Mismatch-Induced Switch of Neocarzinostatin Attack Sites in the DNA Minor Groove†

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ABSTRACT: Based on the finding that the wobble G·T mismatch 5′ to the *C* of AG*C*·GCT results in switching of the attack chemistry by neocarzinostatin chromophore (NCS-Chrom) on the deoxyribose moiety of *C* from C-1′ to C-4′ [Kappen, L. S. & Goldberg, I. H. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 6706–6710], a series of mismatches has been explored for their effect on the chemistry of damage at the *T* of AG*T*·ACT in oligodeoxynucleotides, a site at which 4′-chemistry ordinarily occurs. Placement of a G·T mispair 5′ to the *T* results in a marked increase in 4′-chemistry, as measured by the formation of breaks with 3′-phosphoglycolate ends and abasic sites due to 4′-hydroxylation. Strikingly, 4′-chemistry is induced at the T on the complementary strand, a site ordinarily restricted to 5′-chemistry. Substitution of dioxygen by the radiation sensitizer misonidazole exerts a pronounced effect on the partitioning of the 4′-chemistry in favor of the 3′-phosphoglycolate product. Both stable T·G and unstable T·C mismatches at the attack site itself are associated with marked inhibition of damage at this site. Whereas placement of the relatively stable G·A mismatch on the 5′-side of the *T* residue (AG*T*) results in substantial inhibition of damage at the *T* without shifting of chemistry, the same mismatch at the 3′-side of the attack site decreases damage only slightly but is associated with the appearance of significant 1′-chemistry. By contrast, no shift in chemistry is found with bleomycin, which attacks at C-4′. These findings provide further support for the role of minor groove microstructure in determining the chemical mechanisms of DNA damage and underscore the usefulness of NCS-Chrom as a probe of DNA microheterogeneity.

The non-protein chromophore of the antitumor antibiotic neocarzinostatin (NCS-Chrom) binds to DNA by intercalation via its minor groove and upon activation by thiol adduction rearranges to a diradical species that abstracts hydrogen atoms from minor groove-accessible carbons of the DNA sugar of each strand of duplex DNA (Scheme I). Molecular oxygen adds onto the carbon-centered radicals generated on the DNA deoxyribose to form peroxy radicals that, usually after reduction, give rise to the final DNA damage products [reviewed in Goldberg (1991)]. Under anaerobic conditions, nitroaromatic radiation sensitizers can substitute for dioxygen (Kappen & Goldberg, 1984; Kappen et al., 1989). Three main attack sites and the consequent damage products have been characterized (Scheme II): (a) Abstraction of a hydrogen atom from the 5′-carbon results mainly in single-strand breaks predominantly at T and A residues with a PO_4 at the 3′- and a nucleoside aldehyde at the 5′-ends of the break (Kappen et al., 1982; Kappen & Goldberg, 1983). (b) 4′-Attack, which occurs predominantly at the T residues of GT steps, especially at AGT sequences, leads either to an alkali-labile, 4′-hydroxylated abasic site (Saito et al., 1989; Kappen et al., 1991; Frank et al., 1991; Dedon et al., 1992) or to a strand break with a phosphoglycolate at the 3′- and a PO_4 at the 5′-termini (Dedon & Goldberg, 1990; Frank et al., 1991; Kappen et al., 1991; Dedon et al., 1992). (c) Abstraction of a hydrogen atom from the C-1′ position of mainly C residues of AGC sequences (Kappen et al., 1990) also results in an alkali-sensitive abasic site (Povirk & Goldberg, 1985; Kappen et al., 1988) having a 2′-deoxyribonolactone residue (Kappen & Goldberg 1989). Deuterium abstraction (Meschwitz & Goldberg, 1991; Meschwitz et al., 1992) and molecular modeling (Galat & Goldberg, 1990) studies indicate that in the formation of bistranded lesions the radical center at C-2 of NCS-Chrom is involved in attack at the C of AGC and the T of AGT, while that at C-6 abstracts hydrogen from the T two bases to the 3′-side on the complementary strand.

Thiol, which has a dual role as an adducting activator of the drug and as a reductant of the DNA damage intermediates, influences the partitioning of the reaction intermediates and hence the final damage products (Kappen et al., 1991, Frank et al., 1991). In addition, the structure of the thiol affects the relative proportions of single- and double-strand lesions (Povirk & Goldberg, 1985; Dedon & Goldberg, 1992; Dedon et al., 1992). While base-specific 5′-attack results mainly in single-strand breaks, sequence-specific 1′- and, to a major degree, 4′-attacks occur as part of a bistranded lesion, which also includes a strand break due to 5′-chemistry on the complementary strand two nucleotides to the 3′-side. Several lines of evidence support the notion that the bistranded lesions that occur at AGC·ACT and AGT·ACT sequences result from the concerted action of a single molecule of NCS-Chrom on both DNA strands (Povirk et al., 1988; Dedon & Goldberg, 1990; Dedon et al., 1992).

Such studies also revealed a unique property of NCS-Chrom: its ability to alter the chemistry of attack, depending upon the microstructure and the local geometry of the drug–DNA complex. This is further shown by the finding that replacement of a G·C pair with an I·C pair in an oligonucleotide duplex makes a significant difference in the extent of attack at C-1′ (Kappen et al., 1988) and at C-4′ (Kappen et al., 1991) by NCS-Chrom. In addition, recent work revealed

