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Specific binding of *o*-phenanthroline at a DNA structural lesion

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ABSTRACT

DNA intercalators are found to recognize a DNA lesion as a high affinity receptor site. This lesion-specific binding is observed when one strand of a DNA double helix contains an extra, unpaired nucleotide. Our assay for binding controls for the effects of sequence with a series of oligodeoxynucleotide duplexes which are identical except for the location of the lesion, an extra cytidine. Scission of the series of oligodeoxynucleotides by the cuprous complex of ortho-phenanthroline (OP-Cu) indicates that OP-Cu binds at the lesion-specific stable intercalation site, suggesting that OP-Cu intercalates into DNA. The dispersion of OP-Cu scission sites over three residues is consistent with scission via a diffusible intermediate. The location of the scission sites, directly on the 3' side of the lesion, is consistent with minor groove binding in B DNA.

INTRODUCTION

There has been considerable success in the design of sequence-specific DNA cleaving agents (1,2). In a complementary approach, we have manipulated the receptor site in DNA to increase the affinity for intercalating agents. Our goals are (a) to develop probes for DNA lesions, (b) to characterize the non-covalent interaction between intercalators and a DNA lesion produced by intercalation, and (c) to constrain reactive intercalators to lesion-specific sites of intercalation, then cleave the DNA from these predetermined intercalation sites. This method yields two constraints (sites of intercalation and strand scission) for modeling DNA/drug complexes and reaction mechanisms.

We have recently reported (3) that three reactive intercalating agents, neocarzinostatin chromophore (NCS-C), bleomycin (BLM) and the ferrous complex of methidiumpropyl·EDTA [MPE·Fe(II)], cause enhanced strand scission near a certain type

of DNA lesion. Such specific binding of DNA intercalators is observed when there is an extra, unpaired nucleotide on one strand of the DNA double helix. An extra nucleotide, known as a bulge, is thought to be an intermediate in frameshift mutagenesis (4,5). Thus, bulges appear to insert or delete residues during DNA replication, recombination or repair. Bulges are stabilized by intercalating agents (6) and the frameshift mutagenicity of intercalators (7,8) appears to result from such stabilization. Our results support a model of bulged DNA with a single site of high stability intercalation, located directly at the lesion (3). Similarly, bulges appear to increase the affinity of RNA for intercalators (9,10). In this report, we demonstrate the utility of this lesion to form a set of predetermined binding sites to study the binding mode and mechanism of action of a well known "artificial nuclease", the cuprous complex of ortho-phenanthroline (OP-Cu) (for a review of the reaction of OP-Cu with DNA, see reference 11).

METHODS

Oligodeoxynucleotides were synthesized, purified, 5'-^{32}P-phosphorylated, and annealed as previously described (3). The sequences and sites of strand scission of all oligodeoxy-nucleotides were confirmed by chemical sequencing techniques (12). OP-Cu reaction conditions. First, 12 ul of freshly prepared 17 uM copper sulfate, 3.3 mM dithiothreitol, and 170 uM 1,10-phenanthroline, then 4 ul of 0.1% hydrogen peroxide in water (v/v) were added to 4 ul of annealed duplex (5 mM base pairs) in 50 mM Tris·HCl, pH 8.0. The reactions were allowed to proceed for one hour at room temperature. Reactions were terminated by the addition of 20 ul of 1.0 mM EDTA, 80% formamide, 0.5 mgs per ml xylene cyanol in water, immediately heated to 92°C for 2 minutes and loaded onto 20% (1:20 crosslinked) denaturing (7 M urea) acrylamide gels. Autoradiography was conducted at -70°C on Kodak, X-Omat AR film.

