

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/20698916>

# Selective strand scission by intercalating drugs at DNA bulges

ARTICLE *in* BIOCHEMISTRY · MAY 1988

Impact Factor: 3.02 · DOI: 10.1021/bi00408a051 · Source: PubMed

---

CITATIONS

73

---

READS

8

2 AUTHORS, INCLUDING:



[Loren Dean Williams](#)

Georgia Institute of Technology

110 PUBLICATIONS 5,076 CITATIONS

SEE PROFILE

- McHenry, C. S. (1985) *Mol. Cell. Biochem.* 66, 71-85.
- Mikhailov, V. S., & Androsova, I. M. (1984) *Biochim. Biophys. Acta* 783, 6-14.
- Mosbaugh, D. W., & Linn, S. (1984) *J. Biol. Chem.* 259, 10247-10251.
- Peneffsky, H. S. (1977) *J. Biol. Chem.* 252, 2891-2899.
- Prelich, G., Kostura, M., Marshak, D. R., Mathews, M. B., & Stillman, B. (1987a) *Nature (London)* 326, 471-475.
- Prelich, G., Tan, C.-K., Kostura, M., Mathews, M. B., So, A. G., Downey, K. M., & Stillman, B. (1987b) *Nature (London)* 326, 517-520.
- Tabor, S., & Richardson, C. C. (1987) *Proc. Natl. Acad. Sci. U.S.A.* 84, 4767-4771.
- Tan, C.-K., Castillo, C., So, A. G., & Downey, K. M. (1986) *J. Biol. Chem.* 261, 12310-12316.
- Tan, C.-K., So, M. J., Downey, K. M., & So, A. G. (1987) *Nucleic Acids Res.* 15, 2269-2278.
- Villani, G., Fay, P. J., Bambara, R. A., & Lehman, I. R. (1981) *J. Biol. Chem.* 256, 8202-8207.
- Wahl, A. F., Kowalski, S. P., Harwell, L. W., Lord, E. M., & Bambara, R. A. (1984) *Biochemistry* 23, 1895-1899.
- Wahl, A. F., Crute, J. J., Sabatino, R. D., Bodner, J. B., Marraccino, R. L., Harwell, L. W., Lord, E. M., & Bambara, R. A. (1986) *Biochemistry* 25, 7821-7827.
- Wang, T. S.-F., & Korn, D. (1980) *Biochemistry* 19, 1782-1790.
- Wierowski, J. V., Lawton, K. C., Hockensmith, J. W., & Bambara, R. A. (1983) *J. Biol. Chem.* 258, 6250-6254.

## Selective Strand Scission by Intercalating Drugs at DNA Bulges<sup>†</sup>

Loren Dean Williams<sup>\*‡</sup> and Irving H. Goldberg<sup>\*</sup>

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

Received September 18, 1987; Revised Manuscript Received December 1, 1987

**ABSTRACT:** A bulge is an extra, unpaired nucleotide on one strand of a DNA double helix. This paper describes bulge-specific strand scission by the DNA intercalating/cleaving drugs neocarzinostatin chromophore (NCS-C), bleomycin (BLM), and methidiumpropyl-EDTA (MPE). For this study we have constructed a series of 5'-<sup>32</sup>P end labeled oligonucleotide duplexes that are identical except for the location of a bulge. In each successive duplex of the series, a bulge has been shifted stepwise up (from 5' to 3') one strand of the duplex. Similarly, in each successive duplex of the series, sites of bulge-specific scission and protection were observed to shift in a stepwise manner. The results show that throughout the series of bulged duplexes NCS-C causes specific scission at a site near a bulge, BLM causes specific scission at a site near a bulge, and MPE-Fe(II) causes specific scission centered around the bulge. In some sequences, NCS-C and BLM each cause bulge-specific scission at second sites. Further, bulged DNA shows sites of protection from NCS-C and BLM scission. The results are consistent with a model of bulged DNA with (1) a high-stability intercalation site at the bulge, (2) in some sequences, a second high-stability intercalation site adjacent to the first site, and (3) two sites of relatively unstable intercalation that flank the two stable intercalation sites. On the basis of our results, we propose a new model of the BLM/DNA complex with the site of intercalation on the 3' side (not in the center) of the dinucleotide that determines BLM binding specificity. It appears that specific scission at DNA bulges can be employed as a general assay for intercalation and binding orientation.

**D**NA double helices that contain single nucleotide bulges are of interest in part because of evidence that bulges are intermediates in the process of frameshift mutagenesis (Streisinger et al., 1966). A bulge, defined as an extra, unpaired nucleotide on one strand of the double helix, is thought to be stabilized by intercalating drugs (Nelson & Tinoco, 1985). Thermodynamic stabilization of bulges may be the basis of the frameshift mutagenic properties of many intercalators (Brockman & Gobin, 1965; Ames & Whitfield, 1966).

We have investigated the scission specificity near DNA bulges of a series of drugs that intercalate into and cleave DNA. The data provide information on binding in the vicinity

of a bulge. The results are consistent with a new model describing intercalation stabilities at several sites in the vicinity of a bulge. From this model of bulged DNA, we have deduced an unexpected orientation of the bleomycin/DNA complex. It appears that specific scission at DNA bulges can be employed as a general assay for intercalation and binding orientation of any agent that cleaves DNA.

The following three drugs have been employed in this study: (1) Neocarzinostatin chromophore (NCS-C) is an antitumor antibiotic that damages DNA by causing O<sub>2</sub>- and thiol-dependent single-strand breaks (Kappen & Goldberg, 1978; Burger et al., 1978). DNA strand scission, with specificity thymine > adenine >> cytosine > guanine (Hatayama et al., 1978; D'Andrea & Haseltine, 1978; Takeshita et al., 1981), occurs via free radical abstraction of a hydrogen from the 5' carbon of the deoxyribose (Kappen & Goldberg, 1985). It has been shown that the substituted naphthoate moiety of NCS-C intercalates into DNA (Povirk et al., 1981), and this is expected to anchor the drug such that the active site is in

<sup>†</sup>This research was supported by American Cancer Society Grant PF-3062 and National Institutes of Health Grant GM 12573.

<sup>\*</sup>Address correspondence to either author.

<sup>‡</sup>Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

close proximity to the 5' carbon of the deoxyribose. The reasons for the base selectivity of strand scission are not understood.