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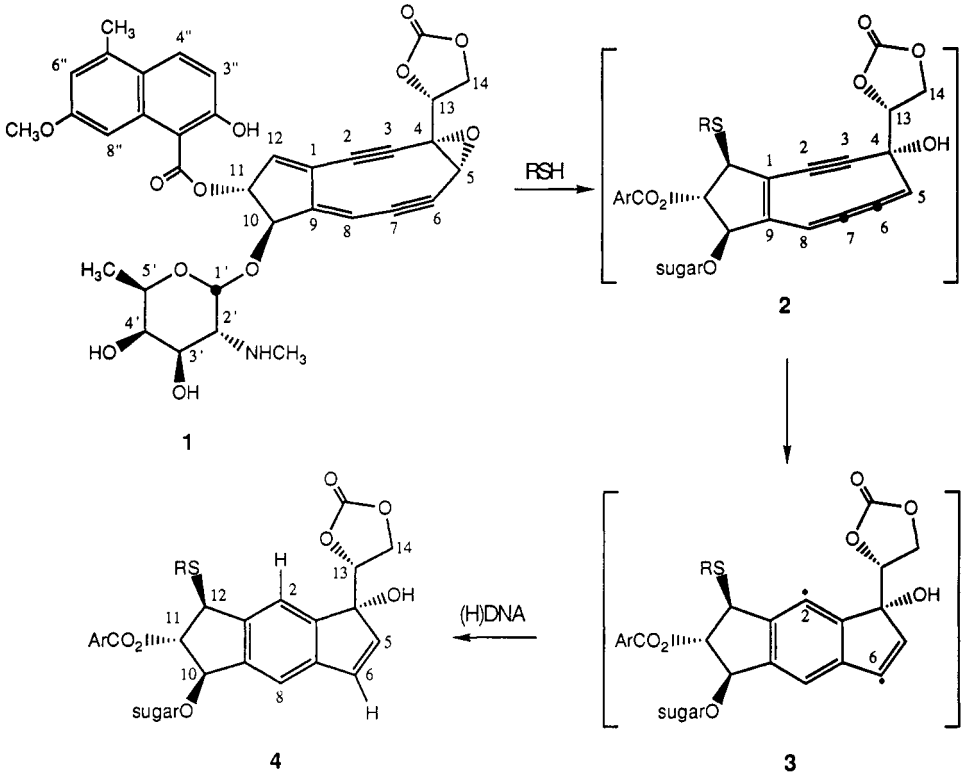
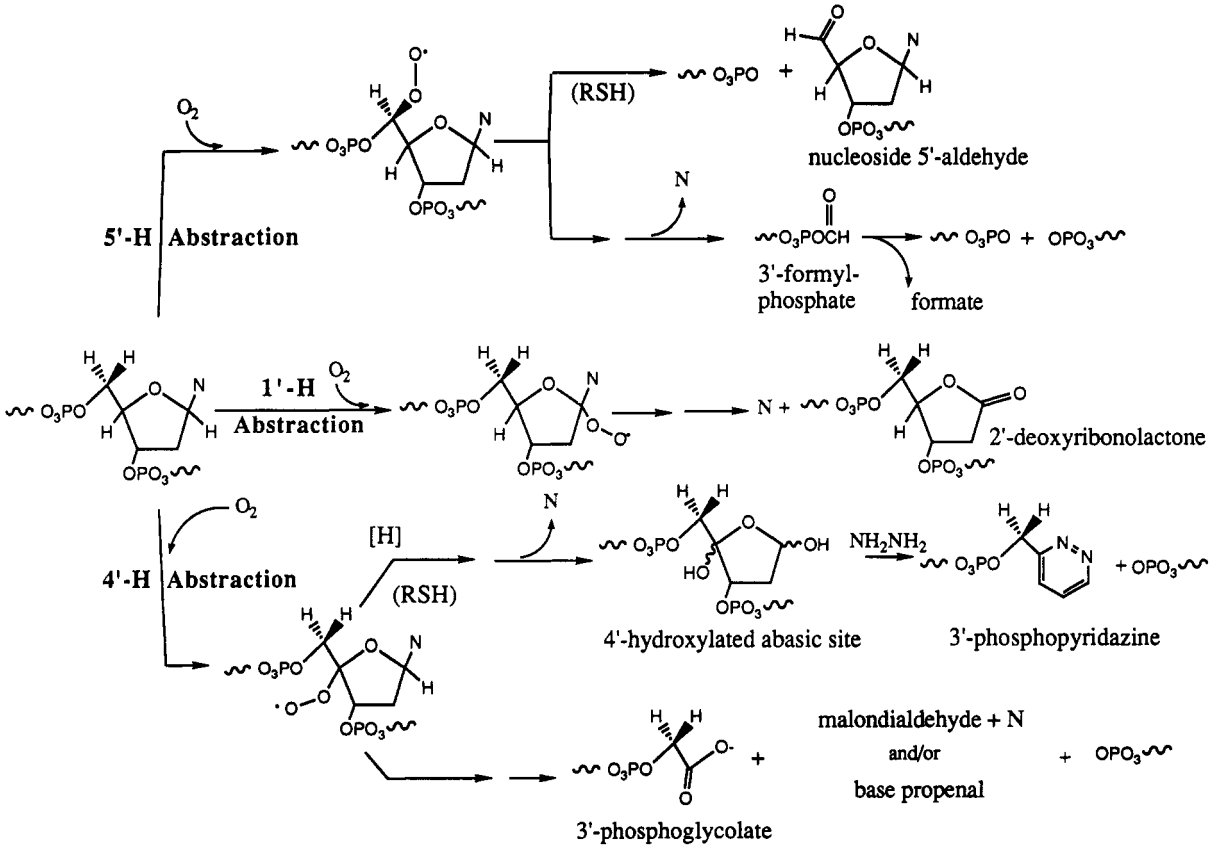
Scheme I: Thiol Activation of NCS-Chrom to the Diradical Species [Based on the Proposal of Myers (1987)]



Scheme II: Major Mechanisms of NCS-Induced DNA Damage at C1′, C4′, and C5′



that placement of a G·T mismatch 5′ to the C of AGC in an oligonucleotide duplex results in switching of the chemistry of attack at the C from 1′ to 4′ (Kappen & Goldberg, 1992). Further, shuttling between attack sites (4′ and 5′) has also been attributed to deuterium isotope selection effects (Kappen et al., 1991). The versatility of its action in response to subtle changes in DNA microstructure makes NCS-Chrom a sensitive tool to probe DNA microheterogeneity. In this study we have explored the influence of DNA microstructure on the chemistry of NCS-Chrom action at AGT·ACT sequences using oligodeoxynucleotide duplexes containing mispaired bases. We find that, depending on the type and location of

the mismatch, the relative amounts of 4′-, 5′-, and 1′-chemistry will fluctuate significantly.

MATERIALS AND METHODS

The materials and their sources are as follows: chemically synthesized oligodeoxynucleotides, Oligos Etc. or Chem Genes; [γ-^{32}P]ATP (3000 Ci/mmol), New England Nuclear; polynucleotide kinase, New England Biolabs; NCS, Kayaku Antibiotics; bleomycin, Bristol Laboratories; misonidazole, Hoffmann-LaRoche.

The oligomers were 5′-end-labeled with [γ-^{32}P]ATP, using polynucleotide kinase, by standard procedures (Maniatis et al., 1982) and purified on a urea-containing 20% polyacrylamide gel. The product from the gel slices was recovered as previously described (Kappen et al., 1991).