RESULTS

A 'moving lesion' assay clearly controls for any sequence-dependence of DNA interactions. This assay for lesion-specific

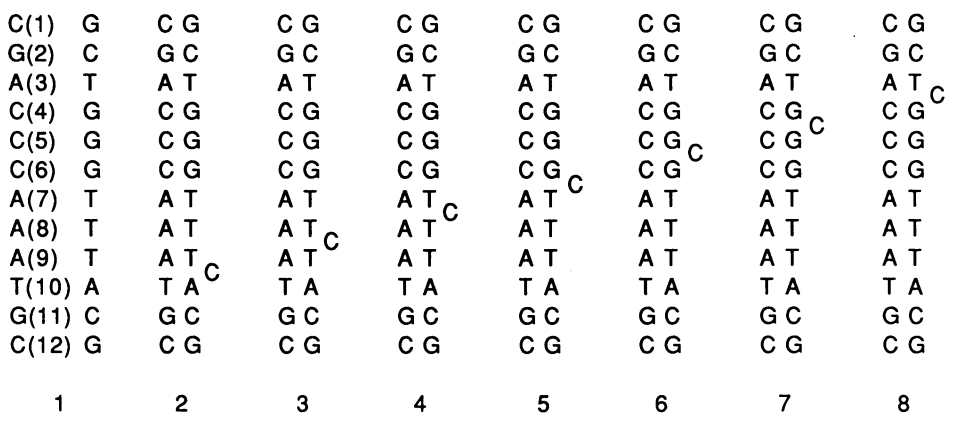

Figure 1. The series of oligodeoxynucleotide duplexes cleaved by OP-Cu.

binding utilizes a series of eight analogous oligodeoxynucleotide duplexes. The sequence of the plus strand is invariant in all eight duplexes. The minus strand of duplex 1 (Figure 1, number 1) is the dodecamer that is complementary to the plus strand and thus, duplex 1 does not contain a bulge. 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. The position of the cytidine bulge has been shifted stepwise in each succeeding member of the series. In this assay for lesion-specific binding, sites of specific binding shift in a stepwise manner in each succeeding duplex of the series.

The plus strand of the analogous series of oligodeoxynucleotide duplexes was 5'-^{32}P endlabeled and the annealed duplexes were treated with OP-Cu. The reaction products of the plus strand were resolved by high resolution polyacrylamide gel electrophoresis.

OP-Cu caused scission at all residues of the normal duplex (duplex 1, lane 1 of Figure 2). However, the intensity of strand scission is greatest in the region centered around T(10). Thus, the scission pattern of the normal duplex is consistent with

