The nature of the Uvr protein-DNA complexes formed on psoralen-DNA interstrand cross-links was analyzed by DNase I footprinting and correlated with the incision efficiency of the UvrABC endonuclease on the cross-links of different DNA sequences. Our results indicate that the repair specificity is dependent on the DNA sequence and the psoralen orientation in the cross-link. On the strand that will be cut, a 30-nucleotide long UvrAB footprint with a DNase I hypersensitive site at the 11th nucleotide 5' to the lesion was observed and subsequently rearranged to a 22-nucleotide long UvrB-lesion footprint. On the strand that will not be cut, the UvrAB-lesion footprint had no 5' DNase I hypersensitive site and did not form the UvrB-lesion footprint. Although UvrABC incision requires the formation of UvrB-lesion complex on the strand which will be cut, the affinities of these complexes do not correlate with the incision efficiencies, suggesting that the overall reaction can be driven forward by a favorable next step such as UvrC incision. A study of the time-dependent interconversion of UvrAB-lesion complex to UvrB-lesion complex on a cross-link revealed a secondary recognition of the UvrB-lesion complex by UvrA(B) proteins in vitro.

Nucleotide excision repair is a major pathway in the removal of bulky DNA lesions. In Escherichia coli, this pathway begins with the UvrABC endonuclease which is a multienzyme complex containing UvrA, UvrB, and UvrC proteins as subunits (for reviews, see Grossman and Yeung (1990), Van Houten (1990), and Sancar and Sancar (1988). This endonuclease has a broad range of lesion specificity, including covalent adducts of mitomycin C, cisplatin, psoralen, pyrimidine dimers, 6,4-photoproducts, N-acetoxy-2-acetylaminofluorene, and noncovalent DNA intercalators like ditercalinium (for review, see Van Houten (1990)). The enzyme makes two nicks in the strand containing a lesion: one at the eighth or ninth phosphodiester moiety 5' to the modified nucleotide (nt); the other at the third or fourth phosphodiester moiety 3' to the modified nt (Sancar and Rupp, 1983; Yeung et al., 1983; Van Houten et al., 1986; Jones and Yeung, 1988).

A brief summary of the mechanism of the UvrABC endonuclease is as follows. UvrA is a lesion-specific DNA binding protein. It has a greater affinity for damaged DNA than for undamaged DNA. UvrA dimerizes (Oh and Grossman, 1989) in solution, to form a UvrA complex. This complex associates with UvrB to form a UvrA-B complex (Orren and Sancar, 1989). Oh et al. proposed that this UvrA-B complex binds to the DNA duplex and translocates along the DNA to the lesion by using the 5' to 3' helicase DNA unwinding activity of the UvrA-B complex (Oh and Grossman, 1987, 1989). Upon encountering a lesion, this forms a UvrA-B-lesion complex. According to one model, UvrA dissociates from the UvrA-B-lesion DNA complex to form a Uvr-B-lesion complex (Orren and Sancar, 1990). The DNA in this complex is nicked by 127' (Shi et al., 1992). The UvrC protein may bind to the UvrA-B-lesion complex or to the UvrB-lesion complex to result in one 5' and one 3' incision on the damaged strand (Visse et al., 1992). From site-directed mutagenesis studies on UvrB and UvrC, Lin et al. (1992) suggested that UvrC is responsible for the 5' incision, but UvrB is responsible for the 3' incision. This difference in protein requirement for the two incision events may further increase the complexity of the sequence specificity of the UvrABC endonuclease.