Drug Reactions. NCS-Chrom was extracted from the holoantibiotic using methanol, as descibed (Kappen & Goldberg, 1985). ^{32}P-Labeled oligodeoxynucleotide (+) strands were first annealed to the (–) strands (1:2 ratio) by heating in the 2× reaction buffer at 90 °C for 2 min and slow cooling. After the addition of thiol and H_2O to make up the final reaction volume, the mixture was chilled in ice before the drug was added. A no-drug control received an equal volume of methanol, the final concentration of which did not exceed 10%. A standard reaction (30–90 μL) contained 20 mM. Tris–HCl, pH 8.0, 1 mM EDTA, 5 mM 3-mercaptopropionate (MPA), 229 μM ^{32}P (+) strand (PO_4), 438 μM (–) strand (PO_4), and 31.7 μM NCS chromophore. The reaction in ice was allowed to proceed in the dark for 30 min.

Anaerobic reactions were performed in a vessel containing a side arm, as previously described (Kappen & Goldberg, 1985). All components except thiol (final concentration, 2 mM) were placed in the main reaction chamber. After removal of oxygen, the reaction was started by addition of thiol from the side arm. When misonidazole (20 mM) was used, it was placed in the main reaction chamber from the start.

Conditions for bleomycin reactions with the duplex oligomers were similar to those for NCS-Chrom except that ferrous iron equimolar to bleomycin (19 μM) was present in the reaction.

Piperidine or Hydrazine Treatment. Duplicate aliquots of the drug reaction (8–20 μL) were dried in a Speed Vac concentrator. One of the resulting pellets was heated in 100 μL of 1 M piperidine at 90 °C for 30 min. After being dried, the sample was dissolved in 15 μL of H_2O and dried. H_2O addition and drying were repeated three times. A third portion of the reaction was treated with hydrazine hydrochloride (50 mM), pH 8.0, for 2 h at room temperature and dried.

Product Analysis. The dried sample pellets were dissolved in 80% formamide–1 mM EDTA containing marker dyes and electrophoresed on a urea-containing 20% polyacrylamide gel with the gel maintained at room temperature. The intensities of the gel bands were quantitated by scanning preflashed, lightly exposed autoradiograms using an LKB Ultroscan laser densitometer.

RESULTS AND DISCUSSION

Effect of G·T Mismatches. On the basis of the earlier findings that NCS-induced lesions at the T of a GT step in oligodeoxynucleotides involves both 5′- and 4′-chemistry (Kappen et al., 1991, Frank et al., 1991) and that AGT·ACT sequences are preferred targets (Dedon & Goldberg, 1990), we chose the 12-mer CCAGT$_5$GCACTGC as the substrate, either as a perfectly matched duplex or as one having mismatched base pairs (Table I). Products of strand scission

Table I: Oligodeoxynucleotide Duplexes Used as Substrates for NCS Chromophore[a]

1.	CCAGTGCACTGC GGTCACGTGACG	5.	CCAG**T**GCACTGC GGTC**C**CGTGACG
2.	CCA**G**TGCACTGC GGT**T**ACGTGACG	6.	CCA**G**TGCACTGC GGT**A**ACGTGACG
3.	CCAGT**G**CACTGC GGTCA**T**GTGACG	7.	CCAGT**G**CACTGC GGTCAAGTGACG
4.	CCAG**T**GCACTGC GGTC**G**CGTGACG	8.	GCAGTGCATTGG CGTCACGTAACC

[a] Mispaired bases are shown in bold letters.

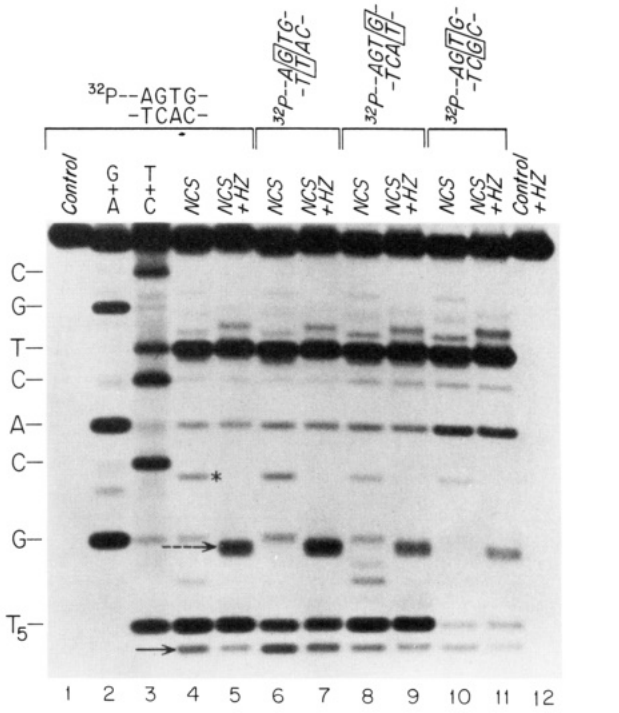


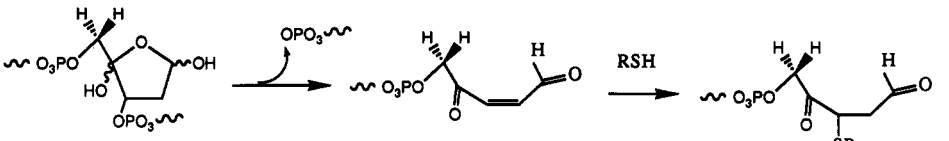
FIGURE 1: Effect of a G·T mismatch on NCS-Chrom-induced lesions at the T of AGT$_5$. 5′-^{32}P-Labeled CCAGT$_5$GCACTGC [(+) strand], either as a perfectly matched duplex or as one having the mispaired base on the (–) strand, was treated with NCS-Chrom and then with hydrazine (HZ) as described in Materials and Methods. G + A and T + C represent Maxam and Gilbert markers. Lanes 1 and 12 are no-drug controls. Solid arrow, "glycolate" and dashed arrow, pyridazine derivative in this and subsequent figures. Asterisks in this and subsequent figures are explained in the text.

for each attack site have been previously characterized, and their distinctive mobilities on a sequencing gel offers a convenient method for their quantitation (Kappen et al., 1991; Frank et al., 1991). 5′-Chemistry generates fragments with 3′-PO_4 termini. The C-4′ attack lesion partitions to produce a 3′-phosphoglycolate-ended DNA fragment and a 4′-hydroxylated abasic site. The latter is measured, after hydrazine treatment (Sugiyama et al., 1988), as a break having a pyridazine derivative at its 3′-end (Scheme II).