(2) Bleomycin (BLM) represents a family of closely related glycopeptides with antitumor antibiotic activity. BLM causes Fe(II)- and O<sub>2</sub>-dependent DNA strand scission (Sausville et al., 1978; Horwitz et al., 1979), preferentially at (5' → 3') purine-pyrimidine sequences (Takeshita et al., 1978; D'Andrea & Haseltine, 1978; Takeshita et al., 1981). Strand scission by BLM is thought to result from free radical abstraction of a hydrogen from the 4' carbon of deoxyribose (Wu et al., 1985). BLM is composed of two domains, the DNA cleaving domain that chelates Fe(II) and the C-terminal fragment that binds to DNA (Chien et al., 1977; Lin & Grollman, 1981; Fisher et al., 1985). Intercalation by at least one of the C-terminal bithiazole moieties may be involved in the stable binding of BLM to DNA (Lin & Grollman, 1981; Fisher et al., 1985; Henichart et al., 1985). The reasons for the dinucleotide sequence selectivity of strand scission are not understood.

(3) Methidiumpropyl-EDTA (MPE), a metal chelator tethered to a DNA intercalator, cleaves DNA in the presence of Fe(II) and O<sub>2</sub> (Hertzberg & Dervan, 1984). In contrast to NCS-C and BLM, MPE-Fe(II) cleaves DNA with little base or sequence preference (Van Dyke & Dervan, 1982). In further contrast to NCS-C and BLM, MPE-Fe(II) causes strand scission via a nonspecific mechanism involving the generation of diffusible hydroxyl radical (Hertzberg & Dervan, 1982). It has been shown that the analogue MPE-Mg(II) intercalates into DNA, although binding outside the helix is preferred (Hertzberg & Dervan, 1982).

For this study we have constructed a series of DNA duplexes that are identical except for the location of a bulge (Figure 1). In each succeeding duplex of the series, the bulge has been shifted stepwise up (from 5' to 3') one strand of the duplex. The series of duplexes was treated with the three DNA cleaving drugs described above. Sites of bulge-specific cleavage or bulge-specific protection from cleavage were expected to shift in a stepwise manner in each succeeding duplex of the series.

## MATERIALS AND METHODS

**Oligonucleotide Synthesis, Purification, and 5'-<sup>32</sup>P-Phosphorylation.** Oligonucleotides were synthesized manually by the solid-phase phosphite triester method (Caruthers, 1982) using fully protected 5'-O-(dimethoxytrityl)-2'-deoxyribonucleoside 3'-(β-cyanoethyl N,N-diisopropylphosphoramidites) (American Bionetics, Emeryville, CA). The capping series of reactions was omitted. Analogous oligonucleotides were synthesized simultaneously in sets of three by linking three 2'-deoxycytidine long chain alkylamino controlled pore glass columns (American Bionetics) in series. To add the bulged out nucleotide, one column was separated from the other two after deprotection and brought through an additional cycle. The columns were then rejoined to continue simultaneous synthesis. After completion of synthesis, the final dimethoxytrityl group was removed by acid treatment, and the oligonucleotides were deprotected and cleaved by base from the solid support (Caruthers, 1982). After extraction with diethyl ether, the oligonucleotides were purified with preparative high-performance reverse-phase thin-layer chromatography (Analtech, Newark, DE) in two successive developments of 15% and then 20% methanol in 0.10 M triethylammonium bicarbonate buffer. Oligonucleotides were visualized with short-wave ultraviolet light and obtained in over 90% purity by extraction of silica scrappings with 50% methanol in 1.0

M triethylammonium bicarbonate buffer. Oligonucleotide 5'-<sup>32</sup>P-phosphorylation with T4 polynucleotide kinase (New England Biolabs, Beverly, MA) and [γ-<sup>32</sup>P]ATP (New England Nuclear, Boston) and final purification by gel electrophoresis were conducted as previously described (Sekiya et al., 1976; Lo et al., 1984). The sequences of all oligonucleotides were confirmed by chemical sequencing techniques (Maxam & Gilbert, 1980).

**DNA Cleavage Reactions.** Equimolar ratios of two complementary strands of DNA were mixed in 100 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 8.0, 5 times the desired final NaCl reaction concentration and a volume of 0.2 mL. In addition, each sample contained approximately 10<sup>7</sup> dpm of one strand of the appropriate 5'-<sup>32</sup>P end labeled oligonucleotide. The DNA was annealed by slow cooling from 92 °C to room temperature, and reactions were performed on 4-μL aliquots that were diluted to 20 μL. Reactions were terminated by the addition of 20 μL of 1.0 mM (ethylenedinitrilo)tetraacetic acid (EDTA), 80% formamide, and 0.5 mg/mL xylene cyanol in water, immediately heated to 92 °C for 2 min, and loaded onto 20% (1:20 cross-linked) polyacrylamide denaturing (50% urea) gels. Autoradiography of the gels was conducted at -70 °C on Kodak X-Omat AR film.

**NCS-C Reaction Conditions.** NCS-C was isolated from the protein component by extraction with methanol as previously described (Povirk et al., 1981; Povirk & Goldberg, 1983) and stored at -70 °C. Aqueous glutathione and then methanolic chromophore were added to the DNA solution to give final concentrations of 150 μM DNA base pairs, 20 mM Tris-HCl, pH 8.0, 5 mM glutathione, and 30 μM NCS-C and a methanol concentration of less than 10%. Reactions were conducted in a volume of 20 μL at 4 °C for 1 h in the dark.

**BLM Reaction Conditions.** BLM (Bleoxane) was a generous gift from Dr. W. T. Bradner of Bristol Laboratories. A typical BLM cleavage reaction contained 150 μM DNA base pairs, 20 mM Tris-HCl, pH 8.0, 10 μM BLM, 10 μM ferrous ammonium sulfate, and 2 mM dithiothreitol. Each reaction was conducted at both 0 and 100 mM NaCl. A freshly prepared solution of ferrous ammonium sulfate was mixed with BLM in 1:1 stoichiometry to make a relatively concentrated stock solution that was quickly diluted and added to the DNA solution. Dithiothreitol was added to the reaction mixture last. The reactions were allowed to proceed for 1 h at room temperature. Some reactions were repeated at 4 °C.

**MPE-Fe(II) Reaction Conditions.** MPE was a generous gift from Dr. Peter Dervan. A typical MPE-Fe(II) cleavage reaction contained 150 μM DNA base pairs, 20 mM Tris-HCl, pH 8.0, 10 μM MPE, 10 μM ferrous ammonium sulfate, and 2 mM dithiothreitol. Each reaction was conducted at both 0 and 100 mM NaCl. Following published procedures (Hertzberg & Dervan, 1984), a freshly prepared solution of ferrous ammonium sulfate was mixed with MPE in 1:1 stoichiometry to make a relatively concentrated stock solution that was quickly diluted and added to the DNA solution. Dithiothreitol was added to the reaction mixture last. The reactions were allowed to proceed for 1 h at room temperature.

## RESULTS

An analogous series of nine oligonucleotide duplexes (Figure 1) has been constructed for the purposes of this investigation. The plus strand, a dodecamer, is d(CGACCCAAATGC) in each oligonucleotide duplex (1-9) of the series.