In this report, we have examined the sequence-specific interactions of UvrAB proteins with psoralen interstrand cross-links. Psoralens are naturally occurring linear furanocoumarins. 4',5',8-Trimethylpsoralen (TMP) is a good example of this class of compounds. In the presence of UV light (360-nm wave length), a double bond of either the furan ring or the pyrone ring of the TMP molecule (intercalates at a 5' TA or 5' AT site) photoreacts with the 5,6-double bond of a thymine residue to form a cyclobutane adducts (Song and Tapley, 1979). The adducts are called furan-side monoadduct or pyrone-side monoadduct, respectively. Only a furan-side monoadduct can undergo further photochemical reaction with the double bond of the thymine moiety in the opposite strand to produce an interstrand cross-link (Cimino et al., 1985). The UvrABC endonuclease incision can be on the furan-side strand or on the pyrone-side strand of the cross-link (Jones and Yeung, 1988; Van Houten et al., 1986). The choice of strand for repair depends on the composition of the bases flanking the cross-link (Jones and Yeung, 1990). In this report, we extend the analysis of this repair specificity to the level of the protein-DNA complex formed in the reaction by examining how different interstrand cross-links interact with UvrA and UvrB proteins in a sequence-dependent manner and how these interactions affect the repair efficiency.

Since the nature of the intermediates formed in the reaction of UvrAB proteins with a DNA lesion can be probed with
DNase I footprinting, we have adopted this method for our current study of site-specific cross-links with Uvr proteins (Van Houten et al., 1987; Bertrand-Burggraf et al., 1991; Munn and Rupp, 1991). Understanding the sequence-specific interactions of UvABC proteins with TMP cross-links may provide some insights to the mechanism of DNA lesion recognition and repair. The results presented in this work show the relationship between the extent of protein-DNA complex formation and the sequences flanking the cross-link in the DNA adduct.

**EXPERIMENTAL PROCEDURES**

**Preparation and Isolation of Specific Cross-links**—The sequence of the 121-bp fragment is shown in Fig. 1. The preparation and isolation of a set of site-specific cross-links is the same as previously reported for 165-bp fragment. 88 µg of 5'-32P-labeled 121-bp fragment (Fig. 1) containing 3' UV5 probe region was used as the starting material (Jones and Yeung, 1990). The 3' labeling of the cross-links on the 117-mer strand was done using the Klenow fragment of DNA polymerase I and [α-32P]dATP as described by Sambrook et al. (1989).

**UvABC Endonuclease Incision Reactions**—About 15 fmol of end-labeled DNA was incubated with 1 pmol of UvrA, 1 pmol of UvrB, and 1 pmol of UvrC (Yeung et al., 1986a) in a volume of 150 µl of UvrABC reaction buffer (85 mM KCl, 50 mM Tris-Cl, pH 7.5, 5 mM dithiothreitol, 10 mM MgCl2, and 2 mM ATP). After 45 min of incubation at 37 °C, the reactions were terminated with the addition of 10 µl of 0.5 M EDTA, pH 8.0. After another 10 min of incubation at 37 °C, the samples were ethanol-precipitated and resolved on a 10% polyacrylamide, 7 M urea sequencing gel at 49 °C. The autoradiograms of the gel were quantified using an AMBIS two-dimensional densitometer (AMBIS Inc., San Diego, CA) from which the percent efficiencies of the UvABC endonuclease 5' or 3' cutting reactions were calculated.

**DNase I Footprinting Reactions**—About 15 fmol (75 pm) of cross-linked DNA was incubated with UvrA or UvrA + UvrB (concentrations as indicated in figure legends) in 200 µl of UvrABC reaction buffer at 37 °C. After 15 min of incubation, 150 ng of pBR322 plasmid DNA was added. After 5 more min of incubation, the reaction mixture was cooled to 24 °C. Two µl of CaCl2 (100 mM) and 2 µl of DNase I (15 ng) were added and the mixture was incubated at 24 °C for 2 min before being terminated by the addition of a 200-µl mixture of 20 mM EDTA, 70 mM sodium acetate, pH 5.2, 1 µg of calf thymus DNA, and 1% SDS. The DNA was ethanol-precipitated. To break the psoralen adduction in the cross-links were identified (data not shown) by the sizes of the fragments generated by the digestion of the 5'-labeled cross-links with the 3' to 5' exonuclease activity of the T4 DNA polymerase (Sage and Mouschacchi, 1987), followed by alkali reversal of the cross-link (Yeung et al., 1988b; Jones and Yeung, 1990).

