Specific Binding of the Biradical Analog of Neocarzinostatin Chromophore to Bulged DNA: Implications for Thiol-Independent Cleavage†

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ABSTRACT: The enediyne anticancer antibiotic neocarzinostatin chromophore generates a single, site-specific break at a bulge in DNA in a thiol-independent reaction, involving intramolecular drug activation under general base catalysis [Kappen, L. S., & Goldberg, I. H. (1993) *Biochemistry 32,* 13138−13145]. As part of an effort to elucidate the three-dimensional structure of the active complex formed between the labile drug and bulged DNA, we have studied the binding of stable drug products generated in the course of the cleavage reaction with oligodeoxynucleotides containing the bulged structure. By use of fluorescence quenching, we have found that one drug product, which is also formed in the absence of bulged DNA and most closely resembles the biradical intermediate in the cleavage reaction, specifically binds bulged DNA with a K_d in the low micromolar range and competitively inhibits the cleavage reaction. Other drug products, including one formed only in the presence of bulged DNA, fail to bind to the bulged DNA. Implications of these results for the proposed mechanism of bulge-specific cleavage and for the role of the DNA bulge in generating a unique drug product are discussed.

The labile nonprotein component of the enediyne antitumor antibiotic neocarzinostatin (NCS-Chrom) damages duplex DNA *in vivo* and *in vitro* following its activation by thiol (Goldberg, 1991, and references cited therein). NCS-Chrom (**1**, Scheme 1) binds in the minor groove of duplex DNA by intercalation of its naphthoate moiety and specific base pair recognition by its 2′-*N*-methyl sugar moiety (Povirk et al., 1981; Dasgupta & Goldberg, 1985; Gao et al., 1995). Cycloaromatization of the enediyne core of NCS-Chrom, upon nucleophilic attack by thiol at C12, is proposed to proceed via formation of a cumulene intermediate **3** to a biradical species **4** of the drug (Scheme 1) (Myers, 1987) that abstracts hydrogen atoms from deoxyribose of DNA to produce strand breaks and abasic sites in the form of single- and double-stranded lesions. In the absence of thiol, duplex DNA is not a target for damage by NCS-Chrom, and the drug decomposes in a base-catalyzed reaction under physiological conditions (pH ≥ 6) to a mixture of inactive forms, previously referred to as chromophore D (Napier et al., 1981; Goldberg, 1991, and references cited therein).

Recently, however, it has been found that NCS-Chrom can efficiently and selectively cleave DNA in a thiol-independent reaction at a specific bulged structure in a reaction involving general base catalysis (Kappen & Goldberg, 1993a,b). Determination of the chemical structures (Hensens et al., 1993, 1994) of the drug products generated in the presence and the absence of bulged DNA has led to the proposal shown in Scheme 2 for their formation (Hensens et al., 1994). In this mechanism, the spirolactone cumulene **1c** is stereoselectively generated via an intramolecular Michael addition at C12 by the enolate anion **1b**, which is a resonance form of the naphtholate anion **1a** of NCS-Chrom, resulting in the formation of the biradical **1d**. This series of reactions occurs spontaneously, and in the absence of bulged DNA, the biradical is quenched by other proton sources (including methanol in the solvent) to produce **2a** and **2b**. Drug product **3** is generated only in the presence of a substrate-bulged DNA. Thus, it has been proposed that in the presence of bulged DNA the cumulene **1c**, which has been implicated as the species that searches for the favored DNA binding site (DeVoss et al., 1990; Hensens et al., 1994; Myers et al., 1994), is in equilibrium between bound and free forms, which lead to **3** and to **2a** and **2b**, respectively, via **1d**. The bulge-cleaving species, presumably **1e**, is formed by quenching of the C2-based radical of **1d** by intramolecular bond formation with C8″. In this scheme, **1e** (and its final product **3**) is formed only in the presence of substrate-bulged DNA, which presumably induces a conformational change in the drug (step 4) so as to bring C8″ of the naphthoate moiety close to the radical center at C2.

An understanding of the exact role played by bulged DNA in this unusual reaction will require elucidation of the structure of the complex formed with the relevant NCS-Chrom intermediate. Given the extreme lability of NCS-Chrom and its proposed intermediates in bulge-specific cleavage, one can approach this goal only by determining first if any of the characterized drug products (**2a**, **2b**, **3**), which resemble the active drug intermediates, can form a sufficiently stable complex with bulged DNA to permit ^{1}H NMR and/or X-ray crystallographic analysis. A similar approach has recently been used successfully to determine the three−dimensional structure by ^{1}H NMR of a complex formed by duplex oligodeoxynucleotide and a postactivated thiol (glutathione) adduct (**5**, Scheme 1) of NCS-Chrom (Gao et al., 1995).