In the perfectly matched duplex containing 5′-^{32}P-CCAGT$_5$-GCACTGC (1, Table I), cleavage at T$_5$ gives a major band coincident with that of the Maxam and Gilbert (1980) marker for breakage at the same T and known to have 3′-PO_4 termini (Figure 1, lane 4). The intensity of this band is a measure of the 5′-chemistry (Table II). The weak band (solid arrow) just ahead of the PO_4 band is due to 3′-phosphoglycolate-terminated fragments ("glycolate"). Hydrazine treatment produces an additional band (dashed arrow, lane 5), representing the pyridazine derivative of the 4′-hydroxylated abasic site. Hydrazine treatment following the drug reaction causes a decrease in the amount of "glycolate", presumably by its reaction with an intermediate in glycolate formation (Dedon

Scheme III: Proposed Mechanisms for the Formation of Thiol Adducts of Sugar Fragments Derived from the Initial 4′- and 1′-Attack Products

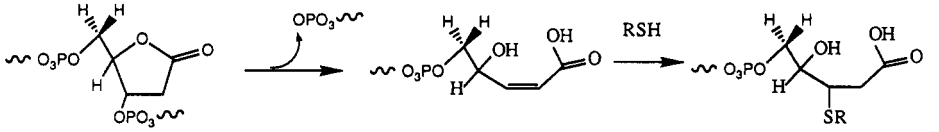
A



4′-hydroxylated abasic site

1 2 3

B



2′-deoxyribonolactone

4 5 6

Table II: Distribution of NCS-Chrom-Induced Chemistry at T in AGT_5 with and without a G·T Mismatch[a]

substrate	total damage	% 5′	% 4′
^{32}P--AGT- -TCA-	1.6	75	25
^{32}P--AGT- -TTA-	1.1	38	62
^{32}P--AGTG- -TCAT-	1.0	75	25

[a] Data are derived from gel analysis of NCS-Chrom-treated oligomer duplexes after hydrazine treatment in Figure 1. The band intensities were determined by densitometry. Total damage comprises 5′-chemistry (PO_4) and 4′-chemistry ("pyridazine" plus "glycolate"). Mispaired bases are shown in bold letters.

et al., 1992). T_5 is thus subject to attack at C-5′ and C-4′, the former being the major lesion. By contrast, in restriction fragments, 4′-chemistry predominates at the T of AGT sequences (Frank et al., 1991; Dedon et al., 1992). Placement of a G·T mispair on the 5′-side of T_5 (2, Table I) causes an inhibition in T_5-PO_4 formation with a substantial increase in both products of 4′-chemistry, i.e., "glycolate" and the pyridazine derivative (lanes 6 and 7; Table II). In contrast, a G·T mismatch on the 3′-side of T_5 (3, Table I) causes inhibition of both 5′- and 4′-attacks (lanes 8 and 9) to the same extent (Table II). While a T·G mismatch at T_5 itself (4, Table I) causes nearly complete inhibition of strand breakage, the small amount of damage that does occur is mainly due to 4′-chemistry (lanes 10 and 11). It must be noted that in this case cleavage at the A residue three nucleotides to the 3′-side of T_5 is enhanced, suggesting that the drug-binding site has shifted. Damage at T_5 was almost completely inhibited (data not shown) when it was mispaired with a C (5, Table I). The lack of any damage at T_5 when it is mispaired with a C is consistent with the reported instability of this mispair (Patel et al., 1984; Ikuta et al., 1987). The low level of damage at T_5 with the stable T_5·G mispair may be due to factors other than stability itself, such as poor binding of the drug and/or an altered orientation of the radical centers of the drug caused by a localized perturbation of some structural attribute required for drug–DNA interaction.

The minor, slowly moving band, designated by an asterisk (Figure 1, lanes 4, 6, 8, and 10) likely represents the thiol adduct (3, Scheme III) of the 3′-terminal unsaturated sugar (α,β-unsaturated aldehyde) (Manoharan et al., 1988; Bailly & Verly, 1988) generated by a single β-elimination of the 4′-hydroxylated abasic site at its 3′-end. As expected, its intensity varies with the extent of the 4′-chemistry. A similar band was observed before upon damage at the T residue of a GT step and shown to exhibit a substantial isotope selection effect on its formation when deuterium was substituted for protium at C-4′ of the T residue. (Frank et al., 1991; Kappen et al., 1991). Most of the abasic site remains in full-length molecules near the top of the gel (Kappen et al., 1988).

Lesions on the Opposite Strand. Since AGT·ACT sequences are preferred sites for NCS-induced double-stranded lesions (Dedon & Goldberg, 1990; Dedon et al., 1992), it was of interest to examine the chemistry of lesions on the opposite strand. Experiments similar to those in Figure 1 were, therefore, performed using 5′-^{32}P-labeled (–) strands. In the Watson–Crick base-paired duplex, cleavage at T_{22} of ACT in the (–) strand [two nucleotides 5′ to the T_5 of AGT in the (+) strand] gives only 3′-PO_4 termini, indicative of only 5′-chemistry (Figure 2, lanes 3–5). The intensity of this band is virtually unaffected by piperidine treatment, nor is there any pyridazine derivative found with hydrazine, confirming the absence of 4′-attack at T_{22}. In the duplex having a G·T mispair on the 5′-side of T_5, there is a PO_4 band at T_{22} (Figure 2, lanes 7–9). In addition there is a significant "glycolate" band (solid arrow). Further, piperidine intensifies the PO_4 band, due to cleavage of the 4′-hydroxylated abasic site, the presence of which is confirmed by its conversion to the pyridazine derivative (lane 9, dashed arrow). Induction of 4′-chemistry also results in the production of a band (asterisk, lane 7) which, as noted above, is the thiol adduct (3, Scheme III) of the unsaturated sugar fragment produced by a single β-elimination at the 3′-end of the 4′-hydroxylated abasic site. 4′-Chemistry at T_{22} accounts for 32% of the total damage in the presence of the G·T mismatch. These data show that the G·T mismatch on the 5′-side of T_5 of the AGT sequence in the (+) stand causes a shift to 4′-chemistry, not only at T_5 but also at T_{22} in the complementary strand opposite the A of AGT. It should also be noted that strand breakage at T_{21} is completely inhibited in the mispaired duplex (compare lanes 7–9 with 10–12).

Since mispairing converts the sequence ACT in the (–) strand of the normal duplex to ATT in the mispaired duplex, we also examined the damage at T_{22} in the 12-mer duplex (8, Table I), having the normal base-pairing ATT·AAT. Attack at T_{22} in this perfectly matched duplex gives only 3′-PO_4-ended fragments; neither piperidine nor hydrazine treatment made any difference in the products (Figure 2, lanes 10–12).