The mobility of cross-linked DNA on a sequencing gel depends on the position of the psoralen adduct in the DNA duplex (Fig. 2A, lanes 1-6) and therefore the cross-links can be separated on a sequencing gel. The duplexes with the cross-links in the middle of the restriction fragment showed the least mobility and the cross-links in the end showed the greatest mobility. The even numbered cross-links were prepared from the DNA duplex in which the 117-mer contained the monoadducts, the odd numbered cross-links were prepared from the DNA duplex in which the 121-mer contained the monoadducts.

**RESULTS**

**Isolation and Characterization of TMP Intersstrand Cross-links**—Psoralen (TMP) can react with the DNA duplex shown in Fig. 1 to form 18 possible cross-links. The positions of the psoralen adduction in the cross-links were identified (data not shown) by the sizes of the fragments generated by the digestion of the 5'-labeled cross-links with the 3' to 5' exonuclease activity of the T4 DNA polymerase. The 3' to 5' exonuclease activity of the T4 DNA polymerase (Sage and Mouschacchi, 1987), followed by alkali reversal of the cross-link (Yeung et al., 1988b; Jones and Yeung, 1990).

The mobility of cross-linked DNA on a sequencing gel depends on the position of the psoralen adduct in the DNA duplex (Fig. 2A, lanes 1-6) and therefore the cross-links can be separated on a sequencing gel. The duplexes with the cross-links in the middle of the restriction fragment showed the least mobility and the cross-links in the end showed the greatest mobility. The even numbered cross-links were prepared from the DNA duplex in which the 117-mer contained the monoadducts, the odd numbered cross-links were prepared from the DNA duplex in which the 121-mer contained the monoadducts.

**Incision of TMP Cross-links by UvABC Endonuclease**—The UvABC cuts for each cross-link were identified by 5'-labeling one of the strands of each cross-link. For example, in Fig. 2A, lanes 7-12 show positions of the UvABC cuts for cross-links 20, 30, 18, 14, 12, and 10, respectively. The pyrone-side cuts for cross-link 20 and 30 are shown in Fig. 2B (lanes 1 and 2). Weak incision on the pyrone-side of cross-link 30 is not seen in Fig. 2B (lane 2) but visible in Fig. 2A (lane 8). The incised sites on the cross-links were identified on a 10% acrylamide DNA sequencing gel by comparing the size of the labeled fragments generated by UvABC reaction with the bands in the reference DNA sequencing ladder.

The cross-links are cut at the ninth phosphodiester moiety 5' to the lesion and at the second or third phosphodiester...
FIG. 2. *UvrABC* cutting of the 117-bp cross-links. Panel A, the autoradiogram of a 10% acrylamide DNA sequencing gel resolving the *UvrABC* cut fragments containing 5'-32P-label on the bottom strand of cross-linked DNA. *Lanes* 1–6 are the control lanes for cross-links 20, 30, 18, 14, 12, and 10, respectively, without *UvrABC* G, G + A, T, and C chemical sequencing lanes are as indicated (Maxam and Gilbert, 1980; Rubin and Schmidt, 1980; Yeung et al., 1988). *Lanes* 7–12, cross-link samples (0.1 nM) in lanes 1–6 treated with 6 nM of *UvrABC* at 37°C for 45 min as described under "Experimental Procedures." The pyrone cuts produced bands of sizes greater than 117 nt in the gel. The position of the modified thymines are circled and *UvrABC* furan-side cuts are shown as breaks (//) between nt corresponding to each cross-link. For cross-link 10, the position of the cut site cannot be assigned, since the bands move close to the top of the gel. Panel B, DNA sequencing gel resolving the *UvrABC* cut fragments from cross-linked DNA containing 5'-32P-label on the top strand. *Lanes* 1 and 2 are pyrone-side strands of cross-links 20 and 30, respectively, treated with 6 nM of *UvrABC* as described. Weak pyrone side incision (<4%) is not visible in this figure.