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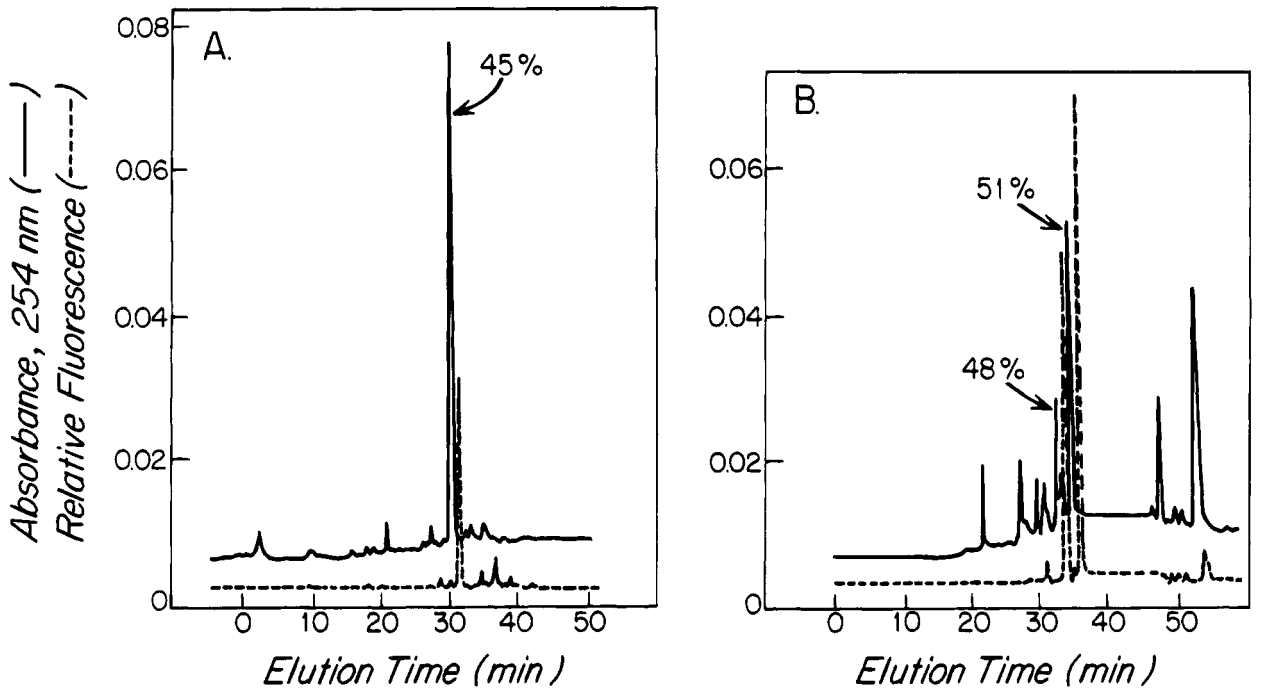
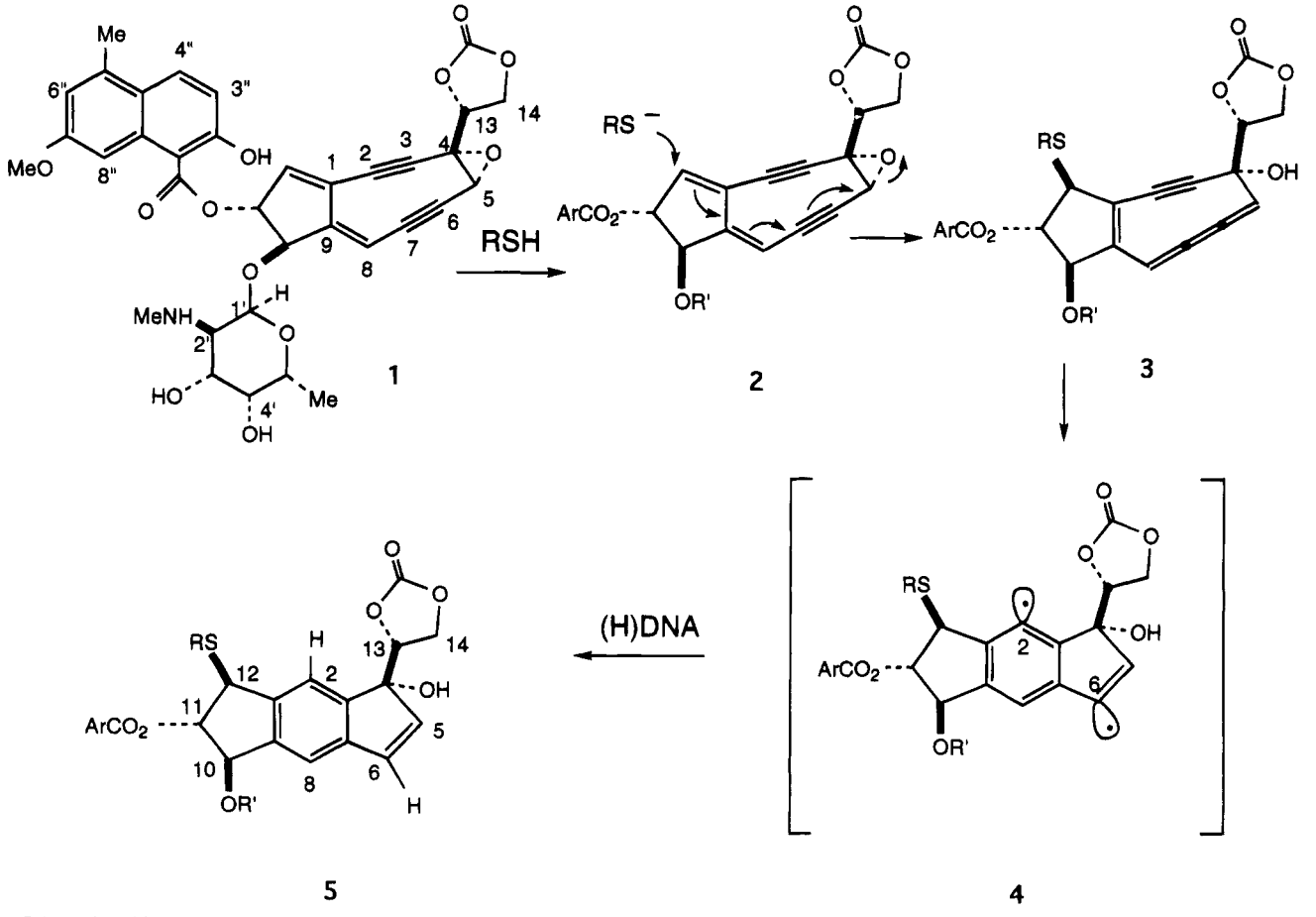


FIGURE 1: HPLC profile of NCS-Chrom products. HPLC of postactivated drug product **3** (A) and **2a** and **2b** (B) generated in standard reactions, described in Materials and Methods. A linear gradient of 40−65% methanol in 5 mM ammonium acetate, pH 4, at a rate of 1 mL/min, was applied. The percent of methanol, at which each drug product is eluted, is shown (see Materials and Methods). Chart recording of fluorescence is displaced (delayed) from that of absorbance by 1 min.

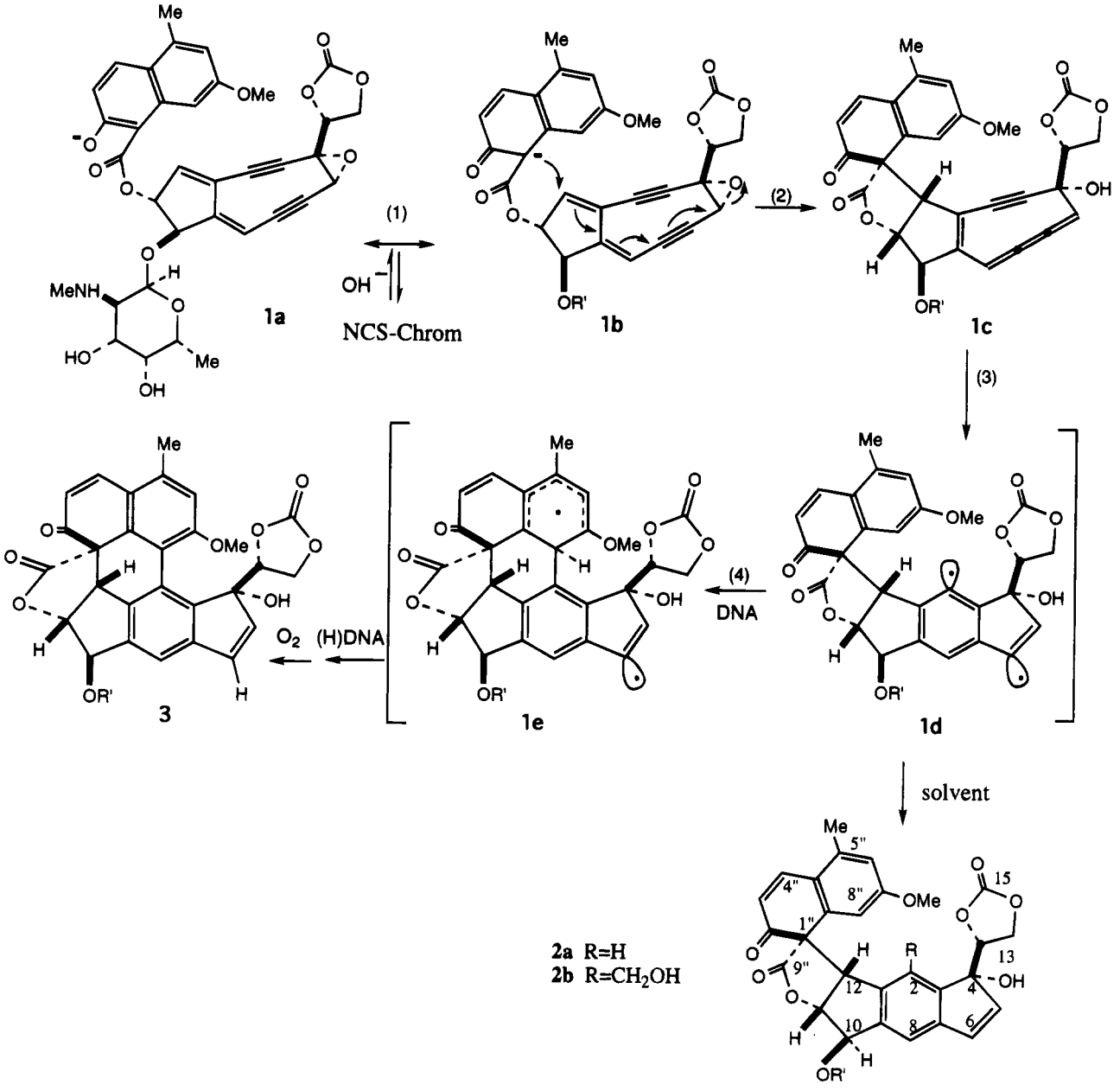
Scheme 1: Proposed Thiol-Dependent Mechanism of NCS-Chrom-Induced Cleavage of Duplex DNA[a]



[a] RSH, glutathione.