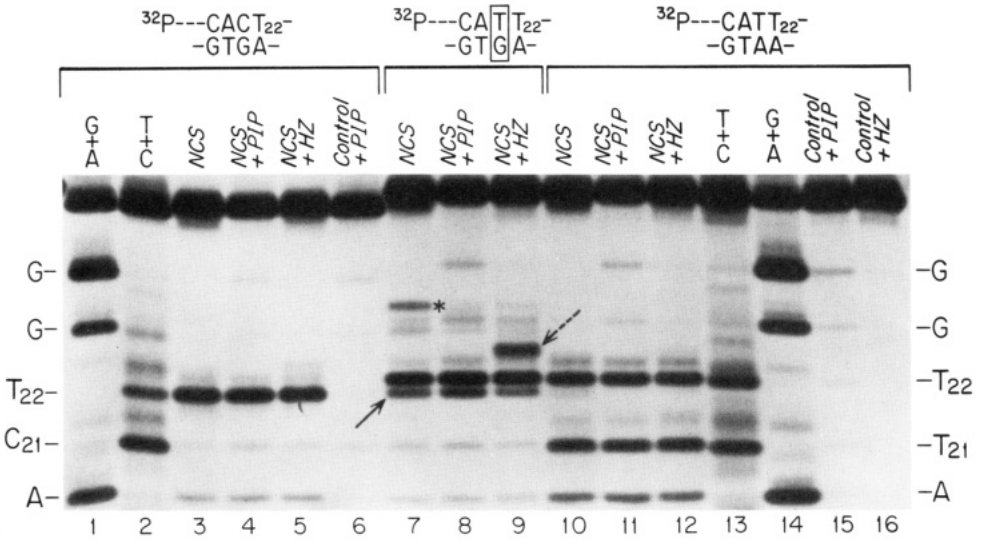


FIGURE 2: NCS-Chrom-induced lesions on the complementary strands of the normal duplex and the G·T mispaired duplex. In experiments similar to those in Figure 1, the (–) strands of the duplexes have 5′-^{32}P label. Controls have no drug. PIP and HZ represent piperidine and hydrazine treatments, respectively, in this and subsequent figures.

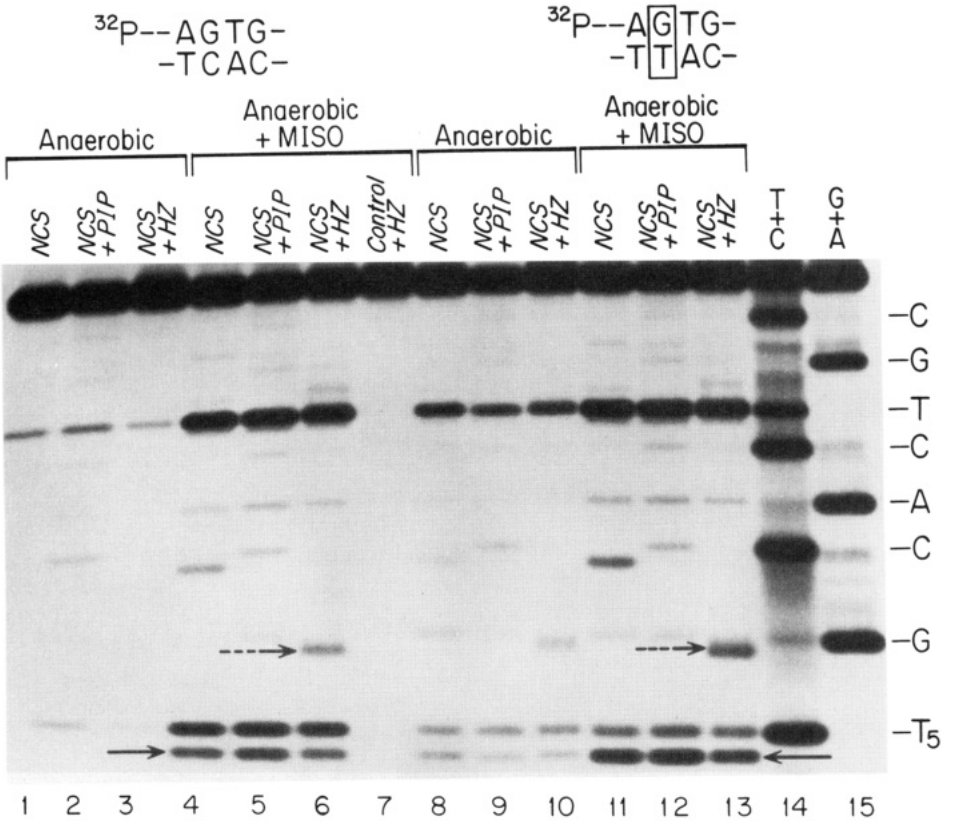


FIGURE 3: Effect of misonidazole on the distribution of NCS-Chrom-induced lesions at the T of AGT_5 in the presence and absence of a G·T mismatch. Reactions similar to those in Figure 1 were performed under anaerobic conditions in the absence and presence of misonidazole (Miso). Control in lane 7 has no drug.

This rules out the possibility that the switch to 4′-chemistry at T_{22} in the mispaired duplex is due to a change in the sequence itself.

Misonidazole-Containing Reactions. Previous studies have shown that substitution of the radiation sensitizer misonidazole (Miso) for oxygen alters the partitioning of the 4′-chemistry products so as to increase the "glycolate" at the expense of the 4′-hydroxylated abasic product (Kappen et al., 1991; Dedon et al., 1992). This observation was used to further confirm the involvement of 4′-chemistry. Experiments similar to those in Figure 1 were performed under anaerobic conditions. In the absence of oxygen, there is very little damage at the T residues in either the perfectly matched duplex or in the mispaired duplex (Figure 3, lanes 1–3 and 8–10). Miso restores the damage reactions. In the Miso-dependent reaction, there is a significant increase in the amount of "glycolate" (lanes 4–6, solid arrow) when compared to the air reaction in Figure 1 (lanes 4 and 5). This increase is even more striking in the duplex having a G·T mismatch at the 5′-side of T_5 (Figure 3, lanes 11–13). In this case, "glycolate" amounts to more than 80% of the total damage at T_5. Preferential formation of "glycolate" in the misonidazole reaction has been attributed to the involvement of an oxy-radical intermediate (Kappen et al., 1991; Dedon et al., 1992).

