Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3638–3642, June 1987
Biochemistry

Helicase properties of the *Escherichia coli* UvrAB protein complex

(DNA repair/incision/ATPase/D-loop DNA)

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Communicated by William Harrington, February 12, 1987 (received for review December 1, 1986)

ABSTRACT **The *Escherichia coli* UvrA protein has an associated ATPase activity with a turnover number affected by the presence of UvrB protein as well as by DNA. Specifically, the structure of DNA significantly influences the turnover rate of the UvrAB ATPase activity. Double-stranded DNA maximally activates the turnover rate 10-fold whereas single-stranded DNA maximally activates the turnover rate 20-fold, suggesting that the mode of interaction of UvrAB protein with different DNAs is distinctive. We have previously shown that the UvrAB protein complex, driven by the binding energy of ATP, can locally unwind supercoiled DNA. The nature of the DNA unwinding activity and single-stranded DNA activation of ATPase activity suggests potential helicase activity. In the presence of a number of helicase substrates, the UvrAB complex, indeed, manifests a strand-displacement activity—unwinding short duplexes and D-loop DNA, thereby generating component DNA structures. The energy for the activity is derived from ATP or dATP hydrolysis. Unlike the *E. coli* DnaB, the UvrAB helicase is sensitive to UV-induced photoproducts.**

Nucleotide excision in *Escherichia coli* requires a number of gene products including the UvrABC protein complex that is involved in the initial incision step of bulky adduct-modified DNAs (1). Of the three Uvr proteins, the UvrA possesses a DNA binding activity that preferentially interacts with damaged DNA and is stimulated by the presence of ATP (2, 3). The UvrA–DNA complex is capable of interacting with the UvrB and UvrC proteins, forming a multiprotein complex that initiates dual incision events (4, 5). The UvrA protein has an associated ATPase activity that is influenced by DNA and by the UvrB protein (ref. 6 and unpublished observations). It has been suggested that the hydrolysis of ATP is linked with translocating the Uvr machinery to damaged DNA sites (2, 6, 7). However, the rate of the ATPase activity of UvrA (in the absence of other factors) seems too slow when compared with that of such other proteins as DnaB and UvrD protein, which translocate on DNA when driven by the energy of ATP hydrolysis (8–10). Furthermore, the UvrA ATPase activity does not require DNA, nor is it activated by DNA. The UvrA ATPase turnover number when measured *in vitro*, however (in isolation of the other auxiliary proteins or other macromolecules) is probably not reflective of the biologically active series of complex nucleoprotein structures. UvrA and UvrB proteins display a number of properties suggesting them in some interactions with one another—properties that include the stimulation of the ATPase activity by DNA, enhanced binding to damaged DNA, and enhancement of unwinding of supercoiled DNA in the presence of ATP analogues.

In this paper, we report that the UvrA and UvrB proteins can associate into a complex that, when accompanied by ATP hydrolysis, displaces D-loop type structures as well as short duplexes. In this sense the complex behaves as a helicase. The UvrAB protein complex, however, exhibits a substrate spectrum distinctly different from that ascribed to other known helicases. The significance of this activity in relation to mechanisms of damage recognition, complex formation, incision, and postincision processes is discussed.

MATERIALS AND METHODS

Proteins and Enzymes. The UvrA and UvrB proteins were purified according to described procedures (11). The *E. coli* single-stranded DNA (ssDNA) binding protein (SSB) and DnaB proteins were generously provided by B. Gomes (The Johns Hopkins University). The UvrD protein was a gift from S. Kushner (University of Georgia) and the T4 phage gene 41 protein was from B. Alberts (University of California, San Francisco). Restriction endonucleases were purchased from New England Biolabs and Bethesda Research Laboratories and were used according to the manufacturers' suggested procedures. Bovine serum albumin (Miles) was dissolved in water and boiled at 100°C for 10 min.

