Accessibility of Epitopes on UvrB Protein in Intermediates Generated during Incision of UV-irradiated DNA by the Escherichia coli Uvr(A)BC Endonuclease*

Oleg I. Kovalsky† and Lawrence Grossman§
From the Department of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205

Structural intermediates generated during incision of damaged DNA by the Uvr(A)BC endonuclease were probed with monoclonal antibodies (mAbs) raised against the Escherichia coli UvrB protein. It was found that the epitope of B2C5 mAb, mapped at amino acids (aa) 171–278 of UvrB, is not accessible in any of the preformed Uvr intermediates. Preformed B2C5-UvrB immunocomplexes, however, inhibited formation of those intermediates. B2C5 mAb seems to interfere with the formation of the UvrA-UvrB complex due to overlapping of its epitope and the UvrA binding region of UvrB. Conversely, the epitope of B3C1 mAb (aa 1–7 and/or 62–170) was accessible in all Uvr intermediates. The epitope of B2E3 mAb (aa 171–278) was not accessible in any of the nucleoprotein intermediates preceding UvrB-DNA preincision complex. However, B2E3 was able to immunoprecipitate this complex and to inhibit overall incision. B2A1 mAb (aa 8–61) inhibited formation of those Uvr intermediates requiring ATP binding and/or hydrolysis by UvrB. B2B9 mAb (aa 473–630) inhibited Uvr nucleoprotein complexes involving UvrB. B2B9 seems to prevent the binding of the UvrA-UvrB complex to DNA. The epitope of the B3E11 mAb (aa 379–472) was not accessible in Uvr complexes formed at damaged sites. These results are discussed in terms of structure-functional mapping of UvrB protein.

Uvr(A)BC endonuclease initiates NER2 in Escherichia coli by catalyzing dual incision of damaged DNA (1, 2). Precise dual incision is achieved by the combined action of three proteins, UvrA, UvrB, and UvrC, comprising the Uvr(A)BC. Remarkably, these three proteins do not seem to physically associate (at least in vitro) but rather act in a sequential manner generating relatively stable intermediates (3–7). The current working model for these intermediate steps include: (i) UvrA2B1 complex formation; (ii) interaction of this complex with DNA and damage recognition; (iii) formation of the UvrB-DNA complex at damaged sites; (iv) binding of UvrC to the UvrB-DNA preincision complex, triggering dual incision (3–8). The binding and/or hydrolysis of ATP by both UvrA and UvrB are required at different stages of the preincision process (3). The correct structural architecture of the macromolecular intermediates of Uvr(A)BC seems to be critical for repair-proficient incision. Amino acid substitutions in the components of Uvr(A)BC as well as artificial in vitro conditions result in reduced or anomalous incision because of the alterations in the preincision complex architecture (8–10). The apparent universality of the “dual incision” principle for NER (11) underscores the importance of understanding the structural determinants of Uvr(A)BC-catalyzed incision of damaged DNA. To approach this problem the anti-UvrA, -UvrB, and -UvrB*2 mAbs were generated and characterized in terms of their applicability for probing the structural intermediates generated by Uvr(A)BC (14, 15). Here we report on studies of the accessibility of epitopes of six anti-UvrB/UvrB* mAbs in some of these intermediates. The results provide insights into the role of the epitope regions of the UvrB protein in some of the intermediates and reveal some of the structural rearrangements of the complexes involved in Uvr(A)BC functioning.

EXPERIMENTAL PROCEDURES

Protein Purification—Uvr proteins were purified and UvrB* prepared and purified as described (13, 14, 16).

Preparation and Purification of Anti-Uvr mAbs—Anti-Uvr protein mAbs were generated and purified according to standard procedures (17) and manufacturer’s recommendations as described (14, 15).

Protein Concentrations—The concentrations of all proteins were determined using the Bio-Rad protein assay kit with bovine serum albumin as a standard.

Buffers Used in Functional Assays—The binding buffer (BB) is composed of 50 mM K MOPS (pH 7.6), 100 mM KCl, 10 mM MgCl2, 1 mM EDTA, 100 μg/ml bovine serum albumin, 1 mM dithiothreitol, and 5% glycerol (v/v). The supercoiling buffer consists of 20 mM HEPES (pH 7.5), 50 mM KCl, 8 mM MgCl2, 1 mM dithiothreitol, 100 μg/ml bovine serum albumin, 4 mM ATP; and the nicking buffer was BB supplemented with 2 mM ATP.