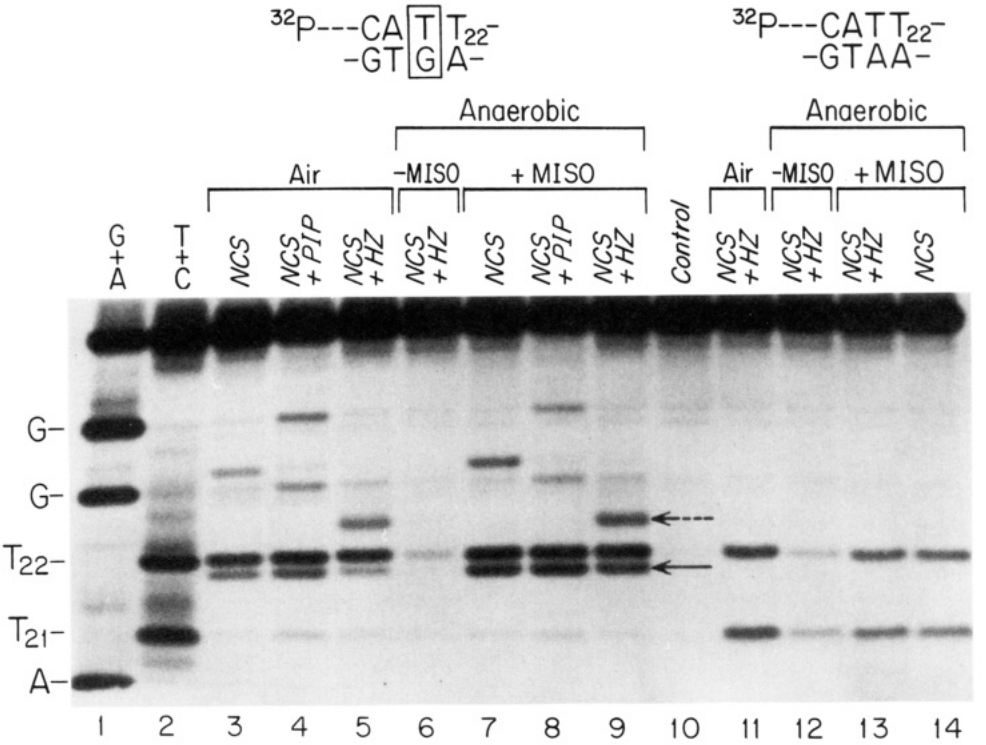


FIGURE 4: Effect of misonidazole on the distribution of NCS-Chrom-induced lesions on the complementary strand of the G·T mispaired duplex and on the same strand in the perfectly matched duplex. Reactions similar to those in Figure 3 were carried out with the G·T mismatched duplex and also with the same ^{32}P (–) strand in the perfectly matched form as substrates. Lane 10 has no drug.

The damage in the (–) strand of the G·T mispaired duplex under anaerobic conditions in the presence of Miso is shown in Figure 4. As predicted, partitioning of the 4′-chemistry at T_{22} is also altered in favor of "glycolate" (solid arrow) in the Miso-dependent reaction when compared to the air reaction (compare lanes 3–5 with 7–9). In the mispaired duplex, 4′-chemistry accounts for 42% of the total damage at T_{22}. As is the case with the air reaction, there are no 4′-attack products at T_{22} when it is base-paired in the perfect match (ATT·AAT) in the 12-mer duplex (lanes 11-14).

Chemistry of Damage in the (+) *and the* (–) *Strands with Different Thiol Activators.* Since the active form of the drug is its thiol adduct, it can be expected that the size, charge, and conformation of the thiol structure will influence the geometry of the drug–DNA complex and hence the chemistry of damage. Further, it has been shown that the partitioning of the DNA damaged products at the T of a GT step depends on the nature of the thiols used in the reaction (Kappen et al., 1991; Frank et al., 1991). Thiols with higher reducing potential, such as dithiothreitol, 2-mercaptoethanol, and 4-hydroxythiophenol, result in mainly 4′-hydroxylated abasic site formation, with little or no "glycolate". Thiols, such as 3-mercaptopropionate and glutathione, produce a significant amount of "glycolate" in addition to the other products. "Glycolate" formation is ordinarily restricted to double-stranded lesions (Dedon et al., 1992).

Since it is clear from the results in Figures 1–4 that only in the G·T mispaired duplex do both T_5 of AGT in the (+) strand and T_{22} of ATT in the (–) strand undergo 4′-chemistry, we sought to compare the lesions at these sites in the presence of two thiols. With 2-mercaptoethanol as the activator for the drug, the damage at T_5 Figure 5A, lane 5) and that at T_{22} (Figure 5B, lane 2) involve 5′- and 4′-chemistry. Without the mismatch, there is no 4′-chemistry at T_{22} of ACT with 2-mercaptoethanol (data not shown). 4′-Chemistry in either strand of the mismatched oligomer gives rise mainly to the pyridazine derivative (dashed arrow, lane 5 in A and lane 4 in B) with little "glycolate". In the presence of 3-mercaptopropionate, 4′-chemistry is increased and partitioned between "glycolate" and the pyridazine derivative (lanes 6 and 7 in A and 3 and 4 in B). These data show that the cleavage pattern and the partitioning of the intermediates in response to the two thiols go in parallel at the target T residues in the complementary DNA strands, independent of the thiol used. The asterisk-marked bands again represent the thiol adducts (**3**, Scheme III) of the sugar fragments produced from the 4′-hydroxylated abasic site, as discussed earlier. As expected for the thiol adduct (Bailly & Verly, 1988), the anionic thiol 3-mercaptopropionate results in a band with a faster mobility than that found for the neutral 2-mercaptoethanol adduct (compare the asterisks in lanes 4 and 6 in A with those in lanes 1 and 3 in B).

Effect of G·A Mismatches. Placement of a G·A mismatch on the 5′-side of T_5 (6, Table I) causes a significant inhibition (70%) of total damage (Figure 6, lanes 7–9). Occasionally an uncharacterized extra band with a mobility between that of phosphate and "glycolate" was seen. In contrast, a G·A mispair on the 3′-side of T_5 (7, Table I) inhibits the total damage at T_5 only slightly (26%), but generates a new product (double asterisks, lane 10) with a mobility that is intermediate between that of the phosphate band and the pyridazine derivative. This band, which amounts to 25% of the total lesion, disappears upon piperidine treatment (lane 11), presumably being converted to the phosphate-ended fragment or to a product with the same mobility. The new band appears to represent the thiol adduction product of the 3′-terminal unsaturated sugar (**6**, Scheme III) generated by β-elimination of an abasic site produced by 1′-chemistry. This conclusion is based on the following observations: (1) Previous drug damage experiments with the sequence AG*C*·GCT showed the appearance of a similar piperidine-sensitive band due to 1′-chemistry at the *C* residue, as indicated by a substantial