moiety 3' to the lesion (data not shown). When *UvrABC* makes two cuts on the labeled furan-side strand, it produces a single-stranded fragment, smaller than 117 nt as shown in Fig. 2A. When the two cuts are on the pyrone-side strand, it produces a fragment of size larger than 117 nt (117 + 12 nt) as shown in Panel A (pyrone cuts). When only one of the two *UvrABC* cuts is made on the furan-side or pyrone-side, it is called an uncoupled cut and is seen below the uncut cross-link DNA (Fig. 2A). A *UvrABC* cut fragment contains a 3'-OH group but a chemical sequencing ladder fragment contains a 3' PO₄ group. Therefore, the 5' labeled *UvrABC* cut fragment moves 0.5 nt more slowly than the corresponding size sequencing fragment.

The efficiencies of repair (3' or 5' side cut) were determined by quantifying the cut bands with respect to the total counts in each lane (Table I). The *UvrABC* incision experiments were done at 6 nM of *UvrABC* proteins, which gave the optimum cutting value for most of the cross-links except for cross-links 29 and 19, which showed improved cutting efficiencies when *UvrABC* concentration was lowered from 6 nM to 2.4 nM. It appears that cross-links which are better *UvrABC* substrates are more easily inhibited by as little as 6 nM of *UvrABC*, while others are not. The cross-links studied in this work contain the psoralen adduct in different positions within the duplex, therefore the main difference among them is in their flanking sequences. The repair efficiencies among these cross-links varied from 0 to 34%. The cross-links 14, 18, and 30 showed a preference for the furan-linked strand (117-mer strand). The converse is true for cross-link 20 which preferred repair on the pyrone-linked strand (121-mer strand). Some of the cross-links studied in this work have identical sequences flanking the cross-link but with the adducted psoralen molecule in opposite orientations at the 5' TA or 5' AT site. These pairs of cross-links which differ only in the psoralen orientation are known as orientation isomers. The pairs compared are cross-links 11 and 12, 17 and 18, 19 and 20, and 29 and 30. The *UvrABC* cutting efficiencies on the same DNA strand for these isomers may be the same or differ by as much
were quantified using the AMBIS two-dimensional densitometer. From these quantitations the percent efficiencies of the UvrABC values were determined by quantitating the band migrating close to the uncut DNA in Fig. 3A acrylamide-7 M urea denaturing gel. The autoradiograms of the gel 488 UurABC Cross-link Repair Specificity

**TABLE I**

| Cross-link no. | Furan cut | Uncoupled cut | Pyrone cut | $K_d$ (UvrA complex in nM) | $K_d$ (UvrB complex in nM) |
|----------------|-----------|---------------|-----------|---------------------------|---------------------------|
| 11             | NA        | 11            |           | <2                        |                           |
| 12             | 1         | 19            |           |                           |                           |
| 14             | 14        | 0             | 0         | <2                        |                           |
| 17             | NA        | NA            | 1         | 6-7                       | 0.5                       |
| 18             | 22 (30)   | 5             | 8-12      | 1-1.6                     |                           |
| 19             | 34        | 0             | 0         |                           |                           |
| 20             | 0         | 0             | 31        | 0.8                       |                           |
| 29             | 18 (2)    | 0             | NA        |                           |                           |
| 30             | 15        | 16            | 4         | 2-6                       |                           |

$a$ Values indicate the percent UvrABC cut obtained on the furan or pyrone strand with 5'-labeled substrate using 6 nM of UvrABC.

The apparent equilibrium dissociation constants ($K_d$) were derived from the footprinting experiments of appropriate cross-links as described in experimental procedures. $K_d$ values are expressed in nM of UvrA monomer. Since for all the cross-links except cross-link 20, the formation of UvrB protein-DNA complex reached a maximum at early UvrA concentration, the $K_d$ values represent the maximum approximate value for cross-link. For cross-link 20, $K_d$ is a more accurate estimate.

$^b$ Not applicable.

$^c$ Values indicate the % UvrABC cut obtained with 3'-labeled substrate using 6 nM of UvrABC.