Preparation of Helicase Substrates. The DNA preparations pBR322 replicative form I, M13 replicative form I (replicative form I is superhelical duplex DNA), and M13 circular ssDNA were prepared according to established procedures (12, 13). Previously described procedures for the preparation of the various helicase substrates were followed implicitly (14, 15). To prepare a DNA sequence for use in the synthesis of these helicase substrates, the 346-base-pair (bp) *Hin*dIII–*Bam*HI fragment from the tetracycline resistance gene of pBR322 was cloned into the *Hin*dIII–*Bam*HI site in the polylinker region of M13mp9 to create M13mp9-pBR. This allows for the hybridization of a variety of restriction fragments from pBR322 to M13 ssDNA circles. To synthesize substrate I (Table 1), the 346-bp *Hin*dIII–*Bam*HI fragment from pBR322 was separated on and purified from 1.5% agarose of low melting temperature (13). One microgram of DNA fragment was dephosphorylated with 260 units of bacterial alkaline phosphatase at 60°C for 4 hr and purified by phenol extraction and ethanol precipitation. The DNA was labeled at 5′-ends with polynucleotide kinase (United Biochemicals) and [γ-^{32}P]ATP (100 μCi, 3000 Ci/mmol: ICN; 1 Ci = 37 GBq). A 5-fold molar excess of labeled DNA fragment, 4.4 pmol (1.0 μg), was mixed with 0.8 pmol (2 μg) of M13mp9-pBR ssDNA in 150 μl of 15 mM sodium chloride/1.5 mM sodium citrate, pH 7.8. The DNA was denatured with NaOH, neutralized with Tris·HCl as described (16) and allowed to hybridize for 1–3 hr at 65°C. The mixture was loaded onto 5-ml 10–35% sucrose gradients (10 mM Tris, pH 7.4/2 mM EDTA/1.0 M NaCl) in a SW50.1 rotor and centrifuged at 40,000 rpm for 5.5 hr at 20°C. Fractions of ≈160 μl were collected, counted using Cerenkov radiation, and the fractions corresponding to ssDNA circles were pooled. The DNA was precipitated with

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Abbreviations: ssDNA, single-stranded DNA; ATP[γS], adenosine 5′-[γ-thio]triphosphate; Ado*PP*[NH]*P*, adenosine 5′-[β,γ-imido]triphosphate; Ado*PP*[CH_2]*P*, adenosine 5′-[β,γ-methylene]-triphosphate.

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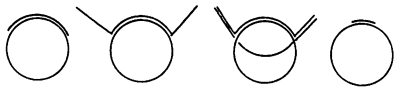
Table 1. *E. coli* helicase specificities

		Fragment release, %			
					
		(I)	(II)	(III) "D-loop"	(IV)
DnaB	(5′ → 3′)	0.9	61.8	20.7	5.4
UvrD	(3′ → 5′)	57.2	72.1	39.7	64.2
T4 gene 41	(5′ → 3′)	1.3	26.2	18.2	33.2
UvrA + UvrB	(5′ → 3′)	0.9	0.1	16.7	37.3
UvrA		0.1	0.0	0.7	0.3
UvrB		0.5	0.2	0.7	0.0

Substrate specificity of the *E. coli* helicases. Displayed are the types of helicase substrates used in this study in the amount (ssDNA circles) of ≈8 fmol. The reaction conditions used for assaying helicase activities have been described, and the release of the product from circular ssDNA is assayed by gel electrophoresis followed by autoradiography and by quantitation of radioactivity in the product and substrate bands. Percent fragment released is calculated as [(cpm product/cpm product + cpm substrate) × 100] after appropriate subtraction for background (9). M_r values and amounts of proteins are as follows: UvrB (76,000 and 1.4 pmol); UvrD (76,000 and 1.6 pmol); DnaB hexamer (300,000 and 3.0 pmol); T4 gene 41 (58,000 and 5.8 pmol); and UvrA (104,000 and 1.0 pmol).

2 vol of ethanol, washed with 0.5 ml of 95% ethanol, lyophilized, and resuspended in 10 mM Tris/1 mM EDTA, pH 8.0 (TE buffer).

To prepare substrate III (D-loop), essentially the same procedure for synthesis of substrate I was followed except a 1107-base-pair 5′-labeled *Hin*cII fragment of pBR322 was used. The fragment thus contains 485- and 276-base-long heterologous ends. M13mp9-pBR ssDNA (0.8 pmol) was exposed to 4–7 pmol (3–5 μg) of 5′ ^{32}P-labeled *Hin*cII fragment during the hybridization. The resulting substrate is probably a mixture of structure III and the hemicatenane type structure described by Bianchi *et al.* (17). The nature of substrate III has not been rigorously proved but is presumed from the methods of synthesis and its behavior upon treatment with nuclease (18–20).

