to a considerable extent by the DNA microstructure at the sites of reaction. Hydrogen atoms at C-5', C-1', and C-4' are all readily accessible to the diradical lying in the minor groove,⁴⁶ but it is, to a considerable degree, the local geometry that determines whether SS or DS lesions ensue and whether C-1' or C-4' is a component of the bistranded lesion. The two types of bistranded lesions result from different chemical mechanisms, depending on whether a C or a T residue is 3' to a G residue. Further, the omission of a 2-NH₂ group from either strand at the site of interaction has profound effects on DS lesion formation. This intimate relationship between DNA microstructure and the chemistry of NCS-Chrom-induced damage makes this agent a potentially useful probe in the study of the nature and function of particular DNA microstructures, such as the extremely narrow and deep minor groove found at a GT step.⁵¹ It must be emphasized, however, that whereas such effects are usually perceived as being sequence-related, it is the local *geometry* of the formed complex that is the critical determinant. In fact, it has been found that cleavage of DNA by NCS-Chrom is especially prominent immediately 3' to a single-base bulge on the opposite strand, *independent of the sequence involved*.⁵²

The use of NCS-Chrom as a specific reagent for generating deoxyribose radical species has made it possible for the first time to perform, on the nitroaromatic compounds, mechanistic studies that have implications for their actions as radiation sensitizers. Misonizadole substitutes for dioxygen, presumably forming, at each of the carbon-centered radicals generated on the deoxyribose by NCS, a nitroxide radical adduct species that undergoes oxy radical formation in a manner similar to that shown in Scheme III for C-5'. Due to the complexity of the lesions induced in DNA by ionizing radiation, involving both base (80%) and sugar (20%) moieties,³² efforts to elucidate its mechanism in this system have met with limited success.⁵³

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Finally, the capacity of NCS-Chrom to generate bistranded lesions with an abasic site (1' or 4' chemistry) on one strand and a direct strand break (5' chemistry) two nucleotides to the 3'-side on the complementary strand results in an unprecedented type of premutagenic lesion.⁴³ The mutagenicity of the abasic site is probably significantly enhanced by the break on the opposite strand. Because of the loss of the duplex character of the DNA at the lesion site, cellular apurinic/apyrimidinic endonucleases appear to be less effective in removing the abasic lesion,^{42a} so that repair

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of the strand break likely occurs first. This process results in the insertion of a wrong base opposite the noncoding abasic site. With the reestablishment of the duplex structure, the abasic site is removed but replaced by a base determined by the mutagenized lesion on the complementary strand. Uniquely, mutagenesis occurs entirely during the repair process in the absence of DNA replication.

I thank my associates, whose names appear in the original articles on which this account is based, for their invaluable contributions to this research effort. This work was supported by Grants CA 44257 and GM 12573 from the National Institutes of Health.

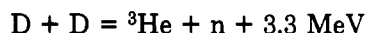
Fusion Reactions in Dense Hot Atom Assemblies Generated by Cluster Impact[†]

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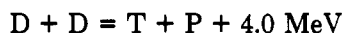
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Received June 1, 1990 (Revised Manuscript Received May 13, 1991)

The nuclear physics of D-D fusion reactions is well understood. The reaction products and the energy liberated have been well characterized. Rates of reaction have been shown to depend on rates of tunneling through the coulomb barrier between the reactant deuteron nuclei. The reactions under consideration are



and



The problem of finding conditions suitable for the practical production of energy from thermonuclear fusion processes has, with the exception of uncontrolled reactions in thermonuclear weapons, so far not been solved. Extensive efforts to develop techniques of magnetic or inertial confinement of dense energetic assemblies of fuel atoms have demonstrated the need for much more research. Additional information is needed in the area of condensed matter science for the solution of the fusion energy problem. An understanding must be developed of the properties of dense systems of atoms capable of sustaining fusion reactions in burning processes analogous to chemical combustion processes. To achieve the energy density and particle density required for ignition of the fusion burning process, matter must be compressed and heated to densities and "temperatures" orders of magnitude larger

than those required for chemical combustion.

To appreciate the difficulty of igniting controlled fusion reactions, one should consider the magnitude of the energy and particle densities needed. The power density required for inertially confined fusion is estimated at about $2 \times 10^{14} \text{ W/cm}^2$.¹ But the delivery of energy to an assembly of fuel atoms is not a sufficient condition for the ignition of thermonuclear reactions. The atoms must be in a state of high density, and most of the available energy must be in atomic motion or in the form of translational energy. The "microbombs" needed for the controlled release of fusion energy are of limited size and lifetime and may not survive long enough to establish thermodynamic equilibrium. Consequently deposition of energy into electronic degrees of freedom rather than atomic translations can be very inefficient. With inertial confinement, the efficiency of energy transfer to fuel atoms can be optimized,¹ if energy is delivered to heat surface elements of a pellet containing the fusion fuel to a temperature of approximately 200 eV (approximately $2 \times 10^6 \text{ K}$). If beams of heavy ions were used to directly heat and compress an inertially confined assembly of fuel atoms, then current pulses of the order of $2 \times 10^{12} \text{ A/cm}^2$ would be needed. Space charge limitations² prohibit the generation of atomic ion beams with 200 eV kinetic energy and this magnitude of current density.

The mutual repulsion of similarly charged ions moving with relatively low velocities has been a major obstacle to efforts to ignite inertially confined systems of fusion fuel atoms. Winterberg³ suggested the use of

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[†]This research was carried out at Brookhaven National Laboratory under contract DE-AC02-76CH00016 with the U.S. Department of Energy and supported by its Division of Chemical Sciences, Office of Basic Energy Sciences.

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