$^d$ For 5'-labeled cross-links 19 & 29, the percent cutting efficiency observed using 2.4 nM of UvrABC was 39% and 34% respectively.

as 34% (Table I). These results suggest that repair specificity depends both on the sequence flanking the cross-link and the psoralen orientation within a cross-link.

**UvrAB Footprint Intensity on Cross-Links Does Not Correlate with Cutting Efficiency**—UvrABC endonuclease mechanism consists of several reaction steps involving different protein-DNA interactions. Previous work by others have indicated that the nature of the Uvr protein-DNA complexes so formed as UvrA-DNA, UvrAB-DNA, and UvrB-DNA complexes each with a distinct footprint (Visse et al., 1992; Van Houten et al., 1987; Bertrand-Burggraf et al., 1991; Orren and Sancar, 1989; Mazur and Grossman, 1991; Munn and Rupp, 1991). Because the UvrAB protein-DNA complexes are not completely stable under gel-shift assay conditions (Visse et al., 1992), we have used the more reliable DNase I footprinting technique as a tool to quantify these Uvr protein interactions on different psoralen cross-links.

Cross-links can be bound with equal affinity to UvrAB proteins and yet have different repair efficiencies. Fig. 3 shows the furan-side and the pyrone-side footprint on two orientation isomers, 18 and 17, respectively (Fig. 3, A and B). Incubation of cross-links 18 and 17 with increasing amounts of UvrA resulted in gradual UvrA dimer binding on these cross-links as shown by DNase I protection on the strand which will be cut by UvrABC. A clear DNase I protection resulted at 54 and 18 nM of UvrA for cross-links 18 and 17, respectively (Fig. 3, A, lane 5, and B, lane 4). This 32-nt long

![FIG. 3. Footprint of UvrAB complex on the 117-mer strand for the orientation isomers of cross-links 17 and 18. The bottom strand of the cross-links 17 and 18 were dephosphorylated and 3'-labeled with [α-32P]dATP and the Klenow fragment of DNA polymerase I. Panel A, lanes 1-5, the cross-link 18 incubated with 0, 2, 6, 18, and 54 nM of UvrA, respectively, and footprinted with DNase I as described under "Experimental Procedures." The DNase I-sensitive site due to the presence of psoralen cross-link is indicated as H1. UvrA footprint is bracketed in lane 5. Lanes 6-8 show the footprint of UvrABC complex with DNase I, after incubating cross-link 18 with 2 nM of UvrA + 20 nM of UvrB, 6 nM of UvrA + 20 nM of UvrB, and 18 nM of UvrB + 20 nM of UvrB, respectively. Lane 9 is DNase I hydrolysis of control sequence without cross-link. The 5' and 3' hypersensitive sites of the UvrB-cross-link footprint are indicated as H3 and H5, respectively. The exposed DNase I-sensitive phosphodiester bond in the middle of the footprint is shown as H4. The position of the cross-link is one base below the hypersensitive site H4. The X and Y regions indicated are the 5' half and 3' half of the footprint with respect to the exposed phosphodiester moiety 5' to the psoralen modified thymine residue. The region Z shows the footprint of the UvrA(B) recognition of the UvrB-lesion complex. G and G + A lanes are the chemical sequencing ladders used as position reference. Panel B, lanes 1-4, the DNase I footprint on cross-link 17 with 0, 2, 6, and 18 nM of UvrA, respectively. Lanes 5-6, the footprints on cross-link 17 with 2 nM of UvrA + 20 nM of UvrB, and 18 nM of UvrA + 20 nM of UvrB, respectively. H1 indicates the 3' DNase I hypersensitive site. X and Y regions are 5' and 3' side regions of UvrB-lesion footprint as indicated. The nonspecific UvrA recognition of UvrB-lesion footprint is shown by Z1 and Z2 regions.

UvrA dimer footprint is similar to the one formed on a HMT monoadduct (Van Houten et al., 1987). We have assigned this footprint as corresponding to UvrA-DNA complex. This UvrA-DNA complex footprint also shows a DNase I hypersensitive site 11 nt 5' to the modified thymine.