Substrate DNA of structure II was prepared using the 844-bp *Ssp* I–*Sal* I fragment from pBR322. After purification from agarose of low melting temperature, 9 pmol (5 μg) of restriction fragment was treated with 260 units of bacterial alkaline phosphatase at 60°C for 4 hr and purified by phenol extraction and ethanol precipitation. The 5′-termini were labeled with polynucleotide kinase and [γ-^{32}P]ATP (100 μCi, 3000 Ci/mmol). The nature of the DNA fragment results in preferential labeling of the strand complementary to the M13mp9-pBR ssDNA circles. The DNA was denatured by boiling 4 min in 30% dimethyl sulfoxide/0.001% bromophenol blue/0.001% xylene cyanole. The strands were resolved by electrophoresis on 4% nondenaturing horizontal polyacrylamide gels (8 × 20 × 0.7 cm; acrylamide/bisacrylamide 30:1) in 45 mM Tris/45 mM borate/1 mM EDTA, pH 8.0 at 100 V for 18 hr (13), autoradiographed, and the slower migrating band corresponding to the strand complementary to M13mp9-pBR ssDNA, eluted from gels by diffusion (21). After ethanol precipitation, the fragment was hybridized to 1.2 pmol (3 μg) of M13mp9-pBR ssDNA circles in 100 μl of 0.1 M NaCl/20 mM Hepes, pH 7.6/10 mM $MgCl_2$ by boiling the mixture at 100°C for 3 min and incubating for 3 hr at 65°C. The substrate was purified on 10–35% sucrose gradients. The resulting substrate contains 222- and 276-base unhybridized termini.

DNA of structure IV was prepared by hybridizing a pentadecanucleotide (15-mer) universal primer (New England Biolabs) and extending it with the Klenow fragment of DNA polymerase I (Bethesda Research Laboratories) in the presence of appropriate nucleotide substrates to form a 22-base-paired duplex region. Two micrograms of M13mp9-pBR ssDNA (0.8 pmol) was mixed with an equimolar quantity (4 ng) of 15-mer primer in 10 μl as described (22). The volume was adjusted to 20 μl so as to include 100 μM dGTP/100 μM dTTP/50 μCi of [α-^{32}P]dATP (3000 Ci/mmol: ICN). The primer was extended with 3 units of Klenow polymerase for 20 min and chased with 100 μM dATP for 15 min. The reaction was quenched with 50 mM EDTA and placed on ice. The samples were brought to 50 μl with TE buffer, and were desalted by passage through two Mini Spin columns (Cooper). The substrate was used directly without further purification. Background contamination with unincorporated [α-^{32}P]ATP was ≈4%.

Helicase Assays. All reactions were conducted in a volume of 20 μl and contained 50 mM 4-morpholinepropanesulfonic acid (Mops), pH 7.6/50 mM KCl/15 mM $MgCl_2$/5 mM ATP/2 mM dithiothreitol/1 mM EDTA and ≈8 fmol of helicase substrate in ssDNA circles (20 ng of helicase substrate) (figure 3 of ref. 15). Fifty micrograms of bovine serum albumin per ml was present in most reactions, but its omission had little effect. The reaction was initiated by the addition of indicated amounts of the UvrA and UvrB proteins and incubated at 37°C for 30 min. Reactions were quenched with 5 μl of 50% (vol/vol) glycerol/1% $NaDodSO_4$/100 mM EDTA. For substrates I, II, and III, the samples were analyzed on 1.2% agarose gels in 90 mM Tris/90 mM borate/2 mM EDTA, pH 8.0 (TBE). The gels were electrophoresed at 1–2 V·cm^{-1}, dried onto DE-81 paper (Whatman), and autoradiographed with Kodak XAR-5 film with a DuPont Cronex Lightning Plus intensifying screen. The radioactivity was quantified by excising individual bands that were counted in scintillation fluid. Analyses of substrate IV release were conducted on 12% nondenaturing polyacrylamide gels (25 × 30 × 0.1 cm; acrylamide/bisacrylamide 30:1) in TBE buffer. Samples were electrophoresed at 120 V for 1.5–2 hr. The gels were covered with plastic wrap and autoradiographed for 1 hr with DuPont Cronex intensifying screen at 4°C. Radioactivity was quantified by cutting out bands and macerating wet gels in a scintillation vial followed by suspension of the substance in 1 ml of H_2O. After overnight incubation at 25°C, the samples were counted using Cerenkov radiation. An ATP-regenerating system, when included in reaction mixtures, consisted of 5 mM phospho*enol*pyruvate and 5 units of pyruvate kinase (Boehringer Mannheim).