Here, we report that only one of the three NCS-Chrom-derived products, **2a**, which most closely resembles the proposed cumulene **1c** and the biradical intermediate **1d**, forms a stable, specific complex with oligonucleotides containing a bulge, which are substrates for the base-catalyzed cleavage reactions.

Scheme 2: Proposed Thiol-Independent Mechanism of NCS-Chrom-Induced Cleavage of Bulged DNA



MATERIALS AND METHODS

Drug. NCS-Chrom was isolated from the holoantibiotic (Kayaku Antiobiotics) with 20 mM sodium citrate in methanol and stored at −70 °C as described (Chin et al., 1987).

Oligodeoxynucleotide Synthesis. Oligodeoxynucleotides were synthesized using the standard β-cyanoethylphosphoramidite methodology with a ABI DNA automatic synthesizer followed by NH_4OH deprotection. Oligonucleotides were isolated by 1-butanol precipitation and were not further purified. Annealing of duplex DNA (1:1) was performed by heating the sample at 90 °C for 5 min and gradual cooling to 0 °C. Oligonucleotides, containing a triethyleneglycol chemical linker, were synthesized by manual incorporation of the chemical linker to the 5′ end of the positive strand and the 3′ end of the other strand. 9-*O*-Dimethoxytrityl triethyleneglycol 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, which was purchased from Glen Research, was used for the linker incorporation.

Preparation of Spontaneous Drug Products. Drug end products **2a** and **2b** were generated by incubation of NCS-Chrom (80−100 μM) in 50 mM Tris·HCl, pH 8.5, 10% methanol in the absence of DNA at 0 °C for 1 h. Following lyophilization of the reaction mixture, the dried sample was redissolved in 100 mM ammonium acetate at pH 4 and subjected to HPLC on a reverse-phase C-18 column of HPLC (Figure 1). Isolation of **2b** (retention time 34 min, 48% methanol) and **2a** (retention time 37 min, 51% methanol) was accomplished by using a linear gradient of 40−65% solvent B/solvent A over a 40-min period [solvent A, aqueous 5 mM ammonium acetate (pH 4); solvent B, methanolic 5 mM ammonium acetate (pH 4); flow rate, 1 mL/min] (Figure 1B).

Preparation of Bulged DNA-Specific Drug Product. A standard reaction contained 50 mM Tris·HCl, pH 8.5, 1 mM EDTA, and a ratio of a bulge-containing oligonucleotide (22-mer, Figure 3)/NCS-Chrom of 1.0:1.1. Methanol concentration was no greater than 10%. The reaction was allowed to

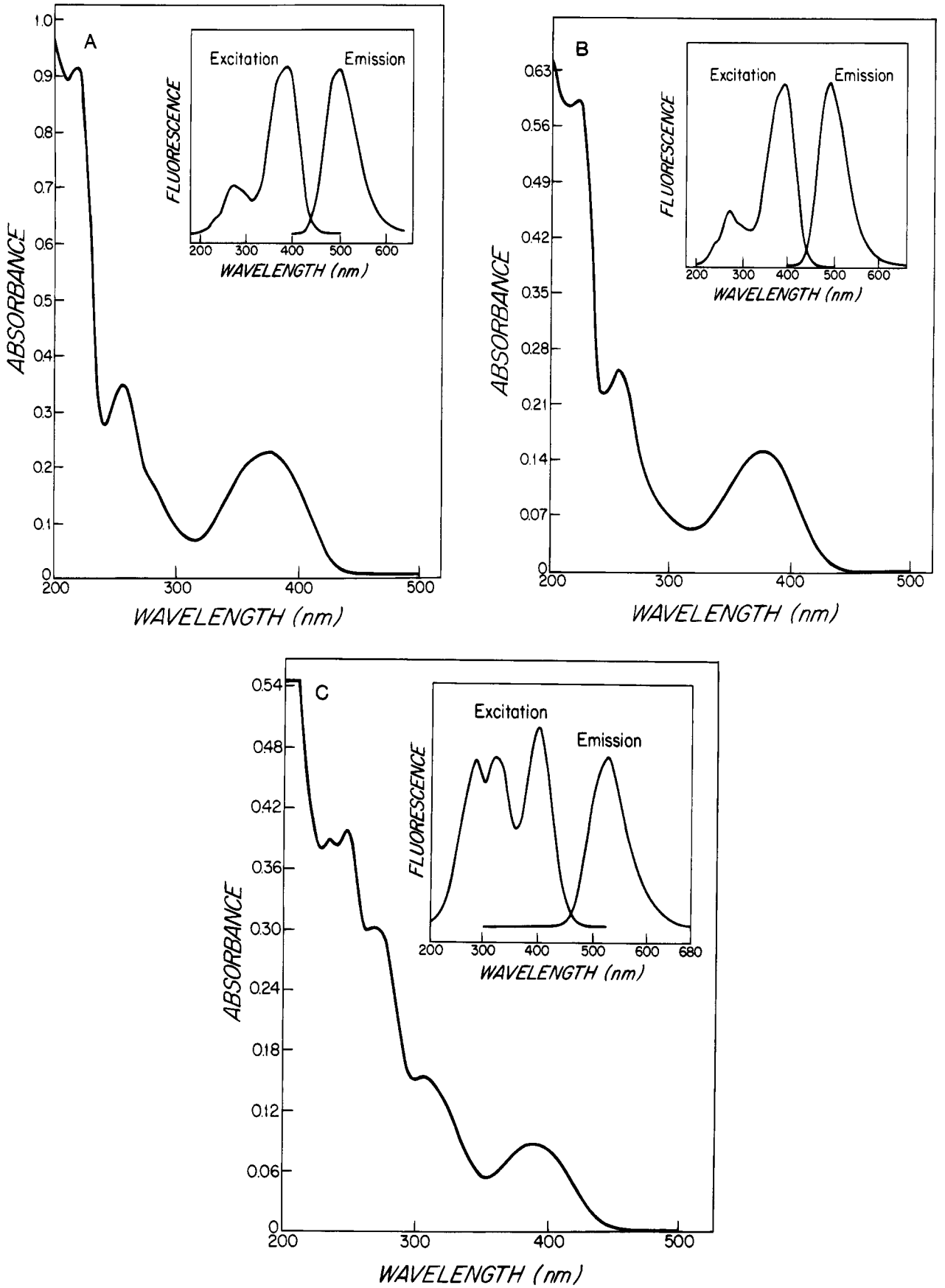


FIGURE 2: Absorption and fluorescence spectra of purified drug products. UV absorption and fluorescence spectra of HPLC-purified **2a** (A), **2b**, (B), and **3** (C) are shown. Fluorescence excitation at 390 nm and emission at 500 nm for **2a** and **2b**; excitation at 400 nm and emission at 530 nm for **3**. Spectra **2a**, **2b**, and **3** were recorded in 5 mM ammonium acetate (pH 4) in 51%, 48%, and 45% methanol, respectively.

proceed in the dark for 1 h in ice. Isolation of the postactivated drug product **3** (retention time 31 min, 45% methanol) (Figure 1A) was performed in the same manner as for **2a** and **2b**.