The minus strand of duplex 1 (Figure 1, number 1) is the dodecamer d(GCATTGGGTCG) that is complementary to the plus strand. Duplex 1 does not contain a bulge and will

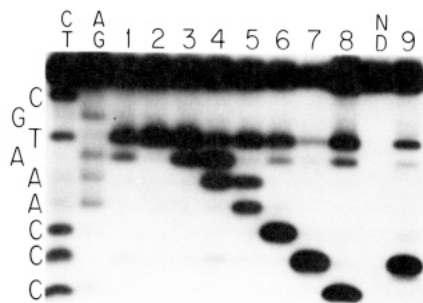
5' 3'									
CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
GC	GC	GC	GC	GC	GC	GC	GC	GC	GC
AT	AT	AT	AT	AT	AT	AT	AT	AT	AT
CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
AT	AT	AT	AT	AT	AT	AT	AT	AT	AT
AT	AT	AT	AT	AT	AT	AT	AT	AT	AT
AT	AT	AT	AT	AT	AT	AT	AT	AT	AT
TA	TA	TA	TA	TA	TA	TA	TA	TA	TA
GC	GC	GC	GC	GC	GC	GC	GC	GC	GC
CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
1	2	3	4	5	6	7	8	9	

FIGURE 1: Series of DNA duplexes used in this study.

5'	3'
C(1)	G(24)
G(2)	C(23)
A(3)	T(22)
C(4)	G(21)
C(5)	G(20)
C(6)	G(19)
A(7)	T(18)
A(8)	T(17)
A(9)	T(16)
T(10)	A(15)
G(11)	C(14)
C(12)	G(13)
3'	5'

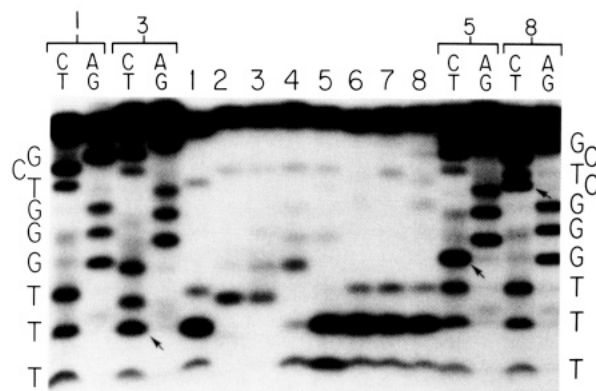
plus strand                      minus strand

FIGURE 2: Oligonucleotide numbering scheme.

FIGURE 3: Autoradiogram of a denaturing 20% acrylamide gel of NCS-C scission reactions as described under Materials and Methods. The plus strands of duplexes 1-9 have been labeled at the 5'-end with  $^{32}\text{P}$ . C+T and A+G, Maxam and Gilbert sequencing reactions; lanes 1-9, reactions of duplexes 1-9, respectively; ND, (no drug) control reaction that contains all reactants and cofactors except NCS-C.

be referred to as the reference duplex. The minus strands of duplexes 2-8 (Figure 1, numbers 2-8) are each a 13-mer that is complementary to the plus strand with the exception of a single extra cytidine residue. Starting with duplex 2, in each successive member of the series the extra cytidine is progressively shifted one residue in the 3' direction. The minus strand of duplex 2 (Figure 1) is complementary to the plus strand with the exception of an extra C between A(15) and T(16) (see Figure 2 for the numbering scheme). The minus strand of duplex 3 is complementary to the plus strand with the exception of an extra C between T(16) and T(17). The same pattern is continued through duplex 8, in which the minus strand has an extra C between G(21) and T(22). The final member of the series is duplex 9, which is similar to duplex 7 except the minus strand has a extra T rather than C between G(20) and G(21).

**Reaction of NCS-C with Bulged DNA.** (1) *Oligonucleotides Are Models of DNA Polymers for NCS-C Scission Reactions.* Products from the reaction of the reference duplex with NCS-C are shown in lane 1 of Figure 3 (plus-strand reaction products) and lane 1 of Figure 4 (minus-strand reaction products). The plus strand of the reference duplex has been cleaved intensely by NCS-C at T(10), the only T in the plus strand. NCS-C cleaves moderately at A(9) and slightly at C(5). No strand scission is observed at G residues

FIGURE 4: Autoradiogram of a denaturing 20% acrylamide gel of NCS-C scission reactions as described under Materials and Methods. The minus strands of duplexes 1-8 have been labeled at the 5'-end with  $^{32}\text{P}$ . C+T and A+G, Maxam and Gilbert sequencing reactions (the numbers on top indicate the correspondence between duplex number and each pair of sequencing lanes; the arrows indicate the bulged out C); lanes 1-8, reactions of duplexes 1-8, respectively. The sequences of duplexes numbers 1 and 8 are shown on the left and right side of the autoradiogram, respectively.

of the plus strand. The minus strand of the reference duplex has been cleaved intensely at T(17), moderately at T(16), T(18), and T(22), and slightly at A(15) [A(15) reaction products not shown]. No strand scission is observed at C or G residues of the minus strand of the reference duplex. The base specificity of NCS-C scission of a normal oligonucleotide reported here ( $\text{T} > \text{A} \gg \text{C} > \text{G}$ ) is the same as the previously reported base specificity of NCS-C scission of DNA restriction fragments (Hatayama et al., 1978; D'Andrea & Haseltine, 1978; Takeshita et al., 1981).

(2) *NCS-C Causes Specific Strand Scission near DNA Bulges.* Products from the reaction of the series of bulged duplexes with NCS-C are shown in lanes 2-9 of Figure 3 (plus-strand reaction products) and lanes 2-8 of Figure 4 (minus-strand reaction products, duplex 9 reaction products not shown). In each bulged duplex (Figure 1, numbers 2-9), the strand opposite the bulge has been cleaved by NCS-C at the residue adjacent to the bulge on the 3' side. Strand scission is observed at T(10) of duplex 2, A(9) of duplex 3, A(8) of duplex 4, etc. The extent of strand scission at this bulge-specific scission site is greater than or equal to that at normal sites of NCS-C scission [i.e., T(10) of the reference duplex].

The identity of the bulged out residue does not appear to be a significant factor in recognition of the bulge by NCS-C. The bulged out C between G(20) and G(21) of duplex 7 has been replaced by a T in duplex 9. The pattern of scission of the plus strand of duplex 7 (Figure 3, lane 7) is virtually indistinguishable from that of duplex 9 (Figure 3, lane 9). As duplex 9 shows the same behavior as duplex 7, duplex 9 will not be discussed further in this paper. The effect of a bulged out purine has not been investigated.