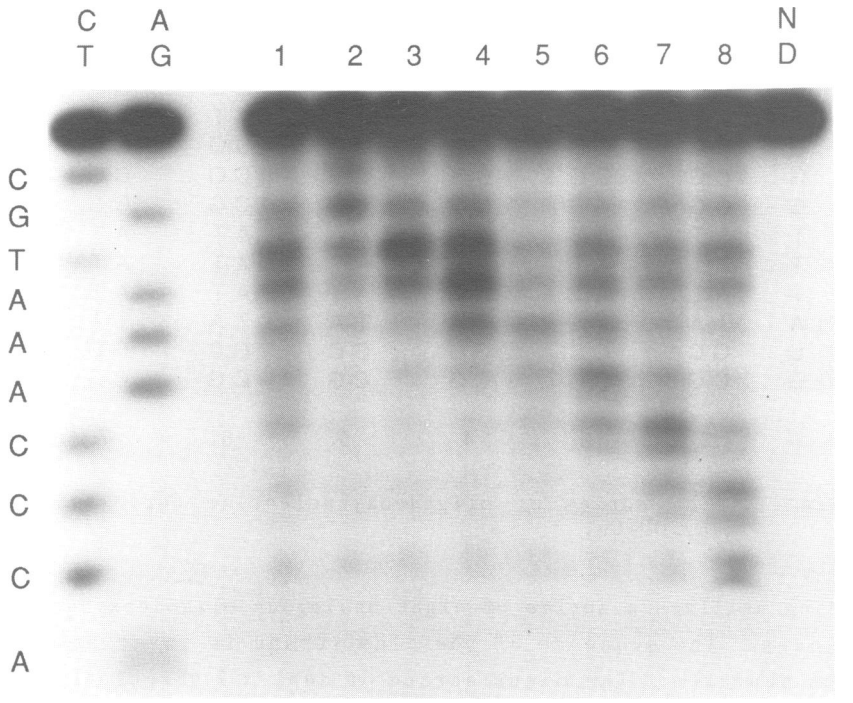

Figure 2. Autoradiogram of a denaturing 20% acrylamide gel of OP-Cu scission reaction products. The plus strands of duplexes 1-8 have been labeled at the 5'-end with ^{32}P. C+T and A+G, Maxam and Gilbert sequencing reactions; lanes 1-8, reactions of duplexes 1-8, respectively; ND, (no drug) control reaction that contains all reactants and cofactors except ortho-phenanthroline.

previous reports describing OP-Cu scission of restriction fragments at all four bases with sequence determined variation in scission intensity (13,14, also see reference 11 for OP-Cu scission of oligodeoxynucleotides). Products from the reaction of OP-Cu with the series of bulged duplexes are shown in lanes 2 - 8 of Figure 2. The sequence-specific scission pattern is maintained in each bulged oligodeoxynucleotide. In addition, a region of bulge-specific scission by OP-Cu covers the first, second and third residues directly on the 3' side of each bulge. This three residue region of bulge-specific scission is centered

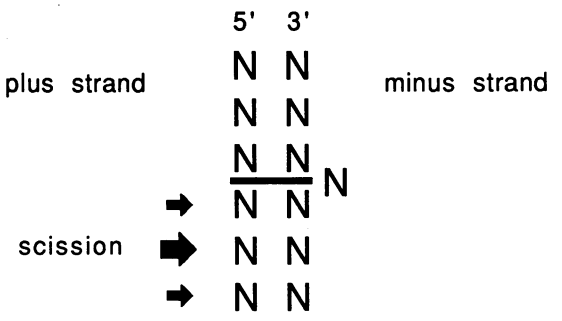

Figure 3. Sites of bulge specific binding and strand scission by OP-Cu.

around, and is most intense at, G(11) of duplex 2, T(10) of duplex 3, A(9) of duplex 4, etc. Further, it appears that sequence specificity is additive with lesion specificity. For example, scission of T(10) is more intense in duplexes 3 and 4 than in duplex 1 or duplexes 5-8.

The major products from reaction of OP-Cu are fragments with 3'-phosphate termini (slower migrating) and the minor products are fragments with 3'-(phosphoro-2"-O-glycolate) termini (faster migrating) (11). Where the resolution is greatest on the gel in Figure 2, both species are clearly observable as products of bulge-specific scission by OP-Cu (for example C(5) duplex 8).

In each bulged oligodeoxynucleotide, a region of bulge-specific protection from OP-Cu scission is observed on the 5' side of the lesion. As can be seen in Figure 2, protection from scission occurs from A(9) through C(4) of duplex 2, A(8) through C(4) of duplex 3, etc. The location of this region of bulge-specific protection is consistent with a model of bulged DNA containing sites of both stable and unstable intercalation in the vicinity of the bulge (3).

DISCUSSION

Mode of Binding. The structure of OP-Cu is analogous to that of MPE·Fe(II) [see reference 15 for a discussion of mode of binding and mechanism of strand scission by MPE·Fe(II)]. Although the planar geometries of these two cleaving agents suggest

intercalative binding to DNA, viscometric studies with an inactive analog of OP-Cu have led to a proposal that intercalation is not the mode of binding of OP-Cu (16). However, our results are consistent with Op-Cu intercalation into DNA (although additional modes of binding cannot be excluded). The 'moving lesion' experiment indicates specific binding of OP-Cu to a previously characterized site of high stability intercalation in bulged DNA (3).

