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Evidence for structural deformation of the DNA helix by a psoralen diadduct but not by a monoadduct

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ABSTRACT

We have investigated the structural change in a double-stranded DNA helix caused by covalent addition of a psoralen. A synthetic double-stranded DNA was constructed to contain either a psoralen furan-side monoadduct or an interstrand diadduct at a specific site. When the unmodified and psoralen modified DNAs were examined by electron microscopy in the presence of distamycin, which stiffens the DNA helix, the DNA containing the psoralen interstrand diadduct appeared bent (or kinked), whereas the furan-side monoadducted DNA appeared similar to the unmodified DNA.

RecA protein from *E. coli* has been shown to preferentially bind UV (ultra violet) irradiated DNA presumably due to alterations in the normal DNA helical structure. Using a nitrocellulose filter binding assay, we have found that the psoralen interstrand diadduct enhances the binding of recA protein to the double-stranded DNA, whereas a furan-side monoadduct has little effect. Thus both the recA protein binding and the electron microscopic data suggest that a psoralen diadduct causes deformation of a DNA helix, most likely by kinking the helix, and that a monoadduct has little effect on the DNA helix structure.

INTRODUCTION

Psoralens are planar heterocyclic compounds capable of intercalating between base pairs in a double-stranded nucleic acid. Upon near UV irradiation (320-380 nm), the intercalated psoralens can photoreact with adjacent pyrimidine bases, mostly with thymidines in DNA and uridines in RNA, to form first monoadducts, which are linked to only one strand of the helix, and then diadducts, which are linked to both strands of the helix (1, 12, 20). In the first step of the photoreaction either a furan-side or a pyrone-side monoadduct can be formed depending on whether the 4',5'-double bond or the 3,4-double bond of an intercalated psoralen reacts with the 5,6-double bond of a pyrimidine base. Upon absorbing a second photon, the furan-side monoadduct can be converted into a diadduct if another adjacent pyrimidine base is available for photoreaction on the other strand (17, 21). The pyrone-side monoadduct cannot be driven to a diadduct because it does not absorb light in the 320-380 nm region. All of these adducts are photoreversible upon exposure to light in their respective absorption regions (2, 16).

The structure of psoralen-modified DNA has also been investigated by optical and X-ray diffraction methods. Using flow linear dichroism, Vigny et al. (25) found that the psoralen moieties of furan-side monoadducts of different psoralen derivatives stack with the base pairs in

duplex DNA. The plane of the psoralen molecule deviates slightly from the normal to the helix axis. An earlier X-ray crystallographic study of the monoadduct formed between thymidine and 8-methoxypsoralen indicated that the thymidine and psoralen rings remain planar and that the interplanar angle is about 50° (14). Based on this data, a structure for a DNA helix containing a psoralen diadduct was proposed in which there is a sharp kink at the diadduct site (9) and this kink was predicted to be greater than that caused by a pyrimidine dimer (13). Although these theoretical studies argued for the presence of a sharp kink at a psoralen diadduct site, direct solution studies using differential decay of birefringence (19) and polyacrylamide gel electrophoresis (8) have failed so far to detect any unusual properties that could be attributed to the presence of psoralen diadducts in DNA.

To further explore the question of whether psoralen diadducts do or do not kink DNA we have constructed 138 base pair (bp) DNA duplexes containing either an interstrand HMT (4'-hydroxymethyl-4, 5', 8-trimethylpsoralen) diadduct (crosslink) or an HMT furan-side monoadduct at a single site and have used this DNA in two different tests. In one test, the unmodified, monoadducted, and the crosslinked DNAs were coded and examined directly by electron microscopy (EM) in a blind test to determine whether any kinking in the DNA could be visualized directly. In a second, indirect test, we utilized the newly recognized ability of the *E. colil* recA protein to detect subtle distortions in the DNA helix. RecA protein is a multifunctional protein essential to homologous recombination and the induction of the SOS response, the latter event possibly involving the binding of recA protein to damaged DNA (4, 7, 10, 26). Recently Lu et al. (11) showed that recA protein will preferentially bind to duplex DNA containing UV lesions. Pinsence, Blaho, Wells, and Griffith (unpublished studies) have found that recA protein shows a two-fold preference for binding at short left-handed tracts in plasmid DNA, and in studies to be reported elsewhere (Thresher and Griffith, 1988) the intercalation of ethidium bromide or acridine orange into duplex DNA was found to strongly nucleate recA protein binding.