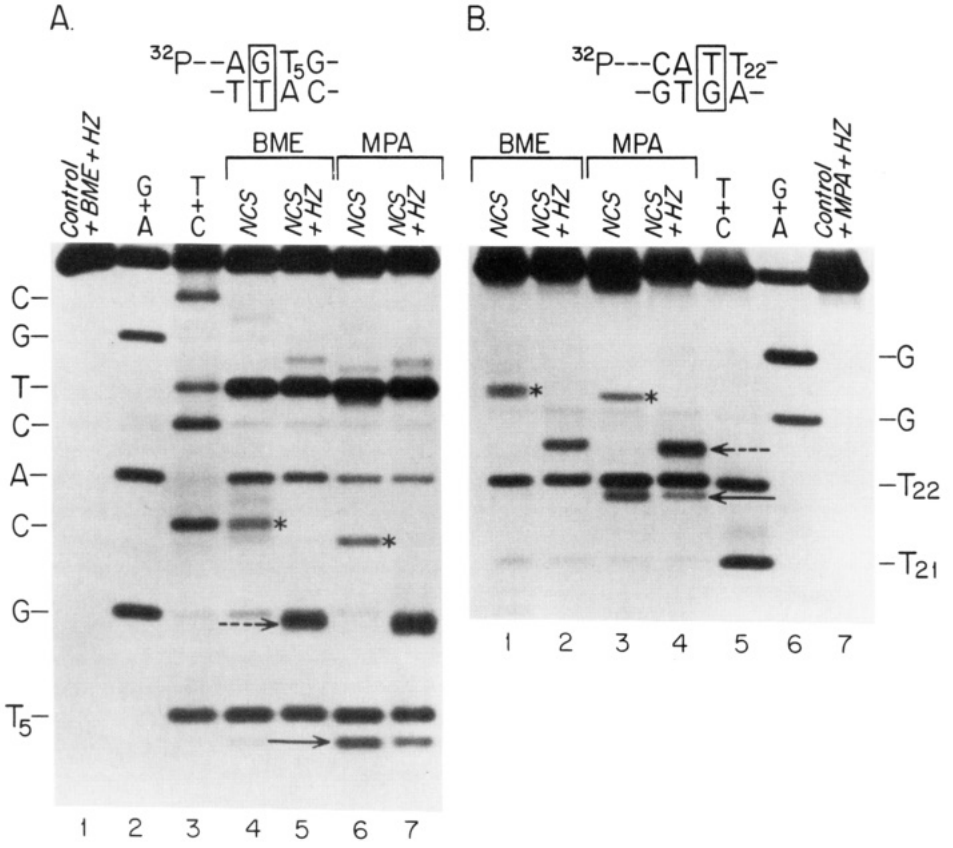


FIGURE 5: Effect of different thiols on NCS-Chrom-induced lesions on the (+) and (–) strands of the G·T mismatched duplex. Reactions similar to those in Figure 1, having 5′-^{32}P label either in the (+) or in the (–) strand of the G·T mispaired duplex, were performed in the presence of the indicated thiol. BME, 2-mercaptoethanol (10 mM); MPA, 3-mercaptopropionate (5 mM). Controls have no drug.

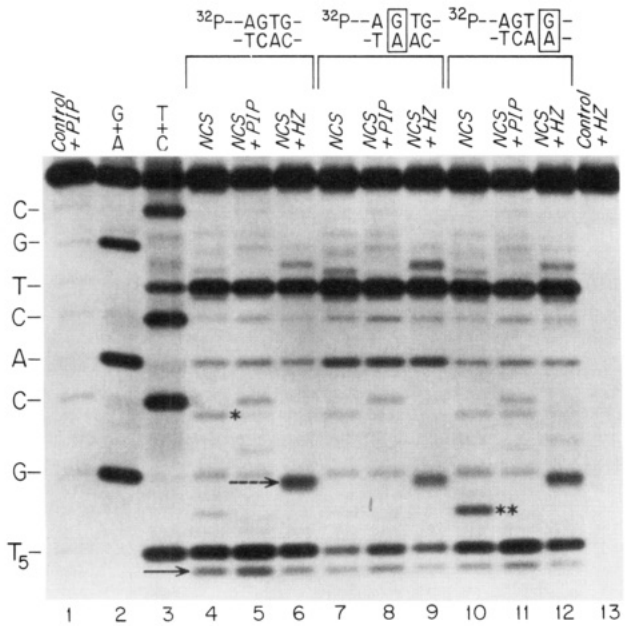


FIGURE 6: Effect of a G·A mismatch on NCS-Chrom-induced damage at the T of AGT_5. 5′-^{32}P-Labeled $CCAGT_5GCACTGC$ (+) strand, either as a perfectly matched duplex or as one having the mispaired base on the (–) strand, was treated with NCS-Chrom under standard conditions, and then with hydrazine (HZ) or piperidine (PIP). Controls have no drug. Asterisk and double asterisks represent thiol adduct derived from 4′- and 1′-lesion intermediates, respectively.

Table III: Summary of Shifts of NCS-Chrom Attack Sites by Mismatched Base Pairs[a]

substrate	deoxyribose attack sites 5′	4′	1′
AG***T***G TTAC	↓	↑	–
AG***T***G TCAT	↓	↓	–
AG***T***G TCGC	↓	↓	–
CAT***T*** GTGA	–	↑	–
AG***T***G TAAC	↓	↓	–
AG***T***G TCAA	↓	↓	↑

[a] The base in italics indicates the site of NCS-Chrom attack under study. Up-pointing arrow indicates absolute increase in attack frequency, down-pointing, decrease in attack compared to the substrate lacking the mismatch. Horizontal line indicates little or no change in attack. Mispaired bases are shown in bold letters.

C-1′ deuterium isotope selection effect on its formation (L.S.K., I.H.G., J. W. Kozarich, and J. Stubbe, unpublished data). (2) This product is not the result of 4′-chemistry, since it is present only in trace amounts under conditions usually associated with significant formation of the 4′-hydroxylated abasic site (Figure 6, lane 4; Figures 1 and 5). In fact, there is an inverse relationship between its formation and the extent of 4′-chemistry. Further, the β-elimination product of the 4′-hydroxylated abasic site has a slower mobility (asterisk, lane 4) than the product of 1′-chemistry, as expected, since the former has an aldehyde moiety where the latter has a carboxylate moiety (Figure 6, compare double asterisks, lane 10, with asterisk, lane 4). (3) Under conditions where 1′-chemistry at the C of AGC is stimulated by placement of an I (instead of a G) opposite the C (Kappen et al., 1988), the intensity of this band is proportionally increased (data not shown). (4) Where a G·T mismatch 5′ to the C in AGC causes a switch from 1′- to 4′-attack (Kappen & Goldberg,