Similarly, analysis of the footprint of 2-54 nM of UvrA + 20 nM of UvrB on cross-links 18 and 17 (Fig. 3, A, lanes 6-9, and B, lanes 5 and 6) showed that UvrB-lesion complexes were formed at 2 nM of UvrA + 20 nM of UvrB (Fig. 3A, lane 6, and B, lane 5). These two 20-nt long footprints showed the key feature of the UvrB-lesion footprint as reported by others for HMT monoadduct and cross-link (Bertrand-Burggraf et
UvrABC Cross-link Repair Specificity

al., 1991; Munn and Rupp, 1991): a strong DNase I hypersensitive site was observed at the 11th and 12th nt 5' to the lesion. The other 5' DNase I hypersensitive site at 18 nt from the lesion, characteristic of UvrA-DNA complex, disappeared in the UvrB-lesion complex. In addition, the footprints showed that the nucleotide next to the modified thymine was not protected from DNase I in this complex. This DNase I sensitivity at the base next to the modified thymine was also seen in other cross-link footprints and suggested that the adjacent bases next to the lesion were not protected by UvrB protein in the UvrB-lesion complex and were accessible from the minor groove. A weakly protected region, Z, in these footprints is due to nonspecific UvrA(B) binding to UvrB-lesion complex and it will be discussed below. Although we have not assigned the nature of the complex independently by other techniques such as gel-shift and supershift assays, we have tentatively assigned this complex as UvrB complex based on the footprint characteristics.

The DNase I protected or hypersensitive band intensities in the autoradiograms of the gels were quantified as described under "Experimental Procedures." From these values, UvrA lesion binding curves were constructed (data not shown). From the UvrA lesion binding curves for cross-link 17 and 18, we have calculated the apparent dissociation constants of the UvrA-DNA complex as the concentration of UvrA corresponding to 50% saturation of DNA lesion sites: maximum \(K_d = 8-12\) nM for cross-link 18; maximum \(K_d = 6-7\) nM for cross-link 17 (Table I).

The UvrA-dependent UvrB binding curve for cross-link 18 (data not shown) was constructed from the DNase I hypersensitive sites of cross-link 18 footprint autoradiogram. From this, we have estimated the apparent equilibrium dissociation constant for UvrA-dependent UvrB binding on DNA as maximum \(K_d = 1.6\) nM (for cross-link 18) and an approximation of the \(K_d\) of cross-link 17 as 0.5 nM (Table I). Therefore the affinities of UvrA or UvrB complexes for cross-link are 5-8 times greater than that of the UvrA complex. This analysis also showed that for a pair of orientation isomers (17 and 18), the affinities of the UvrB complexes formed on the cut strand are similar (\(K_d = 1-2 \times 10^9\)) irrespective of whether the UvrABC incision will be on the furan- or pyrone-linked strand. Although the binding of UvrA and UvrB proteins to these two cross-links on the same strand were similar, the UvrABC incision efficiency for these cross-links were different. It appears that the differences in the subsequent binding and cutting by UvrC after UvrAB-lesion complex formation must be an additional parameter controlling the overall incision efficiency of a cross-link.

UvrB-Lesion-specific Footprint Is Formed Only on the Strand That Is Cut—Fig. 4, A and B, shows the difference in footprints of the UvrAB-DNA complex formed on the two strands of cross-link 20 in which only the top strand will be cut by UvrABC. Incubation of this cross-link with increasing concentrations of UvrA (0-13.5 nM, Fig. 4A, lanes 1-6) did not show a UvrA-specific DNase I footprint on the bottom strand (117-mer strand, furan-linked strand). This absence of UvrA footprint is expected because at least 27 nM of UvrA was required to produce the UvrA footprint for cross-link 18. Under the same conditions but in the presence of only 3 nM UvrB, a 30-nt footprint emerged on this uncut strand even at a low concentration of UvrA (0.8 nM). However, this footprint did not contain a DNase I hypersensitive site at the 11th phosphodiester moiety 5' to the lesion and the footprint did not change in size with increasing concentrations of UvrA or UvrB. This footprint also contained a DNase I-sensitive site at the 28th phosphodiester moiety 5' to the lesion. Because the appearance of this footprint on this strand required both UvrA and UvrB proteins, this footprint corresponds to a UvrAB-lesion complex. The absence of repair on this strand may be related to failure to form the lesion-specific UvrB complex.