In the studies reported here, addition of one psoralen interstrand diadduct was found to enhance the binding of recA protein whereas the addition of one psoralen furan-side monoadduct had little effect. Furthermore, direct EM visualization of these DNAs showed that the 138 bp duplex containing the diadduct appeared kinked in the presence of distamycin, in contrast to the unmodified and monoadducted 138 bp DNAs, which did not.

EXPERIMENTAL PROCEDURES

Materials

HMT was a gift from HRI Associates Inc. (Berkeley, CA). RecA protein from *E. Coli* was generously supplied by Chi Lu (Dept. of Mol. Biol., UC Berkeley). The stock recA protein solution was diluted before use with 20 mM TrisHCl, 10% glycerol, 0.1 mM EDTA, and 1 mM DTT, pH 7.5. Adenosine 5'-[γ-thio]triphosphate (ATP-γ-S) was obtained from Boehringer Mannheim Biochemicals. Distamycin was obtained as a gift from P. Englund (Johns Hopkins University).

Preparation of Double-stranded 138mers

A synthetic double-stranded 138 bp DNA was prepared as described (18) to contain either no modification (UM-138mer, see figure 1), an HMT furan-side monoadduct attached to T-75 of the top strand (M_{Fu}-138mer), or an HMT interstrand diadduct attached to T-75 of the top strand and T-76 of the bottom strand (XL-138mer).

Electron Microscopy

Samples were prepared for EM as described by Griffith and Christiansen (5). In brief, the DNA was adsorbed directly onto a very thin carbon film in the presence of a buffer containing 2 mM spermidine and 0.15 M NaCl. The samples were washed sequentially with water and water-ethanol solutions, air dried and rotary shadowcast at 10^{-7} torr in a fully cryopumped vacuum system. The samples were examined at 20 kV in a Philips EM 400 instrument.

Nitrocellulose Filter Binding Experiment

The binding of recA protein to double-stranded 138mers (UM-138mer, M_{Fu}-138mer, and XL-138mer) was quantified by nitrocellulose filter binding experiments following the procedures of Lu et al. (11). 5'-^{32}P-labeled double-stranded 138mer (3.3 nM) and recA protein (0-6 μM) in 10 μl of reaction buffer (20 mM TrisHCl, 10 mM $MgCl_2$, 1 mM EDTA, 30 mM NaCl, and 1 mM DTT at indicated pH) were preincubated at 37°C for 5 min followed by the addition of 1 μl 2 mM ATP-γ-S in the reaction buffer and incubation at the 37°C for 10 additional min. The binding reaction was stopped by adding 100 μl of reaction buffer (4°C). Each solution was then filtered through nitrocellulose filter (BA85, 8.2 mm diameter, Schleicher and Schuell, Inc., presoaked in 1 mM ATP for at least 30 min) at a flow rate of 2 ml/min. The filter was washed twice with 200 μl of the reaction buffer (4°C) at the same flow rate. The filter was then dissolved in 5 ml of scintillation fluid (mixture of 12 g PPO-BisMSB from ICN Radiochemicals, 2 l toluene, 1 l triton X-100, and 333 ml H_2O) and counted. A sample for each reaction set counted without filtration was used as the control to determine the total counts. The background binding of DNA to the filter was determined by the counts from samples without recA protein. The unmodified and psoralen modified DNA gave similar but negligible backgrounds. The binding was expressed as a percentage of the total counts retained on the filter. Most of the data were averages of two independent experimental results.