UV-Irradiation of DNA. Irradiation of the DNA was conducted as previously described (23). DNA in 10 mM Tris/1 mM EDTA, pH 8.0 was placed in an open sterile Petri dish and irradiated at 2 J·m^{-2}·sec^{-1} from a 15-watt germicidal lamp. The rate of photoproduct formation for pBR322 has been previously determined to be 0.041 per sec (9).

RESULTS

In analyses of the ATPase activity of the UvrA, UvrB, and UvrC protein complexes, it was found that UvrAB ATPase activity was stimulated 20 fold by ssDNA (unpublished data). We have observed that the UvrA protein effects localized unwinding of undamaged supercoiled DNA in the presence of adenosine 5′-[γ-thio]triphosphate ATP[γS] whereas UV-damaged DNA is demonstrably unwound in the presence of ATP (23). Furthermore, the unwinding in the presence of ATP[γS] by UvrA protein is increased by 100% through the addition of the UvrB protein. These observations are consistent with the notion that localized unwinding represents the initiation of a helicase-like activity for the UvrAB protein complex. As a consequence of these results, we examined the action of Uvr proteins on potential helicase substrates. The assay employed in these studies relies upon the release of

DNA (single- or double-stranded) stably hybridized to a circular ssDNA (15). Substrate I (Table 1), which contains flush ends, was unaffected by the UvrAB protein complex or by the DnaB protein, a helicase requiring unannealed single-stranded ends (9). T4 gene 41 protein, a helicase involved in T4 phage replication was ineffective, releasing a small percentage of the labeled fragment only with increasing amounts of protein (14). The UvrD protein, a type II helicase known to effectively utilize flush-ended substrates (10), was able to promote release of the 346-base fragment.

The requirement for unannealed single-stranded ends was studied by preparing structure II, a substrate containing 222- and 276-base unannealed ends. The 844-base-long product was released by the DnaB and UvrD proteins. The T4 gene 41 helicase effectively used this substrate when compared with the flush-ended substrate. The UvrAB protein complex, however, is unable to use this substrate and is, therefore, incapable of unwinding a duplex region of 346 bases in length.

A D-loop type helicase substrate (structure III) was constructed by using a DNA fragment with noncomplementary termini longer than the complementary region. This structure is presumably formed by the invasion of ssDNA into the complementary region in the double-stranded DNA fragment (18). The resultant structure should contain paranemic joints similarly formed during recombination reactions generating topologically unlinked structures. This structure is, however, more stable at 37°C than those structures formed by the RecA protein (17). The topologically linked substrate, a hemicatenane (17), probably constitutes another structure present in this substrate mixture. The D-loop structure population should consist of a distribution in lengths hybridized to circular ssDNA from a range of 346 bases to whatever minimum length is stable during formation of such structures. Substrate III is unstable to treatment with type II restriction endonucleases—in that ≈50% of the label is released by cleavage within the D-loop region, a characteristic of such structures (20). The D-loop structure is an effective substrate for not only the DnaB, gene 41, and UvrD proteins, but for the UvrAB protein complex as well. The released fragment behaves as a double-stranded structure because it migrates on agarose gel electrophoresis more slowly than its single-stranded counterpart, it is resistant to S1 nuclease digestion, and is susceptible to digestion by type II restriction endonucleases (unpublished data). Neither the UvrA protein nor the UvrB protein can act independently to release the labeled fragment. Furthermore, the *E. coli* ssDNA binding protein is effective neither in releasing the product nor in enhancing product release by the UvrAB proteins (unpublished data).