Assay of ATPases Associated with Uvr Protein—Uvr protein complexes were preincubated with excess mAb (100 nm) in BB buffer for 30 min at 37 °C followed by the addition of UvrA (75 nm) and ATP (final concentration 108 μM) containing [3H]ATP (Amersham Pharmacia Biotech). In those cases where the ATPase is associated with Uvr nucleoprotein complexes, DNA or uvDNA (plasmid pTZ18R DNA at 30 μM base pair concentration, containing 0 or ~12 cyclobutane pyrimidine dimers per circle) was also added at this point. The hydrolysis of ATP was assayed as described (18).

Supercoiling Assay—The experiments were conducted essentially as described previously (14, 19).

Immunoprecipitation—The immunoprecipitation of UvrA-UvrB com-

‡ On leave from the N.D. Zelinsky’ Institute of Organic Chemistry of the Russian Academy of Sciences, 117913 Moscow, Leninsky pr. 42, Russia.
§ To whom correspondence should be addressed: Dept. of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health, 615 N. Wolfe St., Baltimore, MD 21205. Tel.: 410-614-4226; Fax: 410-955-2926; E-mail: lg@welchlink.welch.jhu.edu.

1 The abbreviations used are: NER, nucleotide excision repair; mAbs, monoclonal antibodies; uvDNA, UV (254 nm)-irradiated DNA; Uvr (A)BC, E. coli Uvr(A)BC endonuclease; PAGE, polyacrylamide gel electrophoresis; BB, basic buffer; MOPS, 4-morpholinepropanesulfonic acid.

2 This work was supported by National Institutes of Health MERIT Award GM 22846 (to L. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received for publication, February 18, 1998, and in revised form, June 15, 1998.

This paper is available on line at http://www.jbc.org
plexes was performed essentially as described (14), except detection of Uvr proteins in the immunoprecipitate Western blotting and the ECL system (Amersham Pharmacia Biotech) were employed. The mAbs B2E2, B2A1, and anti-UvrA m Ab A2D1 (14, 15) were used for the probing of blots. Immunoprecipitation of UvrA-UvrB-DNA complexes was performed as follows: UvrA (2 nm), UvrB (50 nm), and 50 fmol undamaged [3H]-pHE6 plasmid DNA (14) were preincubated for 30 min in the presence of 1 mM ATP for 30 min. Then mAbs and excess unirradiated pTZ18R plasmid DNA were added followed by immunoprecipitation (14).

Band Shift Analysis—The formation of Uvr protein-uvDNA complexes was assessed by band shift assays (9, 20). In some cases Uvr nucleoprotein complexes were preformed by a "reverse order of addition," UvrA, UvrB, and uvDNA were first incubated in BB plus 2 mM ATP followed by the addition of UvrC. Final concentrations of UvrA, UvrB, and UvrC are 5, 10, and 20 nM, respectively; final reaction volume was followed in 20 min by the addition of UvrC. Final concentrations of UvrA, UvrB, and UvrC were analyzed by 4% PAGE with 1 mM MgCl2, and 1 mM ATP in the gel and running buffers as described (14). In some cases Uvr nucleoprotein complexes were preformed by a 20-min incubation period of UvrA, UvrB, and uvDNA. Then mAbs were added and incubation continued for an additional 20 min. Samples were then treated and electrophoresed as above.

Incision of UV-irradiated DNA by Uvr(A)BC—UvrB was preincubated with excess anti-UvrB/UvrB* mAb (200 nm) in BB plus 2 mM ATP for 20 min at 37 °C. Then immunoreactions were mixed with uvDNA (32P-labeled fragment described under "Band Shift Analysis") and UvrA followed in 20 min by the addition of UvrC. Final concentrations of UvrA, UvrB, and UvrC are 5, 10, and 20 nM, respectively; final reaction volume is 10 μl. In experiments involving "reverse order of addition," UvrB, UvrA, and uvDNA were first incubated in BB plus 2 mM ATP followed by the sequential addition of anti-Uvr mAb and UvrC. The incision reactions were stopped 10 min after the addition of UvrC by 2 μl of 0.5 M EDTA and placing the reaction tubes on ice. DNA was precipitated with ethanol and analyzed by 10% PAGE. The positions of 5'-incisions by UvrABC were assigned by comparison with those generated by the Micrococcus luteus UV endonuclease (14) (data not shown).