On the top strand of cross-link 20, the strand which will be cut by UvrABC endonuclease in the cross-link (121-mer, pyrone-linked strand), concentrations up to 13 nM of UvrA (Fig. 4B, lanes 1-6) did not result in a characteristic UvrA-DNA footprint. However, at 3 nM of UvrB and 0.75 nM of UvrA, a weak UvrA footprint with the characteristic 5' DNase I hypersensitive site was formed (Fig. 4B, lane 7). As the concentration of UvrA was increased from 0.75 to 13.5 nM, the footprint shrank from 34 to 23 nt long (Fig. 4B, lane 12). Thus the lesion-specific UvrB footprint with 5'-hypersensitive site at the 11th phosphodiester moiety is formed only on the strand which will be cut by the UvrABC endonuclease. These footprinting experiments on two strands of the same cross-link illustrate the difference in the nature of the protein-DNA interactions for the strand to be cut and the strand not to be cut.

From the binding curve constructed from the densitometer scans of the autoradiogram of this experiment, we have estimated the equilibrium dissociation constant of UvrB-lesion complex (\(K_d\)) as 0.75 nM (Table I). The \(K_d\) values of cross-links 20, 18, and 17 are comparable (\(K_d = 1-2 \times 10^9\)).

To verify whether the formation of a UvrB footprint, characterized by a hypersensitive site at the eleventh phosphodiester moiety 5' to the lesion, is a prerequisite for UvrABC cutting, we have footprinted the furan-side of the cross-links 12, 14, and 30 with UvrA and UvrB proteins. The footprint on cross-link 12 is an example of a lesion positioned only 10 nt away from the 3' termini. The UvrB complex formed on this lesion protected only 6 nt on the 3' side and 11 nt on the 5' side (data provided to the reviewers but not shown). We estimated the maximum \(K_d\) of UvrB-lesion complex for cross-link 12 to be <2 nM. Similarly the cross-links 14 and 30 produced UvrB footprints with the 5' hypersensitive site on the UvrABC cut strand (furan-linked, 117-mer strand, data not shown). Therefore, it seems likely that most of the cross-links which will be incised by UvrABC endonuclease will form UvrB footprints with a 5' DNase I hypersensitive site on the strand to be cut.

Kinetics of UvrB-Lesion Complex Formation, and Detection of Secondary Recognition of UvrB-Lesion Complex by UvrAB Complex in Vitro—The kinetics of UvrB-lesion complex formation were investigated by DNase I footprinting using cross-link 19. The densitometer tracings of the footprints (autoradiograms not shown) obtained for various aliquots withdrawn after incubation times of 0, 5, 10, 15, 25, 35, and 60 min are shown in Fig. 5. The tracings are divided into two segments (I and II) to aid analysis. The footprint obtained at 5 min showed some protection of segment I. By 9 min, a portion of the bands in this protected region (43 nt long) reappears on the 3'-side (Fig. 5, segment I, peaks 2-8, 10-min time point) with the simultaneous appearance of the 5'-DNase I hypersensitive sites at the 11th and 12th phosphodiester moieties 5' to the lesion (Fig. 5, segment II, peaks 12 and 13, 10-min time point), suggesting the presence of a strong UvrB-DNA complex footprint between nt positions 56 and 41 (Fig. 5, between peak 9 and peak 12 in the 15-min time point tracing). By 60 min, this footprint extended toward the 3'-direction to become 35 nt long, suggesting a secondary UvrAB recognition of the first UvrB-lesion complex in vitro (Fig. 5, between peak 6 of segment I and peak 12 of segment II in the 60-min time point tracing). In this experiment, the UvrA concentration
used was low. No additional UvrA was added after the formation of the UvrB-lesion complex, therefore the natural progression of the protein complexes was not disturbed.