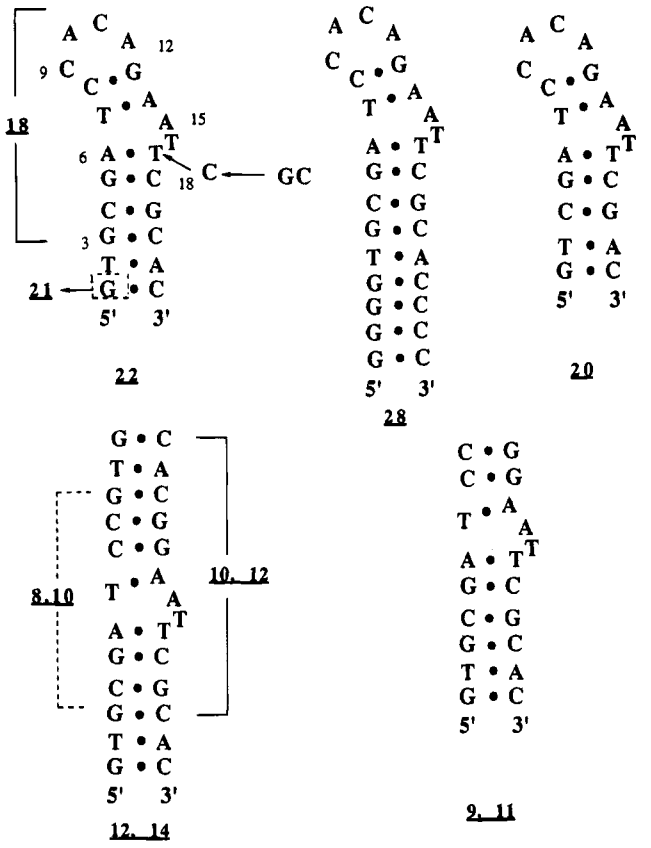


FIGURE 3: Bulge-containing oligomer structures and mutations.

Fluorescence Studies. Fluorescence measurements in the binding studies were performed on a Perkin-Elmer 512 spectrofluorometer at 5 °C. Excitation was at 400 nm, and emission was recorded from 420 to 600 nm. Fluorescence quenching was measured by adding aliquots of a concentrated oligonucleotide solution to the cuvette containing 1.2 μM drug product in a total volume of 600 μL of 5 mM sodium acetate, pH 5.2, with 1% methanol. The concentration of **2a**, **2b**, and **3** is an estimate, assuming an UV extinction coefficient (λ = 375 nm, ϵ = 8, 800 M^{-1} cm^{-1}) similar to that for NCS-Chrom, since only small amounts of the former have been available. The UV and fluorescence spectra of **2a**, **2b**, and **3** in the solvent in which each was eluted are shown in Figure 2.

Inhibition of NCS-Chrom-Induced Cleavage by Drug Products. NCS-Chrom-induced cleavage of bulged DNA (22-mer) was performed in 50 mM Tris·HCl, pH 8.5, and 1 mM EDTA, with 5 μM ^{32}P-5′-end-labeled oligomer 22-mer and 10 μM NCS-Chrom. Drug products **2a** and **3** were added prior to NCS-Chrom to study inhibitory effects on cleavage. The 22-mer was 5′-end-labeled with ^{32}P by standard procedures (Maniatis et al., 1982). The reactions were allowed to proceed in the dark for 1.5 h in ice. The reaction mixtures were separated by electrophoresis in a 20% polyacrylamide denaturing gel. The intensities of the radioactive bands corresponding to cleaved DNA and to the intact DNA were measured with volume integration using a Molecular Dynamics PhosphorImager with Image Quant software version 3.22.

Single-Site Binding Equation. Data were analyzed assuming only one type of binding site, and the dissociation constant (K_d) was calculated from fitting the Scatchard plot

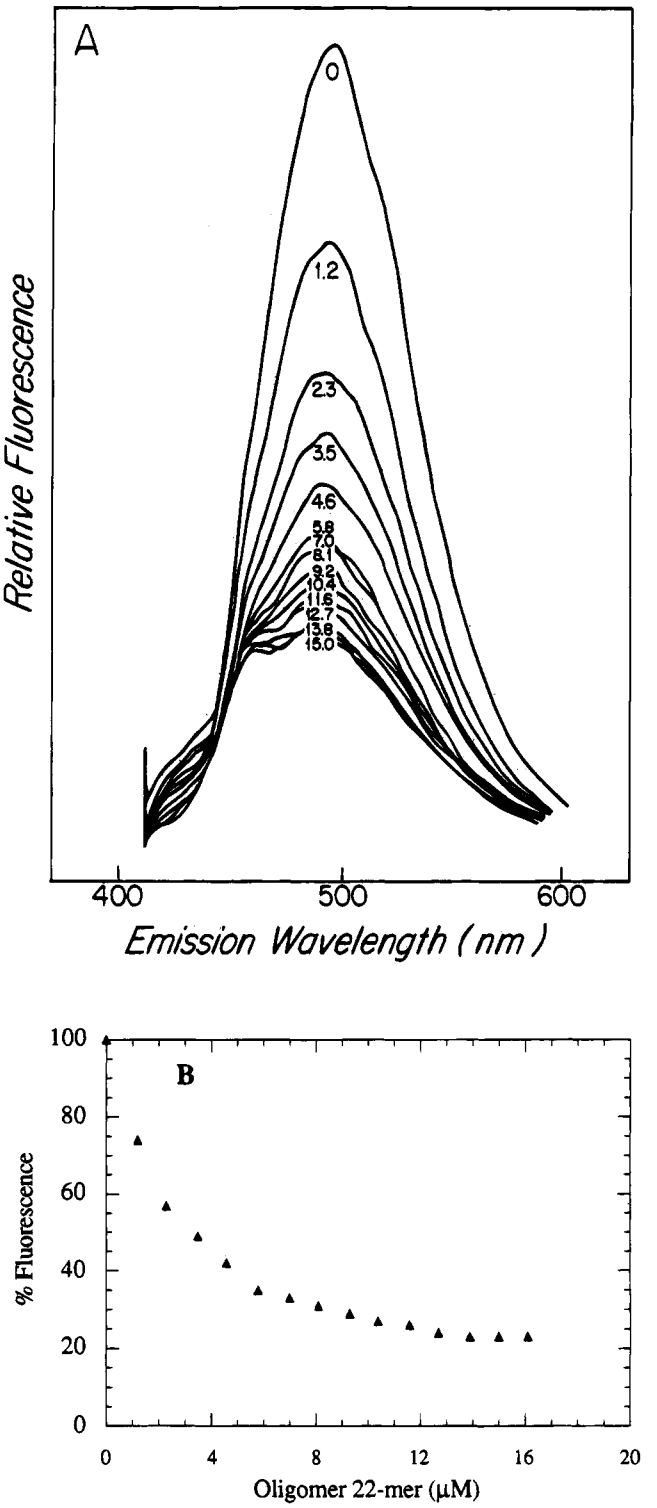


FIGURE 4: Binding of **2a** to bulged DNA. (A) Quenching of **2a** fluorescence by bulged 22-mer. Various amounts of 22-mer were added to a solution of **2a** as described in Materials and Methods. Emission fluorescence intensity (excitation at 400 nm) was measured at 5 °C. Oligomer concentrations in micromolarity are indicated for each spectrum. (B) Fluorescence decay curve of **2a** as a function of the amount of DNA present.