RESULTS

The Influence of mAbs on UvrA-UvrB Complex Formation—UvrA and UvrB in the presence of ATP interact with an apparent stoichiometry of UvrA2B1 (21, 22). Formation of this complex results in a 30–50% inhibition of the UvrA-associated ATPase (23). If the immunocomplex of mAb and UvrB interferes with the formation of the UvrA-UvrB complex then this inhibitory effect should not be observed provided the mAb itself does not inhibit UvrA-associated ATPase. It was found that none of the mAbs in question significantly inhibited UvrA-associated ATPase (data not shown). Further, incubation of ATP with mAbs alone did not result in its hydrolysis above background level (data not shown). Only mAb B2C5 reduced the inhibitory effect of the UvrB binding on UvrA-associated ATPase (Table I). Immunocomplexes of other mAbs did not interfere with the specific inhibitory effect suggesting that the respective mAbs do not interfere with UvrA-UvrB complex formation. These results were consistent with those obtained by immunoprecipitation of preformed UvrA-UvrB complexes (Fig. 1). B2C5 was unable to immunoprecipitate these complexes; only UvrB was detected in the immunoprecipitate even when both proteins were in the reaction mixture (Fig. 1, B2C5, lane 3). Moreover, an excess of UvrA significantly reduced the amount of precipitated UvrB (Fig. 1, B2C5, cf. lanes 2 and 3), suggesting that it blocks access to the epitope. Conversely, B3C1 mAb interacted with preformed UvrA-UvrB complexes, judging from its ability to co-precipitate UvrA (Fig. 1, B3C1, lane 3). B2A1 mAb was also able to immunoprecipitate UvrA-UvrB complexes, although less efficiently than B3C1 (Fig. 1, cf. lane 3). Under these conditions UvrA alone was not precipitated (Fig. 1, lane 1). B*2E3 mAb only marginally immunoprecipitated UvrB alone but not in complex with UvrA. It, however, efficiently interacted with UvrB* and its complex with UvrA (Fig. 1, B*2E3, lanes 4 and 5). These observations are consistent with findings that: (i) B*2E3 has a much higher affinity for UvrB* than for UvrB in solution, although it efficiently recognizes the denatured forms of both species on Western blot (15); (ii) UvrB*, the proteolytic fragment of UvrB, is able to interact with UvrA (cf. Refs. 8, 12, 14, and 21). Immunoprecipitation with B*2E9 and B*3E11 failed to generate a significant UvrB signal in the precipitates regardless of the presence of UvrA (data not shown).

The Influence of mAbs on the Uvr Nucleoprotein Complexes-associated ATPase—The next step in the functioning of UvrA/B is initiated by binding of the UvrA-UvrB complex to undamaged regions of DNA (3, 13). The formation of such a complex is manifested in several ways. In particular, the ATPase associated with this complex is catalytically more ac-

---

**Table I**

| UvrA only | UvrA + UvrB |
|-----------|-------------|
| No mAb    | B2A1        | B2C5        | B3C1        | B2E2        | B*2E9       | B*2E3       | B*3E11      |
| 51 ± 5    | 34 ± 3      | 27 ± 3      | 48 ± 5      | 38 ± 4      | 37 ± 4      | 42 ± 4      | 36 ± 4      | 34 ± 3      |