RESULTS

Visualization of Psoralen-Modified DNA by Electron Microscopy

To investigate the effects of different psoralen adducts on the helical structure of double-stranded DNA, a synthetic double-stranded 138mer was constructed to contain either no psoralen (UM-138mer), or a site specific psoralen adduct. As shown in figure 1, M_{Fu}-138mer contained an HMT furan-side monoadduct attached specifically to T-75 of the top strand and XL-138mer contained an HMT interstrand diadduct attached to T-75 of the top strand and T-76 of the bottom strand through its furan and pyrone ends, respectively. The unmodified and two

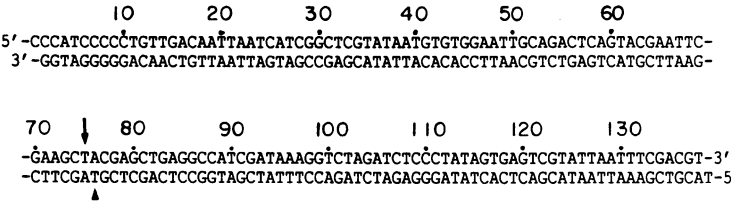

Figure 1. Sequence of the double-stranded 138mer. The arrow indicates the thymidine residue on the top strand to which an HMT furan-side monoadduct is attached in the M_{Fu}-138mer. The solid triangle indicates the thymidine residue on the bottom strand to which the pyrone end of the HMT is attached in the XL-138mer. The furan end of the HMT in the XL-138mer is attached to the thymidine residue indicated by the arrow on the top strand.

psoralen-modified 138mers were prepared and examined by EM in coded form to eliminate any bias. No significant difference could be observed among the samples whether they were prepared for EM at 20°C or 5°C. This appeared to result from the inherent flexibility of the 138 bp molecules which was sufficient to obscure the presence of any single drug-induced kink, since both the UM-138mer and the psoralen modified 138mers appeared bent. To reduce the flexibility of the DNA, we utilized the drug distamycin, a long molecule which binds in the minor groove of

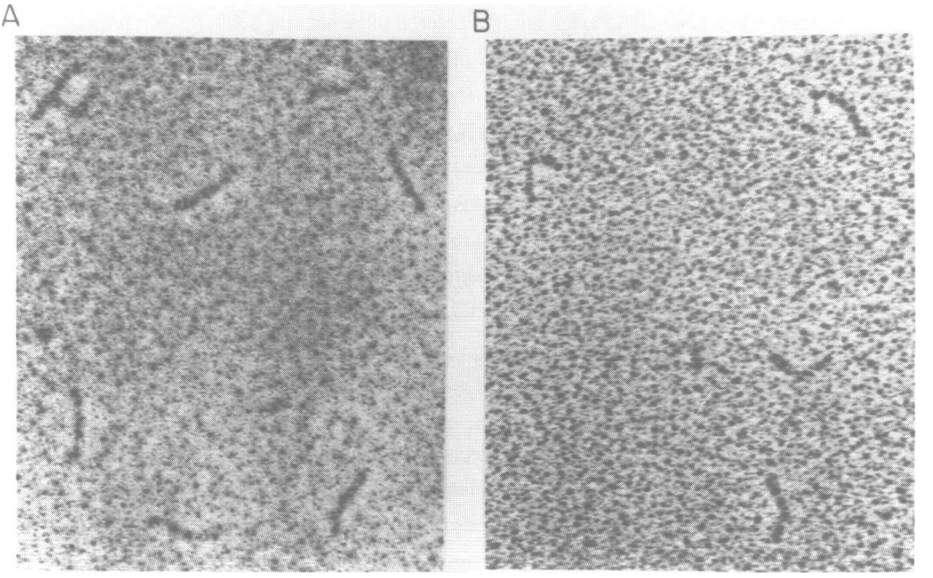

Figure 2. Visualization of synthetic 138 bp DNA molecules with or without a single psoralen adduct. 138 bp DNAs lacking any psoralen adduct (A) or containing a single psoralen diadduct at their center (B) were mixed with distamycin (0.75 μg drug per μg DNA) and prepared for EM by rotary shadowcasting with tungsten (see Materials and Methods).

Table I. Fraction of the Molecules Scored Having a Stiff Bend (Kink) 40-50% from One End of the DNA and in Which the Bend Measures between 30-52°. (Expressed as percent relative to the fraction scoring as straight.)