(3) *NCS-C Causes Specific Strand Scission in Some Sequences at a Second Site near DNA Bulges.* In bulged duplexes 3-5, the strand opposite the bulge has been cleaved by NCS-C at a site located two residues to the 3' side of the bulge. In duplex 4, residue A(9) has been cleaved intensely, while in the reference duplex A(9) has been cleaved only moderately. In duplex 5, residue A(8) has been cleaved moderately, while in the reference duplex A(8) has not been cleaved. In duplex 3, residue T(10) has been cleaved intensely; however, the data from duplex 3 are ambiguous because T(10) has also been cleaved intensely in the reference duplex.

The extent of scission at this second bulge-specific scission site (in duplexes 3-5) is similar to that observed at the first

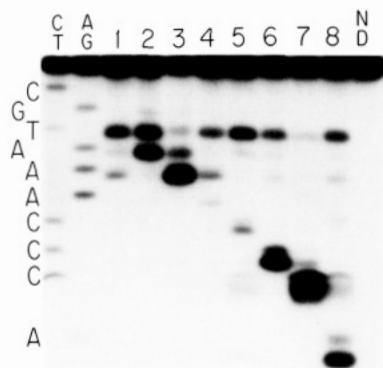


FIGURE 5: Autoradiogram of a denaturing 20% acrylamide gel of BLM scission reactions in 0 mM NaCl at room temperature as described under Materials and Methods. The plus strands of duplexes 1–8 have been labeled at the 5'-end with  $^{32}\text{P}$ . C+T and A+G, Maxam and Gilbert sequencing reactions; lanes 1–9, reactions of duplexes 1–9, respectively; ND, control reaction that contains all reactants and cofactors except BLM.

bulge-specific scission site (as discussed above). However, the second bulge-specific scission site is not observed in duplexes 2 and 6–8. In these duplexes, the plus strand has not been cleaved by NCS-C at the site located two residues to the 3' side of the bulge.

NCS-C does not cause specific scission of the strand with the bulge. Sites of intense scission of the minus strand of duplexes 2–8 correspond to sites of intense scission in the reference duplex (Figure 4, lanes 1–8). In contrast to the scission pattern of the plus strand, there is no prominent fixed (relative to the bulge) site of scission on the minus strand of duplexes 2–8.

(4) *Bulged DNA Is Protected from NCS-C Scission at Specific Sites.* A site of NCS-C strand scission that shows decreased intensity in the proximity of a bulge is considered to be protected by the bulge from strand scission.

On the plus strand of duplexes 2–8, one specific site of protection from strand scission by NCS-C is located three residues to the 3' side of the bulge. None of the bulged duplexes have been cleaved intensely at this site by NCS-C (Figure 3). The extent of scission at T(10) in the reference duplex is considerably reduced in duplex 4. Although this reduction in T(10) scission may not be obvious in Figure 3, it can be clearly seen by direct observation of the autoradiograph. Similarly, the moderate degree of scission at A(9) in the reference duplex has been eliminated in duplex 5.

Another specific site of protection on the plus strand is located adjacent to the bulge on the 5' side. None of the bulged duplexes have been cleaved intensely at this site by NCS-C. The moderate strand scission of A(9) in the reference duplex has been eliminated in duplex 2. A longer exposure (not shown) of the film in Figure 3 reveals that the light scission at C(5) in the reference duplex is eliminated in duplex 6.

On the strand with the bulge, the site adjacent to the bulge on the 5' side is not, in general, accessible to scission by NCS-C. Residue T(16) has been cleaved moderately in the reference duplex (Figure 4, lane 1) but has not been cleaved in duplex 3 (Figure 4, lane 3). Residue T(17) has been cleaved intensely in the reference duplex but has been cleaved only slightly in duplex 4 (Figure 4, lane 4), while T(18) has been cleaved moderately in the reference duplex but has not been cleaved in duplex 5 (Figure 4, lane 5). In addition, the bulged out residue has not been cleaved by NCS-C.

*Reaction of BLM with Bulged DNA. (1) Oligonucleotides Are Models of DNA Polymers for BLM Scission Reactions.*

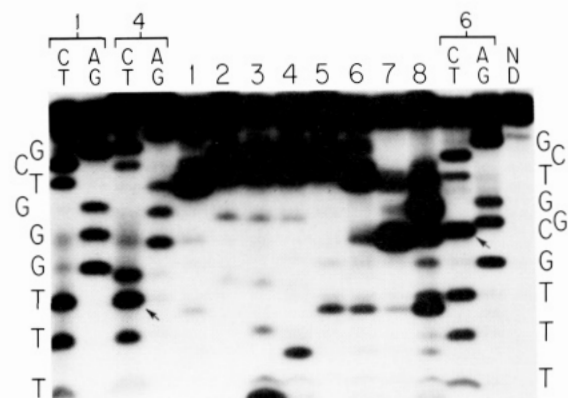


FIGURE 6: Autoradiogram of a denaturing 20% acrylamide gel of BLM scission reactions in 0 mM NaCl at room temperature as described under Materials and Methods. The minus strands of duplexes 1–8 have been labeled at the 5'-end with  $^{32}\text{P}$ . C+T and A+G, Maxam and Gilbert sequencing reactions (the numbers on top indicate the correspondence between duplex number and each pair of sequencing lanes; the arrows indicate the bulged out C); lanes 1–8, reactions of duplexes 1–8, respectively. The sequences of duplexes numbers 1 and 6 are shown on the left and right side of the autoradiogram, respectively.

Products from the reaction of the reference duplex with BLM are shown in lane 1 of Figure 5 (plus-strand reaction products) and lane 1 of Figure 6 (minus-strand reaction products). The plus strand of the reference duplex is cleaved intensely at T in the sequence A(9)–T(10), while the minus strand is cleaved intensely at T in the sequence G(21)–T(22). Minor sites of BLM scission in the reference duplex are at the second residues in the dinucleotides A(7)–A(8) and A(8)–A(9) of the plus strand and T(22)–C(23) and G(19)–G(20) of the minus strand.

BLM has been shown previously to cause strand scission in DNA restriction fragments primarily at pyrimidines in the sequence (5'→3') Pu–Py (D'Andrea & Haseltine, 1978; Takeshita et al., 1978, 1981). In particular, AT and GT dinucleotides are sites of intense BLM scission in DNA restriction fragments. Minor sites of scission in the reference duplex also correspond to minor sites of scission in DNA restriction fragments. Therefore, the dinucleotide specificity of strand scission of oligonucleotides by BLM is the same as the specificity of scission of DNA polymers.

The primary products from BLM scission of restriction fragments are known to migrate more rapidly during gel electrophoresis than the products of Maxam and Gilbert chemical sequencing reactions (Takeshita et al., 1978). BLM scission produces 3'-(phosphoro-2''-O-glycolic acid) termini (Giloni et al., 1981), whereas chemical-sequencing reactions produce 3'-phosphate termini. As can be observed in Figures 5 and 6, products from the reaction of BLM with both normal and bulged oligonucleotides also migrate more rapidly than the products of chemical-sequencing reactions.