using:

$$\frac{\nu}{[\mathrm{DNA}]} = \frac{1}{K_d}(N - \nu) \qquad (1)$$

where K_d is the dissociation constant of the DNA−**2a** complex, ν is the fraction of the total DNA bound to drug,

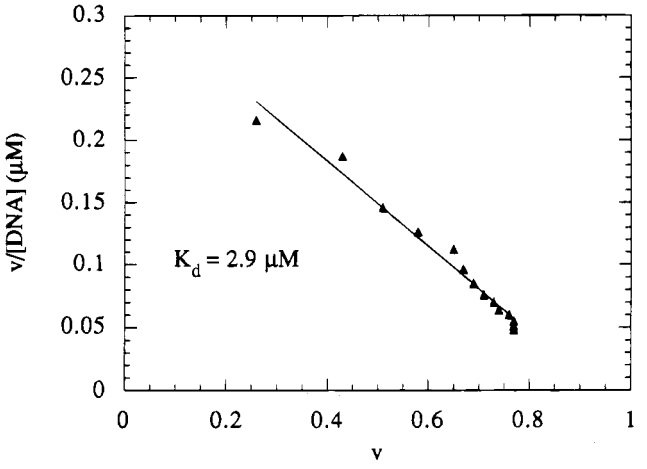


FIGURE 5: Scatchard plot of fluorescence quenching data for **2a** and 22-mer. From the slope of the linear fit using eq 1, the dissociation constant K_d was determined.

and N is the number of binding sites. Assuming there is one binding site, $N = 1$.

RESULTS AND DISCUSSION

Binding of **2a** *to Bulged DNA.* The two spontaneous end products of NCS-Chrom, **2a** and **2b**, and the bulged DNA drug product **3** were isolated by HPLC (Figure 1) and tested for their ability to bind to the bulge-containing 22-mer (Figure 3) by fluorescence quenching. Fluorescence quenching has been shown to reflect the binding of native NCS-Chrom and its thiol postactivated forms to duplex DNA (Povirk et al., 1981) and to be correlated with the protection of native chromophore from base-induced decomposition (Povirk & Goldberg, 1980). Quenching of fluorescence is more pronounced for sequences that are known to be better cleavage substrates for the chromophore (Stassinopoulos & Goldberg, 1995). As shown in Figure 4, increasing amounts of oligonucleotide resulted in proportional increases in the extent of quenching of the fluorescence of **2a** until saturation was reached. There is no shift in the peak emission wavelength. Since the base-catalyzed cleavage reaction gave a single lesion in high yield (Kappen & Goldberg, 1993, 1994), one binding site per bulged DNA can be assumed. Determination of the binding constant by Scatchard analysis from eq 1 revealed that the K_d (2.9 μM) value is of the same magnitude as the one for NCS-Chrom binding to double-stranded DNA (Povirk & Goldberg, 1980; Dasgupta et al., 1985). A linear Scatchard plot (Figure 5) suggests one mode of binding.

By contrast, the DNA bulge-specific drug end product **3** showed no fluorescence quenching by the 22-mer (Figure 6B), and 10 μM **3** failed to inhibit **2a** binding. Models of **3** show it to have a highly planar and relatively rigid structure, very different overall from the one expected for **2a**. It is not surprising, therefore, that it exhibits no binding. Also, **2b**, which differs from **2a** only by having a CH_2OH moiety at C2 instead of a hydrogen, does not bind to the 22-mer as indicated by fluorescence quenching (Figure 6A). A different conformation of the dehydronaphthoate ring system (an alternative, flattened half-chair conformation for ring A of the naphthoate) has been proposed for the **2b** product (Hensens et al., 1994), which will change its overall shape compared to **2a**, especially the naphthoate moiety that is one of the two possible fluorophores on **2a**. This might explain how this seemingly small difference results in such a drastic change in properties between the two compounds. It seems possible that the DNA bulge structure induces a conformational change in **1d** (or initially **1c**) from a conformation similar to that of **2a** to one resembling that of **2b** in which C2 and C8″ are closer together [see Figure 3 in Hensens et al. (1994) for depictions of proposed conformations for **2a** and **2b**]. In such an "averaged" conformation, the exact pucker of the two cis-fused five-membered rings largely dictates the proximity of the two centers C2 and C8″, which must be held sufficiently close under the influence of the bulged DNA for the quenching reaction involving the naphthoate and the radical at C2 to occur with C2−C8″ bond formation. Further, it is also possible that the shape of the binding site on the bulged DNA is modified by the binding of **2a**—induced fit—in such a way as to facilitate the quenching reaction. The ability of the C8″ of the naphthoate to approach C2 of the core under the influence of the bulged DNA will also depend on its flexibility and whether access to C2 is hindered. Thus, the mere existence of the hydroxymethyl group may interfere with the proposed conformational change that is proposed to result in the formation of the C2−C8″ bond and product **3** during the base-catalyzed reaction on the bulged oligonucleotide.

Bulged DNA Structural Requirement for **2a** *Binding.* In order to probe the structural basis for **2a** binding to bulged DNA, an array of different single-stranded DNAs based on the 22-mer (Figure 3), containing an apical stem-loop hinged to a region of duplex structure via a two-nucleotide bulge, were examined. From this study, several simple trends are obvious: (a) the cleaving efficiency and presumably the binding of the cutting species follow the same order as the binding of **2a**, as determined by fluorescence quenching; (b) the binding of the cleaving species is higher than that of **2a**, since oligos that are too small to be stable enough for binding to be observed, e.g., the 16-mer containing the bulge arrangement, are still substrates for the cleavage reaction (Kappen & Goldberg, 1993b); (c) a DNA species that binds **2a** will be a substrate for the cleavage reaction; (d) nonsubstrates for the cleavage reaction will not bind **2a**; (e) features that make the bulge pocket more stable will increase cutting and **2a** binding, e.g., length, position of bulge vs the end; (f) the loop is not important for cutting of single-stranded DNA *per se* but is for the stability of the cleavage pocket and can be replaced by an artificial linker or by sufficient duplex regions on either side of the bulge in bistranded DNA (see later); (g) the base-catalyzed cleavage reaction and binding of **2a** are specific for the bulged structures and are not observed for double-stranded DNA (of the same sequence). Thus, the structures shown in Figure 3, which have been shown earlier to be substrates for single-site cleavage at T_{16} by NCS-Chrom in the thiol-independent reaction, bind **2a**. When T_{17} was replaced by a C residue and the A_6•T_{17} base pair was replaced by a G•C base pair, substrate competence for cleavage was lost (Kappen & Goldberg, 1993b) and no binding was observed (Figure 6D,E). As shown in Figure 7A and Table 1, the tightness of binding of **2a** depends on the length of the base-paired region of the oligonucleotide, presumably reflecting the stability of the bulged structure.