**Fig. 1.** Immunoprecipitation of UvrA proteins and UvrA-UvrB/ UvrA-UvrB* complexes by anti-Uvr mAbs. Three kinds of samples were subject to immunoprecipitation analysis with each of the mAbs: lane 1, 600 ng UvrA; lane 2, 300 ng of UvrB; and lane 3, 600 ng of UvrA + 300 ng of UvrB. In the case of B*2E3 mAb, two additional samples were analyzed: lane 4, 300 ng of UvrB*; lane 5, 600 ng of UvrA + 300 ng of UvrB*. lane 6, standards of UvrA, UvrB, and UvrB* (100 ng each), no immunoprecipitation. The composition of the immunoprecipitates was analyzed by 8% SDS-PAGE followed by Western blotting and ECL (Amersham Pharmacia Biotech) detection of Uvr proteins with mAbs (IgG h.c.), as well as some other unidentified immunoreactive material, as can also be seen.
Probing Uvr(A)BC Incision Intermediates

The influence of the anti-UvrB mAbs on the UvrA-UvrB-DNA- and UvrA-UvrB-uvDNA-associated ATPases. UvrB was preincubated in BB buffer in excess mAb (200 nM) to form immunocomplexes. Then UvrA (final concentrations of UvrB and UvrA were 25 and 75 nM, respectively) and ATP (108 μM) were added, and the initial rates of ATP hydrolysis were analyzed in the absence of DNA (left bars) and in the presence of either unirradiated DNA (middle bars) or uvDNA (right bars).

The stimulation of the ATPase associated with the UvrA-UvrB-DNA complex is even more profound when DNA is damaged (Ref. 23 and Fig. 2, cf. left and middle bars). The immunocomplexes formed with mAbs resulted in a decrease of the stimulating effect of undamaged DNA, whereas other mAbs had little or no significant effect (Fig. 2, middle bars). This suggests that B2C5, B*2B9, and B2A1 interfere with either the formation of the UvrA-UvrB-DNA complex or with its ATPase activity.

The stimulation of the ATPase associated with the UvrA-UvrB-DNA complex is even more profound when DNA is damaged (Ref. 23 and Fig. 2, cf. middle and right bars). As with undamaged DNA, the B3C1 and B*2E3 mAbs had no significant effect on stimulating the ATPase activity by uvDNA, whereas B2A1, B2C5, and B*2B9 mAbs significantly reduced that stimulation (Fig. 2, right bars). However, B*3E11, having no effect on the stimulation by intact DNA, inhibited the additional stimulation attributed to damage (Fig. 2, B*3E11, cf. middle and right bars).

The Influence of the mAbs on Positive Supercoiling—The accumulation of positive supercoils in closed circular plasmid DNA, when incubated with UvrA and UvrB in the presence of E. coli topoisomerase I, reflects the DNA helix-tracking activity of the UvrA-B complex (19). This activity is thought to be essential for damage recognition and/or prepriming (3, 19).

Examining the effect of mAbs on the extent of positive supercoiling provides some insight into the state and accessibility of respective epitopes in this intermediate UvrA-UvrB DNA helix-tracking complex (14). It was found that preincubation of UvrB with B*2E3 mAb had no significant effect on the extent of positive supercoiling (Fig. 3). However, preincubation with B2A1, B2C5, and B*2B9 inhibited positive supercoiling, whereas B3C1 and B*3E11 mAbs significantly stimulated positive supercoiling (Fig. 3). The absence of the effect of immunocomplex on positive supercoiling suggests that B*2E3 mAbs do not interact with the DNA helix-tracking complex; the decrease in positive supercoiling implies that B2A1, B2C5, and B*2B9 mAbs inhibit formation or functioning of these complexes, and stimulation of positive supercoiling indicates that B3C1 and B*3E11 can interact with translocating UvrA-UvrB-DNA complexes (14). Positive supercoiling was not observed when only mAbs or only one of each of the Uvr proteins was present in the reaction mixture (data not shown).