The specificity of DNA scission by BLM is not significantly altered by a change in NaCl concentration from 0 to 100 mM or by a change in temperature from 0 to 25 °C. We have observed similar patterns and intensities of cleavage of both normal and bulged DNA by BLM at both NaCl concentrations and both temperatures (not shown).

(2) *BLM Causes Specific Strand Scission near DNA Bulges.* Products from the reaction of the series of bulged duplexes with BLM are shown in lanes 2–8 of Figure 5 (plus-strand reaction products) and lanes 2–8 of Figure 6 (minus-strand reaction products). In each bulged duplex, the strand opposite the bulge is cleaved by BLM at the residue



adjacent to the bulge on the 5' side. Strand scission is observed at A(9) of duplex 2, A(8) of duplex 3, A(7) of duplex 4, etc.

Although strand scission is clearly observable throughout the series of bulged duplexes at this bulge-specific BLM scission site, the extent of scission is highly variable. Duplexes 2, 3, and 6–8 have been cleaved intensely at the bulge-specific BLM scission site, while duplexes 4 and 5 have been cleaved only moderately. In fact, the intensity of scission at the bulge-specific site is reflected in the sequence selectivity observed in the reference duplex. A longer exposure of the autoradiogram in Figure 5 (not shown) reveals that all residues of the reference strand have been cleaved to some extent by BLM, with A(7) being the site of least intense scission. Similarly, duplex 4 [which has A(7) at the bulge-specific scission site) shows the least intense bulge-specific scission.

(3) *BLM Causes Specific Strand Scission in Some Sequences at a Second Site near DNA Bulges.* In bulged duplexes 2–4, BLM has cleaved the strand opposite the bulge at the site adjacent to the bulge on the 3' side. The extent of scission at A(9) is increased in duplex 3 compared to the extent of scission of this residue in the reference duplex. In duplexes 2 and 4, residues T(10) and A(8), respectively, have been cleaved significantly; however, the data from duplexes 2 and 4 are ambiguous because T(10) and A(8) are also sites of scission in the reference duplex.

Scission at this second bulge-specific site is observed in duplexes 2–4 but not in duplexes 5–8. On the basis of the sequence dependence of scission at the second NCS-C bulge-specific site, BLM scission is expected at A(7) of duplex 5. As shown in the previous section, however, A(7) is particularly resistant to BLM cleavage.

In general, sites of intense scission of the minus strand of duplexes 2–8 correspond to sites of intense scission of the reference duplex (Figure 6, lanes 1–8). In contrast to the scission pattern of the plus strand, there is no bulge-specific site of scission on the minus strand. Duplexes 7 and 8 are anomalous and show intense scission of the minus strand at the residue adjacent to the bulge on the 5' side.

(4) *Bulged DNA Is Protected from BLM Scission at Specific Sites.* On the plus strand of duplexes 2–8, one site of protection from scission by BLM is located two residues to the 3' side of the bulge. None of the bulged duplexes have been cleaved intensely at this site by BLM (Figure 5, lanes 2–8). The intense scission at T(10) in the reference duplex has been reduced in duplex 3. The moderate scission at A(8) and A(9) in the reference duplex (Figure 5, lane 1) has been eliminated in duplexes 5 and 4, respectively (Figure 5, lanes 5 and 4).

Another site of protection is on the strand opposite the bulge at the second residue to the 5' side of the bulge. The moderate scission of A(8) in the reference duplex is eliminated in duplex 2. Similarly, a longer exposure of the autoradiograph shows that the slight amount of scission of C(4) in the reference duplex has been eliminated in duplex 6. On the minus strand, the bulged out residue has not been cleaved by BLM.

*Reaction of MPE-Fe(II) with Bulged DNA.* (1) *Oligonucleotides Are Models of DNA Polymers for MPE-Fe(II) Scission Reactions.* Products from the reaction of MPE-Fe(II) with the reference duplex are shown in trace 1 of Figure 7 (plus-strand reaction products) and trace 1 of Figure 8 (minus-strand reaction products). MPE-Fe(II) caused scission at all residues of both the plus and the minus strand of the reference duplex. The intensity of strand scission appears to be slightly greater toward the center of the duplex. Our results are consistent with the previously reported lack of sequence

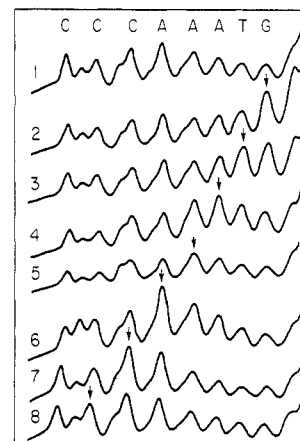


FIGURE 7: Densitometer scan of autoradiogram of a denaturing 20% acrylamide gel of MPE-Fe(II) scission reactions in 0 mM NaCl as described under Materials and Methods. The plus strands of duplexes 1–8 have been labeled at the 5'-end with  $^{32}\text{P}$ . Traces 1–8, reactions of duplexes 1–8, respectively. The arrows indicate sites of enhanced scission.

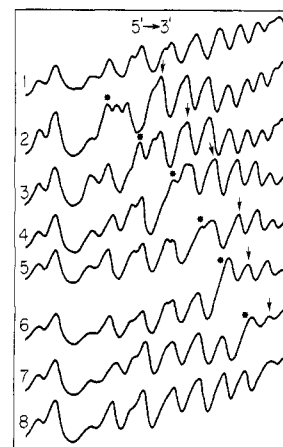


FIGURE 8: Densitometer scan of autoradiogram of a denaturing 20% acrylamide gel of MPE-Fe(II) scission reactions in 0 mM NaCl as described under Materials and Methods. The minus strands of duplexes 1–8 have been labeled on the 5'-end with  $^{32}\text{P}$ . Traces 1–8, reactions of duplexes 1–8, respectively. The arrows indicate sites of enhanced scission. The asterisks indicate the bulged out cytidine.

or base specificity of scission of DNA restriction fragments by MPE-Fe(II) (Van Dyke & Dervan, 1982, 1983).