So long as an oligomer duplex, formed by two separated strands, can form the bulge with a similar sequence environment to the 22-mer, it is a substrate for the thiol-independent cleavage reaction (Kappen & Goldberg, 1993b) and binds **2a**. Thus, the double-stranded structure containing the bulge

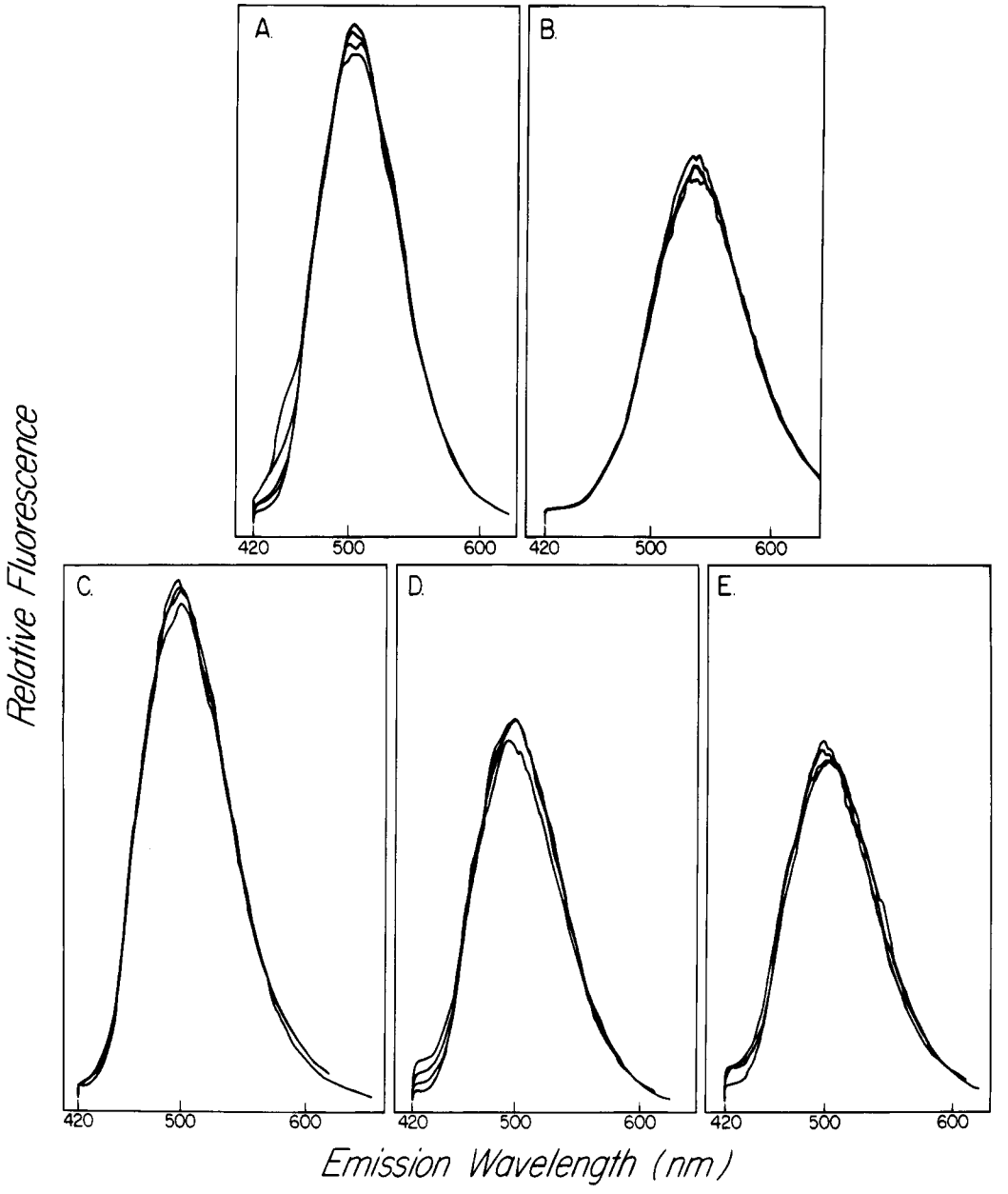


FIGURE 6: Effect of different DNAs on NCS-Chrom product fluorescence intensity. Various amounts of 22-mer (1−30 μM oligomer) were added to a solution containing 1.2 μM (A) **2b** and (B) **3**. Various amounts of (40−320 μM phosphate) calf thymus DNA (C), (1−30 μM) GC-substituted 22-mer (D), and (1−30 μM) C-substituted 22-mer (E) were added to 1.2 μM **2a**.

Table 1: Binding Parameters for **2a**−DNA Interaction[a]

DNA	K_d(× 10^{-6} M)	DNA	K_d (×10^{-6} M)
28-mer	2.1	12,14	3.4
22-mer (+)	2.9	10, 12	9.6
22-mer (−)	3.5	8, 10	NB
21-mer	7.5	6-L-8	103
20-mer	18.0	6-L-8B	NB
18-mer	16.0	7-L-9	15.0
16-mer	NB[b]	8-L-10	3.9

[a] Experiments were carried out at pH 5.2 (5 mM sodium acetate buffer containing 1% v/v methanol) and 5 °C. K_d was determined by Scatchard analysis. [b] NB, no binding.

formed by annealing of the 14-mer and 12-mer (12,14-duplex) shown in Figure 3 is a strong binding substrate (Figure 7B). Again, the stability of the duplex structure is critical in determining the binding affinity, as shown by the decreased binding to the 10,12-duplex (Figure 7B) and the absence of binding to the 8,10-duplex (data not shown). These data clearly show that, as was true for the cleavage reaction, binding of **2a** does not require a loop structure. On the other hand, duplex DNAs lacking a bulge are not substrates for cleavage (Kappen & Goldberg, 1993b) or binding. Double-stranded calf thymus DNA failed to bind **2a** (Figure 6C). Similarly, duplex DNA made by annealing the 22-mer with its complementary sequence does not bind **2a** (Figure 8). When the 22-mer was incompletely annealed with its complement (in the presence of 25 mM Tris·HCl), binding was intermediate between the results found with complete annealing (100 mM Tris·HCl) and with the single-stranded 22-mer. As expected, the complementary (negative) strand of the 22-mer is able to form the same bulge structure and accordingly binds **2a** tightly (K_d = 3.5 μM), although not quite as well as the positive strand. This result follows because the bulge is symmetric compared to its ends; the situation is different than for double-stranded DNA where there is a specific directionality connected with the known binding sites. These results are in agreement with earlier cleavage experiments (Kappen & Goldberg, 1993a,b).