The results suggest that DNA/drug complexes in bulged DNA are similar to those in normal DNA. Support for this hypothesis that introduction of this lesion does not radically alter the structure of a complex or its mechanism of action is provided by the following observations:

(a) We expect that a highly specific mechanism of strand scission is related to a highly specific conformation. BLM cleaves normal DNA by selective abstraction of a 4' hydrogen (17) to produce 3'-(phosphoro-2"-O-glycolate) termini (18). Similarly, BLM cleavage at bulges produces fragments with 3'-(phosphoro-2"-O-glycolate) termini (3). NCS-C cleaves normal DNA by selective abstraction of a 5' hydrogen to produce 3'-phosphate termini (19). Similarly, NCS-C cleavage at bulges produces fragments with 3'-phosphate termini (3). Thus, similarities in reaction products from bulged and normal DNA suggest similarities in structures of the DNA/drug complexes.

(b) Conformational information is also provided by the location of the sites of OP-Cu attack relative to the bulge. The observed sites of strand scission are offset 2 to 3 residues to the 3' side of the bulge (Figure 3). Minor groove binding to B DNA is expected to direct strand scission 2 to 3 residues to the 3' side of the binding site. Such a 3'-offset scission pattern has previously been reported for OP-Cu binding to normal DNA (20, also see reference 21 for other evidence of minor groove binding by OP-Cu). The 3'-offset scission pattern in both bulged and normal DNA suggests that the complexes are structurally similar.

(c) Sequence-specific stabilization is additive with lesion-specific stabilization. This should be true only if the sequence-specific interactions are maintained in the bulged complex.

Bulges appear to provide a useful assay for intercalative

binding. A DNA binding agent 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). However, none of these parameters is uniquely characteristic of intercalation. Further, it is difficult to observe such changes in DNA properties when the intercalator also cleaves the DNA. Specific scission of bulged oligodeoxynucleotides suggests that OP-Cu binds by an intercalative mode. In a control experiment, hydroxyl radical generated by EDTA·Fe(II) did not cause bulge-specific strand scission (unpublished).

Mechanism of Strand Scission. The cofactor requirements and metal chelation of both OP-Cu (22,23) and MPE·Fe(II) (15) suggest mechanisms of strand scission involving diffusible hydroxide radical. Yet, hydroxyl radical traps do not inhibit strand scission by OP-Cu (23). Site-specific binding can help determine mechanisms of strand scission. For example, if OP-Cu cleaves DNA via a diffusible intermediate, we would expect to observe scission over a dispersion of sites from a single binding site [as observed for MPE·Fe(II)]. If OP-Cu cleaves DNA via a non-diffusible intermediate, we would expect to observe scission at a single site from a single binding site (as observed for BLM and NCS-C).

The scission pattern by OP-Cu covers the three residues directly on the 3' side of the binding site (Figure 3). This dispersion of scission sites is thus consistent with a diffusible intermediate in the scission reaction. The bulge-specific scission pattern by OP-Cu is analogous to the pattern observed from sequence-specific iron chelators (1). From a single binding site, such iron chelators generate a diffusible intermediate causing strand scission over 3-5 residues. In comparison, OP-Cu causes a tighter pattern of scission, sharply focused on 3 residues. Presumably, the greater dispersion of scission sites by sequence-specific iron chelators results from the relatively long and mobile tether used to bind the iron. The tight pattern of scission by OP-Cu results from immobilization of the copper deep within the minor groove. Thus, steric exclusion and close

proximity between riboses and the copper could explain the lack of inhibition of the OP-Cu reaction by hydroxyl radical traps (23) and other observations which have led to a contrary proposal that the intermediate is not freely diffusible (21). Our results are consistent with OP-Cu scission of DNA via a freely diffusible intermediate that is generated proximal to the sites of attack. However, we cannot exclude OP-Cu scission of DNA via more than a single mechanism.

In conclusion, this report demonstrates the utility of a bulge as a high affinity receptor for the study of DNA cleaving agents. Lesion-specific scission by OP-Cu has provided evidence for a diffusible intermediate in the scission reaction and for intercalation as the mode of binding.

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