Because the ability of the UvrAB protein complex to act as a helicase might be limited to short duplexes, a helicase substrate with a 22-base-paired duplex region was examined (structure IV, Table 1). The UvrD and gene 41 helicase effectively use this substrate, whereas the DnaB helicase is enzymatically ineffective due to the absence of unannealed single-stranded termini in this structure. The UvrAB protein is capable of catalyzing the release of the labeled fragment. In this case, as well as for others, the separate UvrA and UvrB proteins are unable to effect independent helicase-like reactions.

Substrate III has allowed characterization of some helicase properties of the UvrAB protein complex. Titration-experimental analyses of the requirement for the UvrB protein on helicase activity at constant UvrA are shown in Fig. 2 (*Upper*). At saturation, a ratio of 1:2 for UvrA/UvrB protein is observed, coinciding with the ratio observed for the effect of UvrB on maximal unwinding of supercoiled DNA by UvrA protein in the presence of ATP[γS] (23). This ratio, however, may not represent the true stoichiometry because the fraction of active protein is unknown. The initial rate of fragment

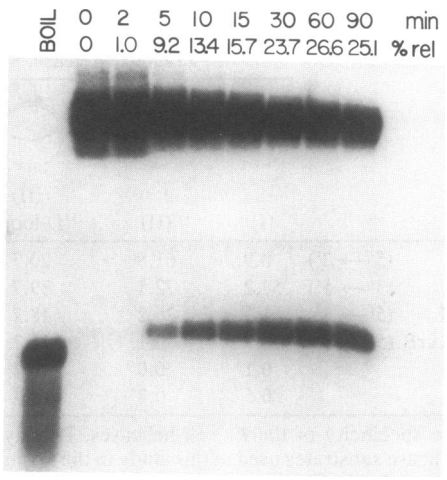


FIG. 1. Autoradiogram of gel analyzing kinetics of release of labeled fragment from substrate III. Reactions were initiated by the addition of 0.6 pmol of UvrA and 1.1 pmol of UvrB and incubated for the indicated amount of time at 37°C. Reactions were quenched with 10% glycerol/0.2% $NaDodSO_4$/20 mM EDTA and then analyzed on 1.2% agarose gels. After drying onto DE 81 paper, the gel was autoradiographed for 12 hr. Percent of product released (% rel) is indicated.

release, as shown in Fig. 1, is rapid and begins to plateau at 30 min. Maximally, 25–30% of the labeled fragment is released after 30 min at 37°C, even with a vast excess of UvrAB protein (Fig. 2 *Lower*). The limited release of fragment is probably due to the presence of other structures in the substrate preparation. Preliminary experiments indicate that a hemicatenane that is probably present in substrate III is inefficiently acted upon by the UvrAB protein (unpublished data).

Nucleotide Requirement. Helicases require nucleoside triphosphate hydrolysis for their action, and different helicases display a different spectrum of cofactor requirements (Table 2). Accordingly, the nucleotide requirements for the UvrAB helicase activity were analyzed. The only nucleotides competently utilized by the UvrAB protein complex are ATP and dATP. The nonhydrolyzable ATP analogues, ATP[γS], aden-

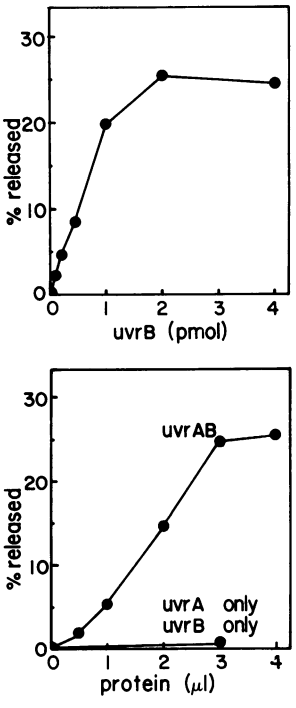


FIG. 2. (*Lower*) Titration curves of UvrA and UvrB proteins unwinding D-loop DNA. One μl of protein solution contained 1.0 pmol of UvrA and 1.4 pmol of UvrB. (*Upper*) Titration of the UvrB protein. The UvrA protein was maintained at 0.6 pmol, and the indicated amount of UvrB protein was added. All reactions were done for 30 min at 37°C.