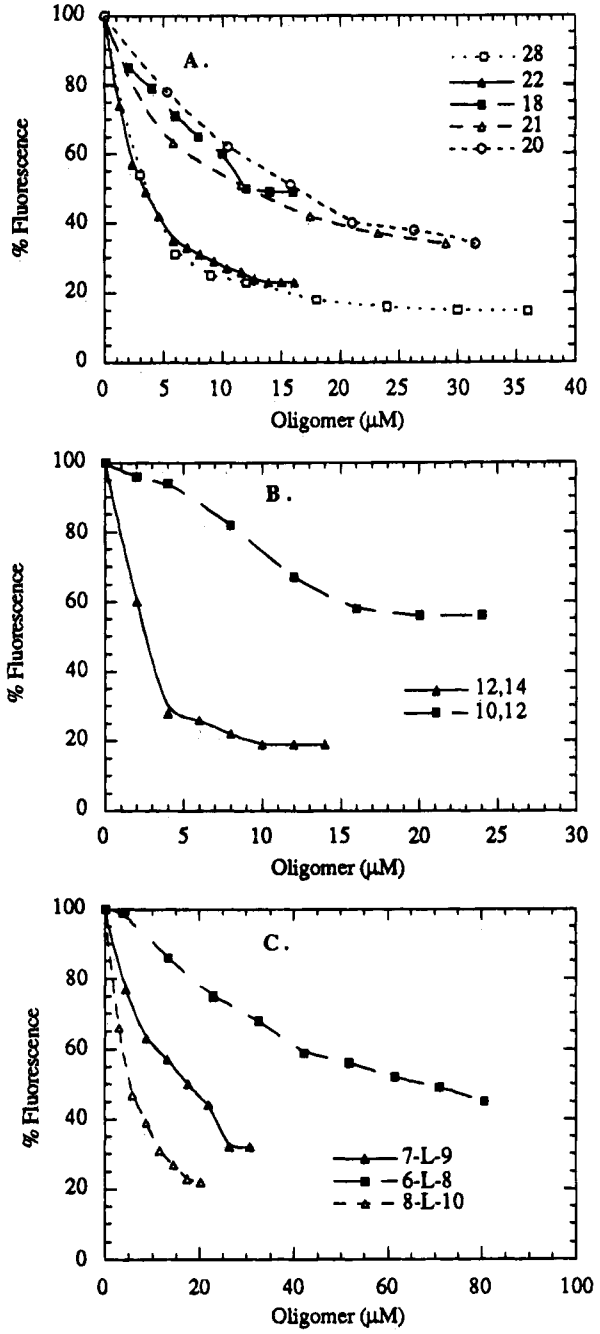


FIGURE 7: Fluorescence quenching of **2a** by bulged DNAs. (A) Single-stranded DNAs, (B) double-stranded DNAs, and (C) linker DNAs were added to 1.2 μM **2a** solution. Experimental conditions were the same as in Figure 4. Concentrations of DNA are as indicated. The structures of the indicated oligomers are shown in Figure 3.

As part of an effort to develop bulged DNA substrates of minimal size and complexity for the study of complex formation by ^{1}H NMR, we have examined the ability of short oligonucleotides joined in the 3′ to 5′ direction by a chemical linker (Figure 9) (Durand et al., 1990) instead of a looped structure to bind **2a**. The linker (triethyleneglycol) contains 9 atoms and is equivalent to 3 nucleotides in length. As shown in Table 1 and Figure 7C, tightest binding was obtained with linked oligonucleotides containing 8 and 10 nucleotides (K_d = 3.9 μM). Removal of base pairs from either side of the linked system (6-L-8, 7-L-9, and 6-L-8B) diminished binding substantially (Table 1 and Figure 7C). It is of significance for the goal of this study that 8-L-10 is about as good a binding substrate as the longer 22-mer and

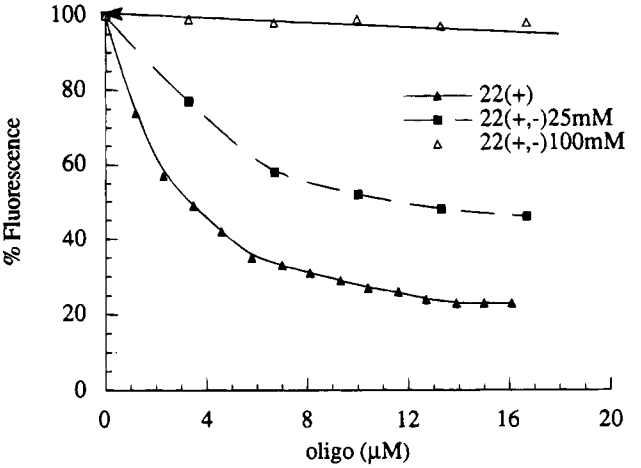


FIGURE 8: Fluorescence quenching of **2a** by single- and double-stranded DNA. Drug product **2a** (1.2 μM) was added to 22-mer (▲), to its complementary strand (□), and to the 22-mer annealed with its complement at a 1:1 ratio at pH 7.0 in 25 mM Tris·HCl (■) and 100 mM Tris·HCl (△).

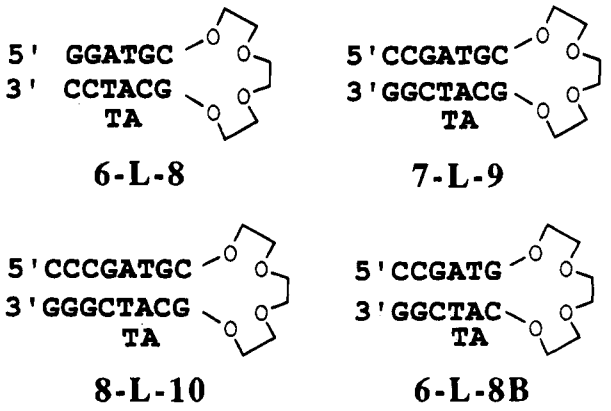


FIGURE 9: Linker oligomer structures.

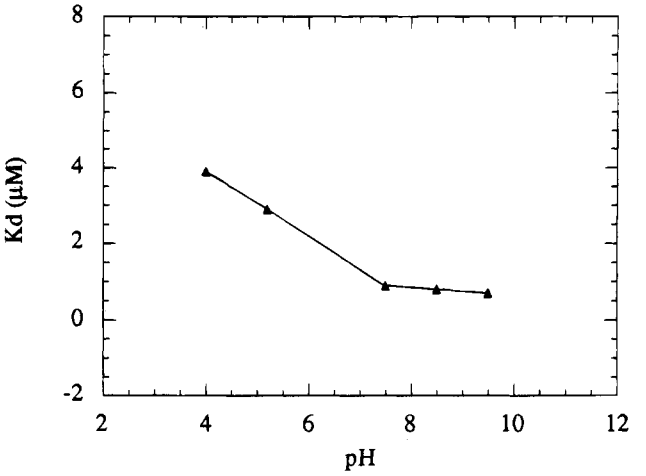


FIGURE 10: Effect of pH on binding. Fluorescence quenching of 1.2 μM **2a** (1% methanol) with DNA 22-mer (1−15 μM) at pH 4.5 and pH 5.5 were conducted in 5 mM sodium acetate, and the assays at pH 7.5, 8.5, and 9.5 were conducted in 5 mM Tris·HCl. Binding constants were calculated from Scatchard plots.

12,14-duplex. Clearly, the presence of the linker enhances the stability of the bulged structure, replacing the effect of the loop in the 22-mer.