Immunoprecipitation of Uvr Nucleoprotein Complexes—The accessibility of epitopes in preformed Uvr nucleoprotein complexes can be assessed directly by immunoprecipitation of these complexes with mAbs. In this type of assay the presence of radiolabeled 3H-pHE6 plasmid DNA in the immunoprecipitate was measured (14). Immunoprecipitation was performed in excess UvrB over UvrA to allow UvrA-UvrB-DNA complexes to prevail over UvrA-DNA complexes (9, 20, 24). With undamaged DNA, the UvrA-UvrB-DNA complexes were not precipitated if ATP was present in the reaction mixture (data not shown). This emphasizes the dynamic nature of Uvr complexes with undamaged DNA in the presence of ATP, stimulated by the promotion of dissociation reactions when catalyzed by ATP hydrolysis (3, 23, 25). Therefore, immunoprecipitation with undamaged DNA was carried out in the presence of the poorly hydrolyzable analog ATPγS. The latter, in excess UvrB over UvrA, results predominantly in the formation of relatively stable UvrA-UvrB-DNA complexes (26). Only mAb B3C1 was able to immunoprecipitate the above complexes (Fig. 4, middle bars).

The Influence of Immunocomplex Formation on the UvrB-DNA Preincision Complex—The localization of the preincision complex at the site of damage results in UvrA dissociation from the damaged site and formation of stable UvrB-uvDNA complexes (9, 20–22). The accessibility of epitopes on UvrB protein in the preincision complex was probed by immunoprecipitation in the presence of ATP and competitor nonradioactive DNA (Fig. 4, right bars). These conditions seem to favor UvrB-DNA preincision complex formation (9, 21, 22). There was no radioactivity in the immunoprecipitates of mAbs B2A1, B2C5, B*2B9, and B*3E11. This suggests that these mAbs...
were unable to interact with Uvr nucleoprotein complexes under these conditions. On the other hand, mAb B3C1 and, surprisingly, B*2E3 were able to precipitate preincision complexes (Fig. 4, right bars).

Band shift techniques were used to detect UvrB-uvDNA complexes (9, 20). Preincubation of UvrB with mAbs B2A1, B2C5, B*2B9, and B*3E11 significantly inhibited UvrB-uvDNA complexes (Fig. 5A). The B*2E3 (Fig. 5A) mAb had almost no effect on such complexes. Preincubation of UvrB with B3C1 mAb resulted in an upward shift and “smearing” of the band corresponding to the UvrB-uvDNA complex (Fig. 5A). If, however, UvrB-uvDNA complexes were first preformed then followed by incubation with mAbs, the inhibitory effects of mAbs B2A1, B2C5, B*2B9, and B*3E11 were significantly reduced, whereas B3C1 and B*2E3 showed almost the same pattern as in the case of immunocomplex preformation (Fig. 5B). Incubation of uvDNA with mAbs alone did not result in any band shift effect (data not shown).

The Influence of Anti-Uvr mAbs on 3'-Incision of uvDNA by Uvr(A)BC—We studied the influence of anti-Uvr mAbs on 3'-incision as a reflection of the overall incision reaction. It was found that the neutralizing effects of mAbs correlate in general with their ability to inhibit UvrB-uvDNA preincision complexes. B2A1, B2C5, B*2B9, and B*3E11 efficiently inhibited incision, whereas B3C1 did not influence the extent of incision if preincubated with UvrB protein (Fig. 6A). Preforming preincision complexes resulted in a decrease of the inhibitory effect of all of the above neutralizing mAbs, although the decrease was less profound in case of B*3E11 (Fig. 6, cf. A and B). Surprisingly, the B*2E3 mAb, which does not seem to interfere with UvrB-uvDNA preincision complexes (Fig. 5), strongly neutralized 3'-incision regardless of the “order of addition” (Fig. 6, cf. A and B).

**DISCUSSION**

The dual incision of damaged DNA by Uvr(A)BC endonuclease is a multistep process involving a number of intermediate Uvr protein-protein and nucleoprotein complexes (3–6). A detailed understanding of the relation between the structural architecture of these intermediates and their role in the incision process is an ultimate goal of structure-functional analysis. In the absence of not only three-dimensional structures of intermediate complexes but even those of individual Uvr proteins, alternative experimental approaches are required to study this problem. mAbs allow one to assess the accessibility of their epitopes in different intermediates and thus to gain

---

**FIG. 4.** Immunoprecipitation of preformed Uvr nucleoprotein complexes. Three types of samples were analyzed with each anti-Uvr mAb: DNA in the presence of only UvrA (2 nM) (left bars); UvrA-UvrB-DNA complexes formed in the presence of UvrA (2 nM), UvrB (50 nM), and 1 mM ATP+γS (middle bars); UvrB-uvDNA complexes formed in the presence of UvrA, UvrB, uvDNA, 2 mM ATP, and excess of undamaged pTZ18R DNA (right bars). The analyzed DNA was 50 fmol of 3H-pHE6 DNA. The percentage of radioactivity found in the immunoprecipitate was taken as a measure of Uvr nucleoprotein immunoprecipitation.