(2) *MPE-Fe(II) Causes Specific Strand Scission near DNA Bulges.* Products from the reaction of the series of bulged duplexes with MPE-Fe(II) are shown in traces 2–8 of Figure 7 (plus-strand reaction products) and traces 2–8 of Figure 8 (minus-strand reaction products). In the series of bulged duplexes, increased MPE-Fe(II) scission intensity on the plus strand is observed at a site located two residues to the 3' side of the bulge. These sites of increased scission have been indicated by arrows in Figure 7. Increased scission is observed at G(11) of duplex 2, T(10) of duplex 3, A(9) of duplex 4, etc. In addition to the increased intensity of scission observed two residues to the 3' side of the bulge, increased intensity of scission can be seen on either side of this site. This is consistent with a report that unlike NCS-C and BLM, from a single binding site, MPE-Fe(II) is expected to cleave DNA over a range of several nucleotides (Hertzberg & Dervan, 1984). The same pattern of bulge-induced cleavage is observed in reactions at both 0 and 100 mM NaCl.

In the series of bulged duplexes, increased MPE-Fe(II) scission intensity on the minus strand is observed at a site located two residues to the 3' side of the bulge. These sites

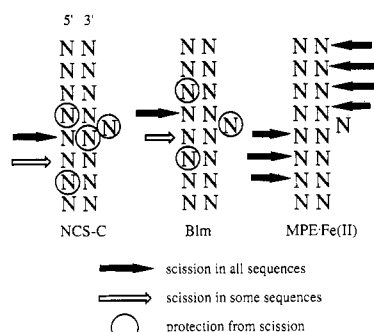


FIGURE 9: Sites of sequence-independent and sequence-dependent strand scission and sites of protection near a bulge.

of increased scission have been indicated by arrows in Figure 8. Increased scission is observed at T(17) of duplex 2, T(18) of duplex 3, G(19) of duplex 4, etc. In general, increased strand scission is also observed on either side of this site. In duplexes 2 and 4, increased scission occurs up to four residues to the 3' side of the bulge. An increase in scission intensity is not observed in duplex 8 due to the proximity of the end of the duplex.

#### DISCUSSION

The results show that NCS-C, BLM, and MPE-Fe(II) cause specific strand scission of bulged DNA (Figure 9). We make the assumption that scission intensity is directly related to binding stability.

A recent paper by Nelson and Tinoco shows that ethidium bromide intercalates with greater stability in bulged DNA than in normal DNA (Nelson & Tinoco, 1985). NCS-C, BLM, and MPE-Fe(II) have radically different structures and mechanisms of action. In the absence of a bulge, NCS-C, BLM, and MPE-Fe(II) cleave DNA with dissimilar base and sequence specificities, suggesting that the three drugs recognize different structural features of DNA. Therefore, it is striking that NCS-C, BLM, and MPE-Fe(II) recognize some common feature of bulged DNA. As the three drugs each contain functionalities that are thought to intercalate into DNA, it is likely that specific strand scission of bulged DNA by NCS-C, BLM, and MPE-Fe(II) results from an increase in stability of intercalation of the three drugs in bulged DNA compared to in normal DNA.

(1) *NCS-C Binds with High Stability to DNA Bulges.* The NCS-C scission pattern (Figure 9) indicates that there is a high-stability NCS-C binding site at a bulge (see below) in all sequences. The stability of NCS-C binding at this site is not significantly affected by the identity of the residue at the scission site, by the identity of the bulged out residue, or by flanking sequence. Further, the stability of NCS-C binding at a bulge is at least as great as the stability of normal binding of NCS-C at thymidine residues. Thus, in bulged DNA, the NCS-C/DNA complex appears to be stabilized primarily by intercalation, indicating that the main determinant of NCS-C scission specificity of normal DNA is relative intercalation stability.

(2) *BLM Binds with Increased Stability to DNA Bulges Compared to Normal DNA.* The BLM scission pattern (Figure 9) indicates that a BLM-binding site at a bulge occurs in all sequences. However, the sequence flanking the bulge also has a significant effect on BLM-binding stability at this site. It appears that intercalation provides a significant contribution to the stability of the BLM/DNA complex. However, other interactions clearly affect BLM-binding stability.

(3) *MPE-Fe(II) Binds with Increased Stability to DNA Bulges Compared to Normal DNA.* The MPE-Fe(II) scission

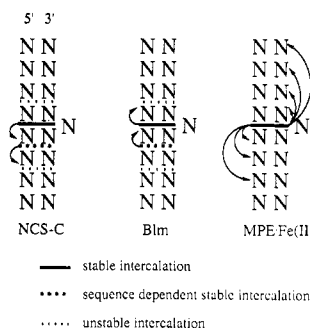


FIGURE 10: Intercalation and cleavage sites near a bulge.

pattern (asymmetric on opposite strands, Figure 9) indicates that there is a single high-stability binding site at a bulge. MPE-Fe(II) scission shows less specificity for bulged DNA than does NCS-C or BLM. A likely reason for the low specificity of MPE-Fe(II) scission is that this drug binds to a significant extent to DNA by two modes: an intercalative mode and a nonintercalative mode (Hertzberg & Dervan, 1982). As MPE-Fe(II) cleaves DNA via a nonspecific mechanism (diffusible hydroxyl radical), strand scission should occur from both types of MPE-Fe(II)/DNA complexes. The nonintercalative MPE-Fe(II)/DNA complex is not expected to bind with greater stability to bulged DNA than to normal DNA. Therefore, bulge-specific scission from the intercalative MPE-Fe(II)/DNA complex is superimposed on a pattern of nonspecific scission from the nonintercalative MPE-Fe(II)/DNA complex.

*Model of Bulged DNA.* We propose that bulged DNA contains a high-stability intercalation site located at the bulge. The location of this primary intercalation site has been determined from two independent observations.

The first observation is that the bulge-specific cleavage pattern of MPE-Fe(II) is analogous to the sequence-specific cleavage pattern of penta(*N*-methylpyrrolecarboxamide)-EDTA-Fe(II) [P5E-Fe(II)]. P5E-Fe(II) is a sequence-specific DNA binding agent (Schultz & Dervan, 1983) with the same DNA cleaving domain as MPE-Fe(II). From one binding site, P5E-Fe(II) cleaves three to five residues of each strand in the same pattern (asymmetric on opposite strands) as that reported here for bulge-specific scission by MPE-Fe(II). CPK models show that the center (in three-dimensional space) of MPE-Fe(II) strand scission of bulged DNA is the bulge site.

The second observation is that, in the absence of a bulge, NCS-C intercalates into DNA and cleaves at the deoxyribose that is directly on the 3' side of the intercalation site (S. H. Lee, L. D. Williams, and I. H. Goldberg, unpublished observations). The site of strand scission of bulged DNA by NCS-C (shown in Figure 9) confines the location of the intercalation site to the bulge (Figure 10).

NCS-C and BLM show sites of both enhanced and depressed strand scission in the vicinity of a bulge (Figure 9). In addition to the single bulge-specific binding site observed for each drug throughout the series of bulged duplexes, a second bulge-specific binding site is observed in some sequences. Further, sites of bulge-specific protection from strand scission by NCS-C and BLM are observed. MPE-Fe(II) is a relatively "low-resolution" strand scission drug and does not yield this type of detailed site-specific information.