Table 2. UvrAB helicase cofactor requirements

Reaction conditions	Fragment released, %
UvrAB + Ado*PP*[CH_2]*P*	0.7
UvrAB + Ado*PP*[NH]*P*	0
UvrAB + ATP[γS]	0
UvrAB + ATP + regenerating system	27.5
UvrAB + ATP	27.4
UvrAB − ATP	1.0
UvrB + ATP	0.8
UvrA + ATP	0.5
Boiled D-loop DNA	100
None	0
UvrAB + dTTP	1.4
UvrAB + dCTP	1.5
UvrAB + dGTP	2.7
UvrAB + dATP	24.7
UvrAB + rUTP	1.5
UvrAB + rGTP	1.5
UvrAB + rCTP	2.5

Nucleotide cofactor requirements of UvrAB helicase when acting on D-loop DNA (substrate III). Amounts of reactants were as follows: UvrA (0.6 pmol), UvrB (1.1 pmol), and substrate (8 fmol); all nucleotides were at concentrations of 5 mM.

osine 5′-[β,γ-imido]triphosphate (Ado*PP*[NH]*P*), adenosine 5′-[β,γ-methylene]triphosphate (Ado*PP*[CH_2]*P*), and also GTP were all ineffective as cofactors in the UvrAB helicase reaction (Table 2). The inclusion of an ATP-regenerating system had no effect on the extent of fragment release.

Effect of Damaged Nucleotides on Helicase Activity. From

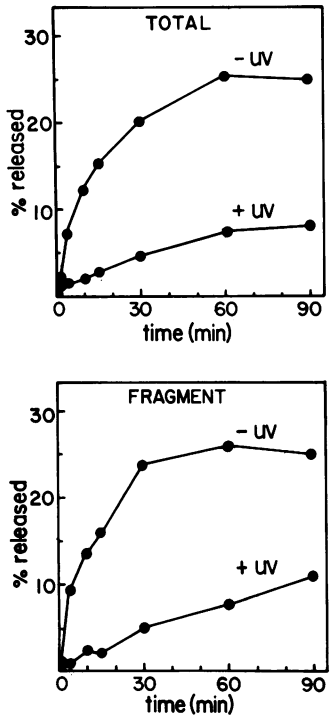


FIG. 3. Kinetics for the release of labeled fragment from D-loop DNA and the effect of UV-irradiation. (*Upper*) **Total** refers to substrate III irradiated for 1800 J·m^{-2} and analyzed at various times for the release of DNA fragment by the UvrAB helicase. (*Lower*) **Fragment** refers to irradiation for 2400 J·m^{-2} of only the *Hin*cII restriction fragment prior to hybridizing to ssDNA circles to form substrate III. The UV damage should therefore be confined to the fragment. The amount of UvrA protein was 0.6 pmol, and the amount of UvrB protein was 1.1 pmol; all substrates were ≈8 fmol in ssDNA circles.

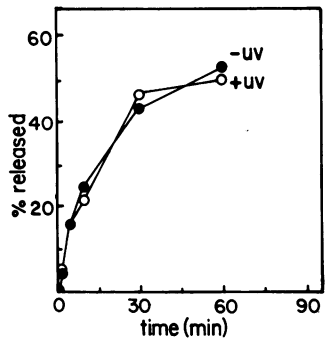


FIG. 4. Effect of UV-irradiated D-loop DNA on DnaB helicase activity. The DNA substrate was unirradiated or irradiated for 1800 J·m^{-2} and assayed for release by 3 pmol of DnaB protein. Reactions were at 37°C for 30 min.

filter-binding experiments, the UvrA protein is found to bind to DNA containing damaged sites. In the presence of UvrB protein and ATP, a UvrAB protein complex is stably bound and is competent to catalyze an incision reaction upon addition of the UvrC protein. For this reason, the effect of UV damage on the helicase substrate III was examined. Since the target size of the hybridized region may be relatively small, the substrate was irradiated for 1800 J·m^{-2}. It can be calculated that approximately three pyrimidine dimers per 346-base-paired region are formed (23). When this damaged substrate was incubated with the UvrAB proteins and compared with untreated substrate, there was significant inhibition of the rate and extent of fragment release (Fig. 3). Since irradiation of the entire substrate, in all likelihood, results in a uniform distribution of photoproducts in the complementary region as well as in the single-stranded region, the restriction fragment was irradiated for 2400 J·m^{-2} prior to hybridization to ssDNA circles. This should localize damage only in the complementary region and the double-stranded ends of the restriction fragment. Helicase analyses of this substrate exhibited virtually identical behavior with totally irradiated substrate. Therefore, the inhibition of fragment release as a consequence of UV-irradiation is not due to damage to the single-stranded circle of the substrate. The effect of UV-irradiation of the substrate on the activity of DnaB helicase was also examined (Fig. 4). Unlike the UvrAB helicase, the DnaB protein is not affected by the presence of UV photoproducts. UvrD protein, on the other hand, seems to be inhibited by UV irradiation of the helicase substrate (unpublished observations).