**FIG. 5.** Band shift analysis of the influence of anti-Uvr mAbs on UvrB-uvDNA preincision complex formation. A, immunocomplexes of UvrB protein (50 nM) and anti-UvrB/UvrB* mAb (200 nM) were first preformed followed by the addition of UvrA (1 nM) and uvDNA; B, preincision UvrB-uvDNA complexes were first preformed by incubating UvrA, UvrB, and uvDNA followed by the addition of mAb.

**FIG. 6.** The influence of anti-UvrB/UvrB* mAbs on the 3'-incision of uvDNA by Uvr(A)BC. A, UvrB protein was first preincubated with excess mAb (100 nM) followed by the addition of two other Uvr protein and uvDNA; B, UvrA and UvrB were first incubated with uvDNA followed by the addition of mAb and UvrC (see “Experimental Procedures”). Incised uvDNA was precipitated and subjected to 10% PAGE, 8 M urea. Concentrations of UvrA, UvrB, and UvrC were 5, 10, and 20 nM, respectively. Autoradiographs of electrophoresed gels are shown.
information about structural rearrangements taking place during the incision process as well as the roles of the protein regions carrying epitopes in the intermediates. mAbs can also be used to probe naturally occurring protein-protein and nucleoprotein complexes under in vivo-like conditions, for instance, in crude extracts or fractionated cells (27).

The anti-Uvr mAbs used in this study exhibit a high affinity for native proteins in solution and are noninvasive in terms of their influence on conformation and/or flexibility of antibodies (15). The latter finding facilitates interpretation of the results of the functional assays of Uvr proteins and their complexes (15, 28).

The accessibility of anti-Uvr-B mAb epitopes on Uvr protein was probed directly by immunoprecipitation of different intermediate Uvr protein complexes and/or indirectly by studying the influence of these mAbs on functional activities associated with those complexes (summarized in Fig. 7). It was found that B2C5 interferes with the first intermediate of Uvr(A)BC, UvrA-UvrB complex. The inhibition of this complex formation appears to result from the steric hindrance created by the B2C5 in immunocomplex. Supporting this conclusion, B2C5 epitope, mapped to amino acids 171–278, overlaps with the N-terminal UvrA binding region of UvrB (amino acid residues 115–250) deduced from mutational analysis (7).

B2C5 mAb does not seem able to access its epitope even after UvrA dissociates from UvrB-uvDNA preincision complex. This suggests that its epitope is either hidden in the UvrB-uvDNA complex or is disrupted because of the conformational change in UvrB upon this complex formation.

B*2B9 mAb is a very potent inhibitor of all functional activities associated with the formation of Uvr nucleoprotein complexes; stimulation of UvrA-UvrB ATPase by DNA and uvDNA, UvrA-UvrB DNA helix-tracking activity, preincision complex formation and overall incision. The B*2B9 epitope region encompassing amino acids 470–630 includes helicase motifs V and VI (29). The latter motifs were found by mutational analysis to be essential for functions associated with UvrA-UvrB nucleoprotein complexes and were implicated in DNA binding (10). Hence, B*2B9 binding could interfere with these structural-functional determinants, thus inhibiting respective complexes. An alternative explanation of B*2B9 inhibitory effects is that it may interfere with UvrA-UvrB complex formation. The epitope of this mAb may overlap with the second UvrB region (amino acids 547–673) implicated in UvrA binding (7). This region, however, seems to be less significant for UvrA binding, because UvrB*- and other truncated proteins lacking this region are still able to productively bind UvrA (Fig. 1 and Ref. 10). Consistently, the B*2B9-UvrB immunocomplex did not result in significant reduction of the characteristic inhibitory effect of UvrB binding on UvrA-associated ATPase. However, we were unable to unambiguously assess accessibility of B*2B9 epitopes on UvrB in Uvr complexes because of the lack of immunoprecipitating ability by this mAb.