NCS-C and BLM both produce cleavage patterns consisting of (from 5' to 3' on the strand opposite the bulge) (1) a site of protection, (2) a site of strand scission in all sequences, (3) a site of strand scission in some sequences, and (4) a second site of protection. The similarities in the cleavage patterns of these two dissimilar drugs provide evidence that NCS-C

and BLM recognize the same set of sites of altered intercalation stability near a bulge (Figure 10). The BLM pattern is offset relative to the NCS-C pattern, presumably because the two drugs cleave at different sites relative to the site of intercalation (see below).

From this information, we propose a model of bulged DNA (shown in Figure 10) with a primary (sequence independent, high stability) intercalation site at the bulge. In addition, there is one secondary (sequence dependent, high stability) intercalation site adjacent to the primary intercalation site. Two relatively unstable intercalation sites flank the two stable intercalation sites.

The model proposed in this paper, with a high-stability intercalation site located at a bulge, differs from a recent model with two high-stability intercalation sites located on either side of a bulge (Nelson & Tinoco, 1985). The two-site intercalation model is not consistent with results obtained from three different intercalating drugs [NCS-C, BLM, or MPE-Fe(II)]. None of these intercalating drugs cleaves bulged DNA at two sites such that the nearest-neighbor exclusion limit is satisfied. The three drugs each cause scission primarily at single sites [MPE-Fe(II) causes scission centered around a single site] near a bulge, consistent with our primary-site intercalation model. Further support for our model is a report of enhanced MPE-Fe(II) strand scission centered around a single, albeit ill-defined, site near a bulge in 16S rRNA (Kean et al., 1985).

**Model of the BLM/DNA Complex.** Although the molecular interactions of BLM with DNA are poorly understood, it has been generally assumed that BLM intercalates between the bases of the dinucleotide that determines binding specificity [for example, see Lin and Grollman (1981)]. If this assumption is correct, BLM attacks the ribose on the 3' side of the intercalation site. This is not consistent with our model of bulged DNA. The bulge-specific BLM scission site observed in all sequences is on the strand opposite the bulge at the residue adjacent to the bulge on the 5' side (Figure 9). In our model of bulged DNA, the primary intercalation site is located at the bulge. We conclude that BLM intercalates on the 3' side of the dinucleotide that determines specificity and cleaves at the ribose on the 5' side of the intercalation site, not on the 3' side of the intercalation site. Therefore, while NCS-C shows 3' attack (relative to the site of intercalation), BLM shows 5' attack of DNA (Figure 10).

**The Moving Defect.** For the purposes of this study we have constructed a series of DNA duplexes that are identical except for the location of a bulge (Figure 1). The bulge is shifted stepwise up one DNA strand (from 5' to 3') in each successive member of the series.

This stepwise series of defective duplexes offers considerably greater sensitivity than the usual comparison of a single defective duplex to a normal duplex [for example, see White and Draper (1987)]. This is true in part because a greater number of experiments yields improved "signal/noise" and in part because the stepwise shift in the position of a defect is expected to produce a predictable (stepwise) shift in the position of defect-specific scission/protection sites. A predictable shifting pattern of defect-specific scission/protection provides a more sensitive assay than single examples of defect-specific scission/protection. Further, with a "moving defect", phenomena that are dependent on the sequences flanking a defect can be distinguished from those that are universal to a defect. Indeed, we have observed both classes of behavior in bulged DNA. The reaction of NCS-C and BLM with the series of bulged duplexes shows there are sites of strand scission and strand protection that follow the bulge up the duplex and are always

observed to occur at the same location relative to the bulge. There are also sites of strand scission that are clearly induced by the bulge but only in certain sequences.

A DNA-binding drug is accepted as an intercalator if binding to DNA results in helix extension (measured by viscosity changes), parallel alignment (as indicated by electric dichroism or X-ray diffraction), or DNA unwinding (as measured by changes in superhelicity). It is difficult to observe such changes in DNA properties which result from the binding of a drug that also cleaves the DNA. It appears that specific binding at bulges is a characteristic of intercalative binding [in the single case investigated, a nonintercalating DNA cleaving agent did not cause bulge-specific strand scission (L. D. Williams and I. H. Goldberg, unpublished observations)]. Specific strand scission near a bulge can be monitored to determine if intercalation is the mode of binding of any drug that cleaves DNA. As shown here for BLM, the relationship between the site of intercalation and the site of strand scission becomes immediately obvious.

#### ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Dr. Seok Ho Lee, who developed the procedure for thin-layer chromatographic purification of oligonucleotides. We thank Jeanne Thivierge for technical assistance and Nidhi Gupta Williams and Dr. Barbara Ramsay Shaw for helpful discussions during preparation of the manuscript.

**Registry No.** NCS-C, 81604-85-5; BLM, 11056-06-7; MPE, 90912-87-1.

#### REFERENCES

- Ames, B. N., & Whitfield, H. J., Jr. (1966) *Cold Spring Harbor Symp. Quant. Biol.* 31, 221-225.
- Brockman, H. E., & Gobin, W. (1965) *Science (Washington, D.C.)* 147, 750-751.
- Burger, R. M., Peisach, J., & Horwitz, S. B. (1978) *J. Biol. Chem.* 253, 4830-4832.
- Caruthers, M. H. (1982) in *Chemical and Enzymatic Synthesis of Gene Fragments* (Gasser, H. G., & Lang, A., Eds.) pp 71-79, Verlag Chemie, Weinheim, FRG.
- Chien, M., Grollman, A. P., & Horwitz, S. B. (1977) *Biochemistry* 16, 3641-3647.
- D'Andrea, A. D., & Haseltine, W. A. (1978) *Proc. Natl. Acad. Sci. U.S.A.* 75, 3608-3612.
- Fisher, L. M., Kuroda, R., & Sakai, T. T. (1985) *Biochemistry* 24, 3199-3207.
- Giloni, L., Takeshita, M., Johnson, F., Iden, C., & Grollman, A. P. (1981) *J. Biol. Chem.* 256, 8608-8615.
- Hatayama, T., Goldberg, I. H., Takeshita, M., & Grollman, A. P. (1978) *Proc. Natl. Acad. Sci. U.S.A.* 75, 3603-3607.
- Henichart, J.-P., Bernier, J.-L., Helbecque, N., & Houssin, R. (1985) *Nucleic Acids Res.* 13, 6703-6717.
- Hertzberg, R. P., & Dervan, P. B. (1982) *J. Am. Chem. Soc.* 104, 313-315.
- Hertzberg, R. P., & Dervan, P. B. (1984) *Biochemistry* 23, 3934-3945.
- Horwitz, S. B., Sausville, E. A., & Peisach, J. (1979) in *Bleomycin: Chemical, Biochemical & Biological Aspects* (Hecht, S., Ed.) pp 170-183, Springer, New York.
- Kappen, L. S., & Goldberg, I. H. (1978) *Nucleic Acids Res.* 5, 2959-2967.
- Kappen, L. S., & Goldberg, I. H. (1985) *Nucleic Acids Res.* 13, 1637-1648.
- Kean, J. M., White, S. A., & Draper, D. E. (1985) *Biochemistry* 24, 5062-5070.