DNA	UM-138mer	M_{Fu}-138mer	XL-138mer
%	31	67	180

B form DNA stiffening it to a degree that eliminates the sequence-directed curvature of kinetoplast DNAs containing phased runs of A's (6, 27). Here the DNA samples were incubated with distamycin (1.5 μg/ml of distamycin per 2 μg/ml of DNA) for 5 min prior to mounting the samples for EM. When this was done, two of the samples appeared relatively straight (figure 2A) while one contained many molecules with a sharp central kink (figure 2B). Upon breaking the code, the latter sample was found to be the XL-138mer while the two straight samples corresponded to the UM-138mer and M_{Fu}-138mer. Analysis of the EM data (Tables I and II) indicated that the XL-138mer was preferentially kinked 2- to 6-fold more often over the unmodified DNA and that the mean angle of the kink for molecules containing a sharp kink within 5% of the known position of the psoralen diadduct was 45 ± 10 degrees. While ti is possible that the kink observed here could be an artifact due to the presence of distamycin, it is unlikely. The effect of distamycin appeared to be removing the curvature due to DNA flexibility, which is important for examining local helical distortions in DNA. Thus, in its presence, the UM-138mer and M_{Fu}-138mer became straight, whereas the XL-138mer remained kinked at the site of psoralen addition.

Preferential Binding of XL-138mer by RecA Protein

The binding of recA protein to the ^{32}P-end-labeled 138mers, facilitated by the presence of magnesium ions and ATP-γ-S was followed by nitrocellulose filter binding assays, which is based on the retention of protein-bound DNA molecules on the filter. The results shown in figure 3A indicate that the UM-138mer was bound by recA protein with a saturation retention of about 30%. The presence of a furan-side monoadduct in the double-stranded 138mer had little effect on the recA protein binding to the DNA. (In fact, we consistently observed a slightly reduced binding of recA protein to M_{Fu}-138mer as compared to UM-138mer.) In contrast, a psoralen diadduct in the 138mer increased the retention by approximately two fold. The effect of the HMT diadduct on the recA protein binding of the DNA was larger than that from irradiating the DNA with 3 kJ/m^2 of

Table II. Electron Microscopic Analysis of 138mers with a Single Psoralen Molecule Bound at the Center. (861 molecules scored.)

DNA	% Molecules Straight	% Molecules with a Bend at 0-30% from one End	% Molecules with a Bend at 40-50% from one End
UM-138mer	35	36	29
M_{Fu}-138mer	26	39	35
XL-138mer	11	36	53

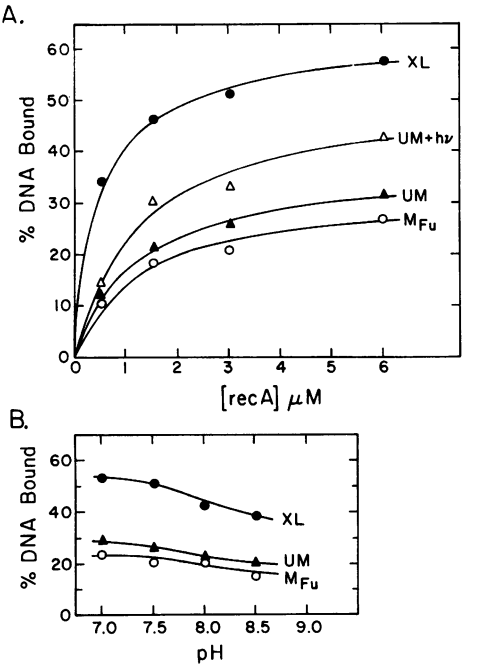

Figure 3. Nitrocellulose filter binding assay. A) Binding of recA protein to 138mers at pH 7.5. UM: UM-138mer; UM + hv: UM-138mer irradiated with 254 nm light (3 kJ/m^2) [7 thymidine dimers per 138mer duplex would be generated based on the number of TT sites and the data of Deering and Setlow (3)]; M_{Fu}: M_{Fu}-138mer; XL: XL-138mer. B) Binding of recA protein (3 μM) to 138mers at different pH.