DISCUSSION

Effective incision of damaged DNA by a multiprotein complex requires a number of discrete molecular events in order to initiate the repair process. The complex must not only bind DNA but must be able to discriminate between undamaged and damaged sites. The ability of the UvrAB complex to bind to and locally unwind superhelical DNA (23) may indicate a phase of the nucleotide excision repair process. We have described in these sets of experiments that the UvrAB proteins possess a distinctive activity that may represent an extension of that event of the repair process. The UvrAB protein complex, in the presence of ATP, functioning as a helicase is capable of unwinding short duplexes and D-loop type structures. The activity occurs unidirectionally in a 5′→3′ manner (unpublished data).

Localized unwinding of DNA occurs when UvrAB protein complex is bound (*i*) to covalently closed supercoiled DNA in the presence of a nonhydrolyzable ATP analogue or (*ii*) to a damaged site in the presence of ATP (23). However, when a ssDNA with a small duplex region or a DNA with a D-loop

configuration is used as the substrate, the hybridized fragment is released by UvrAB protein concomitant with ATP hydrolysis. When presented with supercoiled DNA, UvrAB protein in the presence of a nonhydrolyzable ATP analogue can bind and locally unwind DNA, but is probably incapable of translocation. Therefore, helicase function is observed only with ATP hydrolysis. Furthermore, the sensitivity of the helicase activity to UV damage and the localized unwinding of UV-damaged DNA seem appropriate for a translocational mechanism that is influenced by damage (Figs. 3 and 4). The sensitivity to damage is a prerequisite for a repair complex capable of differentially binding to undamaged and damaged DNA.

The requirement for both UvrA and UvrB proteins for helicase action is suggested from many observations demonstrating their interaction. Helicases such as DnaB, UvrD, and the T4 gene 41 protein function independently of other proteins. Accessory proteins such as DnaG (primase), however, influence the helicase activity of the DnaB protein (9) and similarly gene 61 protein affects the activity of the gene 41 protein (14, 24). Thus multiprotein interactions affecting helicase activity are not uncommon.

The UvrAB helicase activity may participate in a number of stages of the repair process. (*i*) The activity may indicate a mechanism of translocation and damage-sensing process whereby the unwinding reaction is a reflection of the mechanism to detect damage-induced perturbations in DNA. The translocation mechanism is sensitive to the presence of DNA damage. The length of DNA scanned with each binding event could be restricted and serves to explain the limitation of helicase action to short duplexes and D-loop DNAs. (*ii*) An unwinding of DNA may be a requirement for formation of a proper incision complex with the UvrC protein in which a locally unwound region is preserved during translocation to prepare for DNA incision by the UvrABC endonuclease. (*iii*) The strand-displacing function of the UvrAB protein complex could conceivably act to allow UvrD function for turnover of the UvrABC complex following incision. It has been previously shown that the UvrABC postincision complex is stably bound to DNA and that UvrD is required to allow turnover of the UvrC protein and subsequent repair synthesis by DNA polymerase I (7, 25).

The importance of this activity in repair will ultimately require a more refined analysis of the interaction of the repair complex with both damaged and undamaged DNAs. The creation of a specifically damaged DNA of suitable length, with damage at single specific loci will, furthermore, be necessary for such analysis. Also the isolation of mutants producing defective proteins at specific steps in the repair mechanism would help define the importance of such activities in DNA repair.

The authors are indebted to Dr. Sharlyn Mazur, Dr. Randy Bryant, Todd Seeley, Lark Claassen, and Paul Caron for sharing their ideas, criticisms, and expertise during the course of this work. This work was supported by grants from the National Institutes of Health (R0-1 GM 31110 and R0-1 GM 22846) and the Department of Energy (DE-AS02-76EV02814).

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