- Lin, S. Y., & Grollman, A. P. (1981) *Biochemistry* 20, 7589-7598.
- Lo, K.-M., Jones, S. S., Hackett, N. R., & Khorana, H. G. (1984) *Proc. Natl. Acad. Sci. U.S.A.* 81, 2285-2289.
- Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol.* 65, 499-560.
- Nelson, J. W., & Tinoco, I., Jr. (1985) *Biochemistry* 24, 6416-6421.
- Povirk, L. F., & Goldberg, I. H. (1983) *J. Biol. Chem.* 258, 11763-11767.
- Povirk, L. F., Dattagupta, N., Warf, B. C., & Goldberg, I. H. (1981) *Biochemistry* 20, 4007-4014.
- Sausville, E. A., Stein, R. W., Peisach, J., & Horwitz, S. B. (1978) *Biochemistry* 17, 2746-2754.
- Schultz, P. G., & Dervan, P. B. (1983) *Proc. Natl. Acad. Sci. U.S.A.* 80, 6834-6837.
- Sekiya, T., Besmer, P., Takeya, T., & Khorana, H. G. (1976) *J. Biol. Chem.* 251, 634-641.
- Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E., & Inouye, M. (1966) *Cold Spring Harbor Symp. Quant. Biol.* 31, 77-84.
- Takeshita, M., Grollman, A. P., Ohtsubo, E., & Ohtsubo, H. (1978) *Proc. Natl. Acad. Sci. U.S.A.* 75, 5983-5987.
- Takeshita, M., Kappen, L. S., Grollman, A. P., Eisenberg, M., & Goldberg, I. H. (1981) *Biochemistry* 20, 7599-7606.
- Van Dyke, M. W., & Dervan, P. B. (1982) *Cold Spring Harbor Symp. Quant. Biol.* 47, 347-353.
- White, S. A., & Draper, D. E. (1987) *Nucleic Acids Res.* 15, 4049-4064.
- Wu, J. C., Kozarich, J. W., & Stubbe, J. (1985) *Biochemistry* 24, 7562-7568.

## Sequence Specificity in Photoreaction of Various Psoralen Derivatives with DNA: Role in Biological Activity<sup>†</sup>

Véronique Boyer, Ethel Moustacchi, and Evelyne Sage\*

Section de Biologie, Institut Curie, 26 rue d'Ulm, 75231 Paris Cedex 05, France

Received June 22, 1987; Revised Manuscript Received December 11, 1987

**ABSTRACT:** The sequence specificity in the photoreaction of various psoralen derivatives with DNA is investigated by using DNA sequencing methodology. The 3'-5' exonuclease activity associated with T<sub>4</sub> DNA polymerase serves as a probe to map the psoralens' photoaddition (monoadducts plus biadducts) on DNA fragments of defined sequence. This approach has already allowed us to demonstrate a strong sequence context effect on the 8-methoxypsoralen photobinding to DNA [Sage, E., & Moustacchi, E. (1987) *Biochemistry* 26, 3307-3314]. The psoralens studied include bifunctional derivatives [8-methoxypsoralen, 5-methoxypsoralen, and 4'-(hydroxymethyl)-4,5',8-trimethylpsoralen] and monofunctional derivatives (angelicin, 3-carbethoxypsoralen, and three pyridopsoralens). Maps of photochemical binding on two DNA fragments of the *lacI* gene of *Escherichia coli* are established for all the derivatives. These maps demonstrate the following general qualitative rules in the photoreaction of the furocoumarins with DNA: thymine residues in a GC environment are cold, adjacent thymines are better targets, 5'-TpA sites are strongly preferred versus 5'-ApT, and alternating (AT)<sub>n</sub> sequences are hot spots for photoaddition. Depending on the chemical structure of the derivatives and on their affinity for DNA, some minor differences in the binding spectrum are detected. A most interesting example is 3-carbethoxypsoralen, which specifically reacts with (AT)<sub>n</sub> sites. Our observations lead us to define two types of target sites: the "strong sites", which are preferential targets for all psoralen derivatives, and the "weak sites", which are targets only for derivatives having a high affinity for DNA. The frequency of DNA lesions is much higher in the former sites. The specific photochemical binding of each derivative is discussed in conformational terms and related to the repair and mutagenesis induced by the studied furocoumarins.

**P**soralens are a class of compounds of particular interest. In addition to their clinical use in the treatment of certain skin diseases, they have proven to be excellent probes for studying chromatin and nucleic acid structure. Moreover, psoralen-DNA photoadducts provide an attractive model to study the influence of DNA sequence in relation to mutagenicity and to analyze basic DNA repair mechanisms.

The photoreaction of psoralens with DNA is a multistep process [see for review Song and Tapley (1979)]. First, the psoralen forms an intercalative noncovalent complex with DNA. Upon exposure to UVA radiation (320-400 nm), cycloaddition occurs to the 5,6 double bond of pyrimidine bases

(mainly thymine) through the pyrone or the furan ring of the psoralen. In the case of bifunctional psoralens, the thymine-furan side monoadduct can absorb a second photon of near-UV light and, when properly located, be converted to a diadduct that yields an interstrand cross-link. The effect of adducts on the local structure of the DNA depends on the psoralen derivatives. Typically, 8-methoxypsoralen (8-MOP)<sup>1</sup> photoadducts provoke an unwinding of the double helix (Wiesehahn & Hearst, 1978).

In addition to natural furocoumarins, several families of psoralen compounds have been synthesized. The studies on

<sup>†</sup> This work was financially supported by the CNRS, the CEA (Saclay, France), the ARC (Villejuif, France), and Contracts 852017 (INSERM) and B16-151-F (CCE, Bruxelles).

\* Author to whom correspondence should be addressed.

<sup>1</sup> Abbreviations: bp, base pair(s); EDTA, ethylenediaminetetraacetic acid; ATP, adenosine triphosphate; 8-MOP, 8-methoxypsoralen; 5-MOP, 5-methoxypsoralen; 3-CPs, 3-carbethoxypsoralen; MePyPs, 7-methylpyrido[3,4-c]psoralen; BCH 394, 11-methylpyrido[3,4-h]psoralen; HMT, 4'-(hydroxymethyl)-4,5',8-trimethylpsoralen; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.