The amount of UvrB-lesion complexes formed was plotted against the time of incubation (Fig. 6A). From the initial slope, the rate constant for the loading of UvrB was calculated using UvrA and DNA concentrations as the limiting factors. This rate constant also agreed with the second order rate constant derived from the slope of the log([free DNA]) vs. time plot. In both these methods, agreed with the $k_{\text{on}}$ values reported for UvrB loading on pyrimidine dimers (Orren and Sancar, 1989).

**DISCUSSION**

**Sequence-dependent Variation in DNA Interstrand Cross-link Structure Can Affect Repair**—The structure of psoralen interstrand cross-link has very interesting structural features. NMR spectroscopy experiments combined with molecular modeling studies revealed that the psoralen interstrand cross-link produces a bend toward the major groove (Tomic et al., 1987). Gel electrophoretic analysis of psoralen cross-linked duplexes containing mismatched bases revealed that the two sides of the helix flanking the cross-link contain domains of different stabilities (Kumaresan et al., 1992). It is likely that these features of psoralen cross-links flanked by different sequences can adopt different DNA conformations in a sequence-dependent manner. Similar observations for the sequence-dependent variations in the conformations of other DNA-bulky adducts are known. Examples include DNA adducts of (+)-anti-benzo[a]pyrene diol epoxide (Roche et al., 1991; Rodriguez and Loechler, 1993) and C-8-guanine adducts of 2-acetyl-aminofluorene (Veaute and Fuchs, 1991; Belguise-Valladier and Fuchs, 1991). The sequence-specific variations in psoralen cross-link conformation may influence the efficiencies of formation of various UvrABC protein-DNA complexes, like UvrA, UvrAB, UvrB, and UvrB(AB) and Uvr(A)BC complexes. The sequence-dependent variations in DNA conformation and their role in DNA-protein interactions has been recently reviewed (Travers, 1989).

Three major points can be inferred from our work discussed in this paper. (i) We have observed that UvrB-lesion footprint is formed only on the strand which is to be cut, but not on the strand to be cut weakly or not cut. The strand-specificity in the cross-link repair may partly stem from the competition for UvrB loading onto cross-links.
between the lesions on the two strands of a cross-linked DNA duplex for Uvr protein-DNA complex formation. (ii) Although we have observed the formation of a footprint corresponding to UvrB complex in all the cross-links on the strand that is to be cut, the strength of UvrB complex binding did not correlate with the UvrABC incision efficiency. This is in accord with the observation that repair efficiency of N-2-acetylaminofluorene modified DNA substrates at different sites of a DNA duplex could not be accounted for by the binding of the UvrAB complex alone (Seeberg and Fuchs, 1990). But this is in contrast with the observation made by Visse et al. (1992) who showed, for a cisplatin substrate present in a defined sequence, that cutting and UvrAB binding can be correlated. (iii) The time-dependent secondary recognition of UvrB-DNA complex by UvrA or UvrAB can lead to UvrB(AB) DNA complex. This may interfere with UvrC binding and cutting. Similarly with increasing concentration of UvrA protein, in the presence of fixed concentration of UvrB protein, Visse et al. (1992) observed that, in addition to the formation of UvrB-DNA complex, another nonspecific UvrAB-DNA complex was formed in their reaction. More indirectly, others have explained the UvrA concentration dependent change in the footprint of UvrB protein-DNA complex as due to a reversal of UvrB-DNA complex to a UvrAB complex (Bertrand-Burgraff et al., 1991); alternatively UvrA can compete with UvrAB for binding to DNA, which results in UvrA-DNA complex (Snowden and Van Houten, 1991). Our approach of using low concentrations of UvrAB proteins in our experiment has allowed us to observe the sequential formation of UvrB complex footprint followed by the appearance of a secondary recognition of the UvrB-lesion complex by another UvrAB complex.

Acknowledgment—We thank Catherine A. Oleykowski for providing the purified UvrABC proteins.

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