Effect of pH on Binding. Although it is clear that the thiol-independent cleavage at a DNA bulge site is general base catalyzed, the possible effect of pH on the binding of the activated drug to its site has not been studied. As seen in

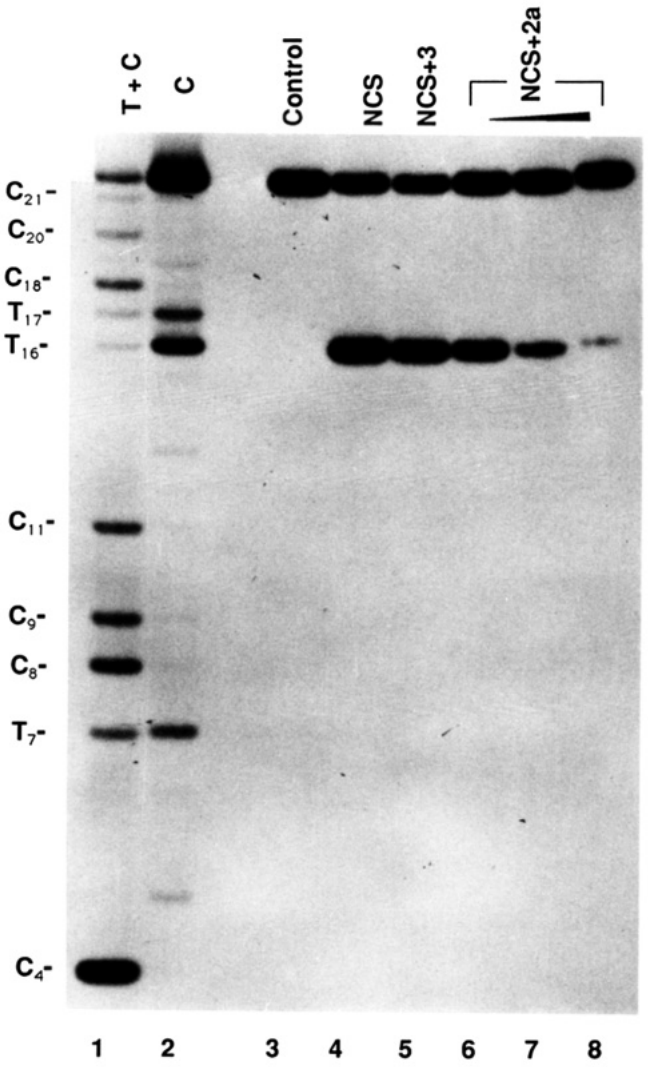


FIGURE 11: Effect of **2a** and **3** on the NCS-Chrom-induced cleavage reaction. 5′-^{32}P-end-labeled 22-mer (5 μM oligomer) was treated with NCS-Chrom (10 μM) in the presence of **3** and **2a** under the conditions described in Materials and Methods. The treated DNA was resolved on a 20% sequencing gel. Lane 1, T+C marker; lane 2, C marker; lane 3, no drug control; lane 4, NCS-Chrom; lane 5, NCS-Chrom in the presence of **3** (50 μM); lanes 6−8, NCS-Chrom in the presence of **2a** (5, 10, and 20 μM, respectively).

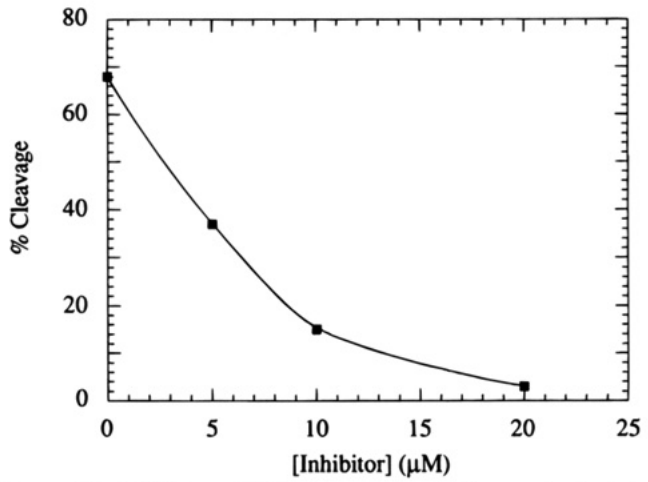


FIGURE 12: Inhibition of NCS-Chrom-induced cleavage by **2a**. The percent cleavage of the DNA in Figure 11, determined by phosphorimager quantitation, is plotted as a function of concentration of **2a**.

Figure 10, the K_d is slightly lower at pHs above 7.5 than in the acidic range. Since the only ionizable group on **2a**, the methylamino moiety on the sugar, has a pK_a on the order of 8.5 (DeVoss et al., 1990), only 5% would be protonated at pH 9.5 where the K_d is at a minimum. In separate experiments, it was found that the fluorescence of **2a** was not diminished at the higher pH over the time period of the experiment. These results are consistent with the pH profile for the cleavage reaction and indicate that the charge on the amino moiety of the sugar is not important for the binding of **2a** and, by inference, for the cleavage reaction.

Specific Inhibition of NCS-Chrom-Induced Bulge Cleavage by **2a**. If **2a** serves as a mimic for the activated form of NCS-Chrom involved in the bulge-specific cleavage reaction, it should compete for binding with this species. Analysis of the extent of cleavage at the bulge site by use of a sequencing gel demonstrates that NCS-Chrom-induced cleavage is almost completely inhibited at about 20 μM **2a**, which is twice the concentration of NCS-Chrom (Figures 11 and 12). Significant inhibition can be seen at 5 μM **2a** (K_I = 5.4 μM). By contrast, as much as 50 μM **3** failed to inhibit the cleavage reaction. The lack of effect of **3** further shows that there is no product inhibition of the reaction and confirms the fluorescence experiments.

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REFERENCES

Chin, D.-H., Kappen, L. S., & Goldberg, I. H. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7070−7074.

Dasgupta, D., & Goldberg, I. H. (1985) *Biochemistry 24*, 6913−6920.

Dasgupta, D., Auld, D. S., & Goldberg, I. H. (1985) *Biochemistry 24*, 7049−7054.

DeVoss, J. J., Hangeland, J. J., & Townsend, C. A. (1990) *J. Am. Chem. Soc. 112*, 4554−4556.

Durand, M., Chevrie, K., Chassignol, M., Thuong, N. T., & Maurizot, J. C. (1990) *Nucleic Acids Res. 18*, 6353−6359.

Gao, X., Stassinopoulos, A., Rice, J. S., & Goldberg, I. H. (1995) *Biochemistry 34*, 40−49.

Goldberg, I. H. (1991) *Acc. Chem. Res. 24*, 191−198.

Hensens, O. D., Helms, G. L., Zink, D. L., Chin, D.-H., Kappen, L. S., & Goldberg, I. H. (1993) *J. Am. Chem. Soc. 115*, 11030−11031.

Hensens, O. D., Chin, D.-H., Stassinopoulos, A., Zink, D. L., Kappen, L. S., & Goldberg, I. H. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 4534−4538.

Kappen, L. S., & Goldberg, I. H. (1993a) *Science 261*, 1319−1321.

Kappen, L. S., & Goldberg, I. H. (1993b) *Biochemistry 32*, 1319−13145.

Maniatis, T., Fritsch, E., & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Myers, A. G. (1987) *Tetrahedron Lett. 28*, 4493−4496.

Myers, A. G., Cohen, S. B., & Kwon, B.-M. (1994) *J. Am. Chem. Soc. 116*, 1670−1682.

Napier, M. A., Holmquist, B., Strydom, D. J., & Goldberg, I. H. (1981) *Biochemistry 20*, 5602−5608.

Povirk, L. F., & Goldberg, I. H. (1980) *Biochemistry 19*, 4773−4780.

Povirk, L. F., Dattagupta, N., Warf, B. C., & Goldberg, I. H. (1981) *Biochemistry 20*, 4007−4014.

Stassinopoulos, A., & Goldberg, I. H. (1995) *Bioorg. Med. Chem.* (in press).

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