254 nm light, a dose which should induce approximately 7 thymidine dimers per 138mer duplex (figure 3A).

The pH dependence of the recA protein binding to 138mers was also investigated. Figure 3B shows the pH dependence at 3 μM recA protein, and similar behavior was observed at other recA protein concentrations. In general increasing the pH reduced both the binding to unmodified double-stranded DNA and the modified DNA. The preferential binding to XL-138mer was observed at all pH values tested. At pH ≥ 9.0, the binding could not be determined due to the high background binding of DNA to nitrocellulose filters in the absence of protein. Thus, the results of the recA binding experiments suggest that the psoralen diadduct distorts the DNA helix, whereas the monoadduct has little effect, consistent with the EM results above.

DISCUSSION

The effects of psoralen addition on the structure of double-stranded DNA were first suggested by a theoretical model of a double-stranded DNA containing a psoralen diadduct. Based on the

X-ray crystallographic data of the 8-methoxypsoralen-thymidine furan-side monoadduct, a psoralen diadduct was predicted to induce a sharp kink in a double-stranded DNA (9). Since then, several independent experiments have been performed to test the model. Two dimensional proton NMR data of a double-stranded deoxyoligonucleotide containing a 4'-aminomethyl-4, 5', 8-trimethylpsoralen-tymidine diadduct shows that the diadduct induces a localized kink and unwinds the DNA helix by about 56° (22). Our data here, especially the EM results, also suggest a kink produced by a psoralen diadduct. However, Sinden and Hagerman (19) did not detect any appreciable kinking due to psoralen addition by the decay of linear birefringence. Similarly, we have failed to detect any evidence for kinking by the diadduct in the XL-138mer by non-denaturing gel electrophoresis (data not shown) in which molecules kinked or bent in a uniform direction show abnormally slow migration as compared to a straight one (27). A possible explanation for these controversial results could be that the DNA helix around a psoralen diadduct is flexible. Therefore, the kinking effect of a psoralen diadduct cannot be detected by dynamic methods such as gel electrophoresis or decay of linear birefringence. In fact, NMR data does suggest significant flexibility on one side of a crosslinked DNA (22). Clearly, the final resolution of the controversy waits further study.

It has been shown that monoaddition of HMT does not destabilize double-stranded DNA. Instead, it stabilizes slightly the helix, suggesting that the psoralen group is stacked with the DNA bases (15). Similarly, using a flow linear dichroism technique, Vigny et al. (25) found that the psoralen moieties of the furan-side monoadducts of different psoralen derivatives are stacked with the DNA base pairs with the plane of the psoralen ring deviating slightly from the normal to the helix axis. Thus the psoralen moiety in a monoadduct is most likely intercalated between the DNA base pairs with one end of the psoralen of the monoadduct attached with one strand and the other end free within the helix. Such a structure might be expected to cause little or no kinking to the DNA helix. Consistent with this, our recA protein binding and EM experiments did not reveal any appreciable difference between the furan-side monoadducted and unmodified DNA fragments.

The preferential binding of recA protein to XL-138mer may have some significance in the repair of psoralen diadducts. Though a psoralen monoadducted DNA can be repaired by excising the short fragment containing a psoralen monoadduct by uvrABC exinuclease followed by filling the gap by DNA polymerase and sealing the break by DNA ligase, uvrABC can only cut around an HMT diadduct on the DNA strand attached to the furan-end of the psoralen, thus leaving the psoralen adducted fragment still attached to the DNA (23, 24). To completely repair a psoralen diadduct, therefore, a more complicated mechanism is required. The preferential binding of recA protein to psoralen crosslinked DNA implicates a possible diadduct repair mechanism, in which recA protein is involved, for example, a mechanism involving homologous recombination as suggested by Van Houten et al. (24).

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ABBREVIATIONS

ATP-γ-S Adenosine 5'-[γ-thio]triphosphate / EDTA Ethylenediaminetetraacetic acid
HMT 4'-hydroxymethyl-4,5',8-trimethylpsoralen / Tris Tris (hydroxymethyl) aminomethane
EM Electron microscopy / bp base pair

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