B2A1 mAb was found to recognize the region of UvrB containing an ATPase site. Moreover, this mAb seems to compete with ATP for UvrB (15). This mAb, however, did not interfere with UvrA-UvrB complex formation in solution, suggesting that ATP binding and/or hydrolysis by UvrB does not seem to be required for UvrA-UvrB interaction in solution. The ATP binding and/or hydrolysis by UvrA, however, seem to be crucial for productive UvrA-UvrB interaction (30). The B2A1-UvrB immunocomaplex did not support formation of any of the successive functional Uvr intermediates as well as the very incision of uvDNA by Uvr(A)BC. ATP binding/hydrolysis was shown to be absolutely required for the formation and functioning of those intermediates (3). Hence, B2A1 mAb seems to prevent the development and functioning of the intermediates and overall incision by blocking the ATP binding site of UvrB. This underscores the importance of ATP hydrolysis/binding by UvrB for the processing of DNA damage by Uvr(A)BC.

B*3E11 seems to inhibit complexes and functions associated with DNA damage; additional stimulation of UvrA-UvrB-associated ATPase by uvDNA, UvrB-uvDNA preincision complex, and the 3'-incision of uvDNA. This is consistent with the B*3E11 epitope being located within amino acids 378–472 of UvrB (15), the region flanked by Phe-365 and Phe-496, which were implicated in direct interaction with DNA bases in the UvrB-DNA preincision complex (7). Binding of B*3E11 to this region may create steric hindrance to the formation of the latter complex. The fact that B*3E11 can still interact with the UvrA-UvrB DNA helix-tracking complex, but fails to interact with UVrB-uvDNA complex, emphasizes the structural rearrangement of the Uvr nucleoprotein complex during UvrB delivery to a damaged site. As a result of this rearrangement at least part of the 378–472 amino acid stretch of UvrB either becomes hidden or changes conformation, so that the “conformational” epitope of B*3E11 (15) is disrupted. It should be noted that this mAb seems to be able to access its epitope in the preformed UvrB-uvDNA preincision complex. The latter, however, seems to destabilize this complex as judged by the persistence of the considerable inhibitory effect of B*3E11 on the UvrB-uvDNA preincision complex and 3'-incision even when preincision complexes were formed before addition of mAb.

B*2E3 mAb is also diagnostic for structural rearrangements.
of Uvr functional intermediates. UvrB is a very poor substrate for this mAb in solution (Fig. 1 and Ref. 15). Consistently, B*2E3 was not able to efficiently interact or interfere with Uvr intermediates involving UvrB. However, it demonstrates the neutralizing effect regardless of the order of protein addition. This suggests that the B*2E3 epitope, barely accessible in UvrB, becomes accessible in the UvrB-DNA preincision complex. Hence, UvrB undergoes conformational changes as a result of preincision complex formation. These conformational changes are compatible with the suggested shift of the C-terminal domain of UvrB, which otherwise sterically hinders binding of B*2E3 to its epitope (15). In support of this, limited immunoprecipitation of Uvr nucleoprotein complexes by B*2E3 was observed under conditions favoring formation of UvrB-uvDNA complexes. The B*2E3-UvrB-uvDNA immunocomplex does not seem to be stable, however, because it was not detected by band shift assay.

The B*2E3 epitope is localized within the same region of UvrB as the B2C5 epitope (amino acids 171–278) (15). However, it should be emphasized that these epitopes are of different types (B2C5 is conformational, whereas B*2E3 is linear) and do not seem to overlap, because both mAbs are able to simultaneously bind UvrB (15). It was found that the region of these epitopes contains one of the two putative coiled coils of UvrB (8). The second putative coiled coil encompassing amino acids 640–673 of UvrB was implicated in the interaction of the UvrB-DNA preincision complex with UvrC (8). The coiled coil located in the region of B*2E3 epitope could also participate in the UvrB-UvrC interaction. This is consistent with the proposed existence of the second dynamic mode of the latter interaction required for the dual incision (7, 8). Hence, presumed binding of B*2E3 to the UvrB-uvDNA complex could interfere with UvrC binding causing the inhibition of incision.