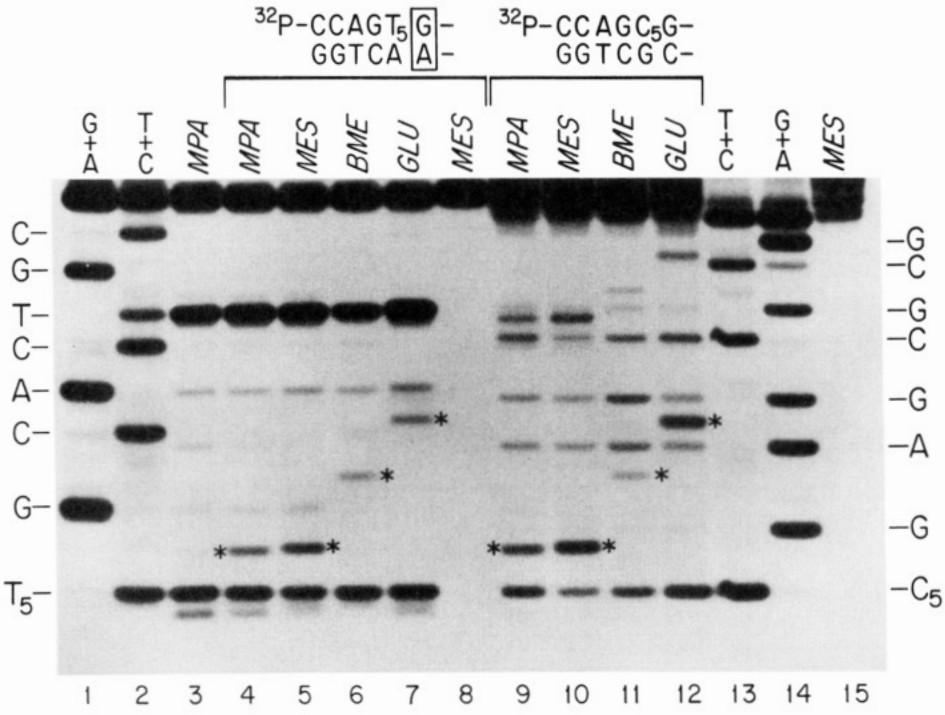


FIGURE 7: Formation of the thiol-adducted 1′-lesion derivative at T_5 of AGT having a G·A mismatch 3′ to T_5. Standard reactions containing NCS-Chrom and 5′-^{32}P-labeled CCAGT$_5$GCACTGC [(+) strand] in duplex form either as perfectly matched (lane 3) or as one having a G·A mismatch 3′ to T_5 (7 of Table 1) (lanes 4–7), or 5′-^{32}P-labeled CCAGC$_5$GAGCGCG as Watson–Crick base-paired duplex (lanes 9–12), were performed in the presence of the indicated thiol. MPA, 3-mercaptopropionate; MES, 2-mercaptoethanesulfonate; BME, 2-mercaptoethanol; GLU, glutathione (BME, 10 mM; all other thiols, 3 mM). Lanes 8 and 15 have no-drug controls.

1992), the intensity of this band decreases. (5) As shown in Figure 7 (lanes 4–7), the mobilities of this thiol addition product vary with the structure of the thiol; bands associated with the anionic thiols 3-mercaptopropionate and 2-mercaptoethanesulfonate have the fastest mobilities. The bulky glutathione adduct has an even slower mobility than that associated with the neutral 2-mercaptoethanol. Further, the mobilities of these bands correspond exactly with related bands produced at the C of AGC (lanes 9–12, Figure 7), a site characterized as primarily a 1′-attack site.

Effect of Mismatches on Bleomycin-Induced Damage. Since bleomycin attacks DNA sugars at C-4′ to generate the 4′-hydroxylation product ("pyridazine") and "glycolate" (Giloni et al., 1981; Rabow et al., 1986; Sugiyama et al., 1988), we have examined the same set of oliogdeoxynucleotide duplexes as described above (Table I) as targets for this agent. Placement of G·A or G·T mismatches 5′ to the attack site and diminishes the extent of damage at T_5 by about 50% and 80%, respectively. By contrast, the G·A mispair on the 3′-side of T_5 significantly stimulates (4-fold) the damage at T_5 (data not shown). In all cases, however, there is no evidence of a switch in chemistry from 4′ to any other site.

In summary, it is clear that appropriately situated stable mismatches, such as the G·T mispair placed on the 5′-side of T_5 of AGT$_5$ and the G·A mispair placed on the 3′-side of the same target nucleotide, lead to shifting of the NCS-Chrom minor groove attack sites (Table III). With the G·T mismtach, 4′-chemistry is enhanced, whereas with the G·A mismatch, 1′-chemistry increases dramatically. When a G·T mismatch was placed 5′ to the C of AGC, 1′-chemistry was markedly inhibited and replaced by 4′-chemistry (Kappen & Goldberg, 1992). This effect could be rationalized on the basis of the localized displacement of the 2-amino group of the G of the mismtach into the minor groove, as observed crystallographically [Hunter et al. (1987); see Modrich (1987)]. In this case, the guanine 2-amino group would be expected to provide steric protection of the deep-lying C-1′, so that the more superficial C-4′ becomes the site of radical-induced hydrogen atom abstraction. Since the radical center at the C-2 of NCS-Chrom abstracts hydrogen from C-1′ (and C-4′), its relationship to the attack site is critical. The role of the guanine 2-amino group is further highlighted by the finding that replacement of the G residue by an I residue at a GT step results in the selective elimination of the 4′-chemistry at the T residue (Kappen et al., 1991). In the case of the G·A mismatch, where C-1′ chemistry is increased, localized conformational pertubation (Gao & Patel, 1988) may permit the radical form of the drug (presumably the radical center at C2) to lie deeper in the minor groove, although alternative binding modes involving other intercalation sites have not been eliminated. Molecular modeling studies are underway to gain insight into the structural basis of this effect. Taken together, these and the earlier studies (Kappen & Goldberg, 1992) show that the chemistry of deoxyribose damage by NCS-Chrom is sensitive to microstructural changes in the DNA minor groove and that, in this sense, NCS-Chrom is a sensitive probe of such alterations in structure. The indacene diradical species of activated NCS-Chrom sits in the minor groove of DNA where it has access to hydrogen atoms at C-5′, C-1′, and C-4′ of the deoxyribose on the complementary DNA strands. The precise attack sites are determined by the local geometry of the drug–DNA complex. That sequence is not the sole determinant of local geometry is shown by the finding of selective strand scission opposite sites of single-base bulges, independent of the specific sequence (Williams & Goldberg, 1988). Finally, it is noteworthy that the attack site flexibility exhibited by NCS-Chrom is not shared by bleomycin, which carries out 4′-chemistry, independent of the extent of the damage reaction.

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