We have recently obtained diffraction quality crystals of UvrB and UvrB*.4 Solution of the protein structures is in progress. The results described here will help to fit the static three-dimensional structure of UvrB into a pattern of complex rearrangements of this protein during incision revealed with the aid of mAbs (Fig. 7).

REFERENCES

1. Yeung, A. T., Mattes, W. B., Oh, E. Y., Yoakum, G. H., and Grossman, L. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 6157–6161.
2. Sancar, A., and Rupp, D. W. (1983) Cell 33, 249–260.
3. Grossman, L., and Thiagarajan, S. (1993) J. Biol. Chem. 268, 16871–16874.
4. Lin, J.-J., and Sancar, A. (1992) Mol. Microbiol. 6, 2219–2224.
5. Van Houten, B. (1990) Microbiol. Rev. 54, 18–31.
6. Lloyd, R. S., and Van Houten, B. (1995) in DNA Repair Mechanisms: Impact on Human Diseases and Cancer (V. J.-M. H., ed.), pp. 25–66, R. G. Landes Company, Austin, TX.
7. Hsu, D. S., Kim, S.-T., Sun, Q., and Sancar, A. (1995) J. Biol. Chem. 270, 8319–8327.
8. Mooenaar, G. F., Franken, K. L. M. C., Dijkstra, D. M., Thomas-Oates, J. E., Visse, R., van de Putte, P., and Gossen, N. (1995) J. Biol. Chem. 270, 30508–30515.
9. Visse, R., de Ruijter, M., Mooenaar, G. F., and van de Putte, P. (1992) J. Biol. Chem. 267, 6736–6742.
10. Mooenaar, G. F., Visse, R., Ortiz-Buysse, M., Gossen, N., and van de Putte, P. (1994) J. Mol. Biol. 240, 284–307.
11. Sancar, A. (1995) J. Biol. Chem. 270, 15915–15918.
12. Caron, P. R., and Grossman, L. (1988) Nucleic Acids Res. 16, 10891–10902.
13. Seeley, T. W., and Grossman, L. (1990) J. Biol. Chem. 265, 7158–7165.
14. Kovalsky, O. I., and Grossman, L. (1994) J. Biol. Chem. 269, 27421–27426.
15. Kovalsky, O. I., Lin, C.-L., and Grossman, L. (1998) Biochim. Biophys. Acta 1397, 91–101.
16. Yeung, A. T., Mattes, W. B., Oh, E. Y., Yoakum, G. H., and Grossman, L. (1986) Nucleic Acids Res. 14, 8553–8556.
17. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY.
18. Thiagarajan, S., and Grossman, L. (1993) J. Biol. Chem. 268, 18382–18389.
19. Koo, H.-S., Claassen, L., Grossman, L., and Liu, L. F. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1212–1216.
20. Snowden, A., and Van Houten, B. (1993) Bioessays 15, 51–59.
21. Orren, D., and Sancar, A. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5237–5241.
22. Orren, D., and Sancar, A. (1990) J. Biol. Chem. 265, 15786–15803.
23. Oh, E. Y., Claassen, L., Thiagarajan, S., and Grossman, L. (1989) Nucleic Acids Res. 17, 4145–4159.
24. Bertrand-Burggraf, E., Selby, C. P., Hearst, J. E., and Sancar, A. (1991) J. Biol. Chem. 265, 27–36.
25. Mazur, S. J., and Grossman, L. (1991) Biochemistry 30, 4432–4443.
26. Ahn, B. (1995) Effect of RNA Polymerase on Nucleotide Excision Repair in Escherichia coli, Ph.D. thesis, The Johns Hopkins University.
27. Lin, C.-L., Kovalsky, O. I., and Grossman, L. (1997) Nucleic Acids Res. 25, 3151–3158.
28. Goldberg, M. E. (1991) Trends Biochem. Sci. 16, 358–362.
29. Gorbalenya, A. E., Koonin, E. V., and Blinov, V. M. (1989) Nucleic Acids Res. 17, 4715–4720.
30. Myles, G. M., Hearst, J. E., and Sancar, A. (1991) Biochemistry 30, 3824–3834.