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Involvement of a cryptic ATPase activity of UvrB and its proteolysis product, UvrB* in DNA repair

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ABSTRACT

The incision of damaged DNA by the *Escherichia coli* UvrABC endonuclease requires ATP hydrolysis. Although the deduced sequence of the UvrB protein suggests a putative ATP binding site, no nucleoside triphosphatase activity is demonstrable with the purified UvrB protein. The UvrB protein is specifically proteolyzed in *E. coli* cell extracts to yield a 70 kD fragment, referred to as UvrB*, which has been purified and is shown to possess a single-strand DNA dependent ATPase activity. Substrate specificity and kinetic analyses of UvrB* catalyzed nucleotide hydrolysis indicate that the stimulation in DNA dependent ATPase activity following formation of the UvrAB complex results from the activation of the normally sequestered UvrB associated ATPase. Using nucleotide analogues, it can be shown that this activity is essential to the DNA incision reaction carried out by the UvrABC complex.

INTRODUCTION

The *E. coli* UvrA, UvrB, and UvrC proteins are involved in the repair of DNA damaged by a number of agents of diverse chemical structures, generally referred to as "bulky" adducts. The in vitro reconstituted UvrABC proteins require ATP to specifically incise damaged DNA (1,2). It has been recently shown that incision of nondamaged DNA by UvrABC can occur at high protein concentration and that this incision is also dependent on ATP hydrolysis (3). The exact role of ATP hydrolysis in carrying out an energetically favorable reaction of phosphodiester bond hydrolysis is not fully clear at this time.

Although specific dual incision of damaged DNA in vitro requires all three Uvr proteins, several protein complexes and protein-DNA complexes which are proposed intermediates in the formation of the UvrABC ternary complex have been identified and characterized. Most notably, a number of partial reactions involving the UvrA and UvrB proteins have been described which are dependent on ATP hydrolysis. These include UvrAB-DNA damage specific complex formation (1,4) and UvrAB catalyzed strand displacement, a helicase-like reaction (5). However, even in these intermediate complexes the significance and function of ATP hydrolysis is unclear.

The DNA sequences of the *uvrA*, *uvrB*, and *uvrC* genes have recently been reported and their respective protein sequences deduced (6-9). The predicted UvrA protein sequence contains a duplication of a region described as a Walker type A nucleotide binding sequence (6). This sequence has been found in many proteins which are known or suspected of catalyzing ATP hydrolysis (10). Properties of the purified UvrA protein are consistent with this finding since this protein possesses an associated ATPase activity (11). Furthermore, two ATP binding sites have

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been identified as components of this protein (S. Thiagalingam, S. Mazur, and L. Grossman, unpublished observations). A similar Walker type A nucleotide binding sequence is present in the UvrB protein (7,8) which has no detectable ATPase activity when analyzed in vitro (12). The only observation which has been noted is a large stimulation in DNA dependent ATPase activity when UvrB binds to UvrA (5,12). This activity enhancement had been thought to be due to the UvrA associated ATPase activity.

Another UvrB sequence homologous with two sites on the *E. coli* Ada protein can be found which are specifically proteolyzed in cell extracts (7,13). Consistent with this observation the UvrB protein is specifically cleaved in stress induced cell extracts to UvrB*, a 70 Kd fragment (12,14). The experiments reported in this paper will show that the cleaved product, UvrB* possesses an ATPase. It will further be shown that this cryptic activity reveals itself in the absence of proteolysis when UvrB interacts with UvrA. This ATPase, furthermore, is essential to completion of the in vitro reconstituted DNA incision reaction.

MATERIALS AND METHODS

Enzymes

The UvrA and UvrB were purified as previously described (15).

Cell growth and lysis

E. coli C600 containing pIS310, a plasmid which contains the *uvrB* gene under control of the λP_l promoter (I. Sandlie and L. Grossman, unpublished) was grown at 30°, induced at 42°, and harvested as previously described (15). The cells were lysed in 0.3 M NaCl, 2 mM DTT, 20 mM EDTA, 0.1 M Tris, pH 8.0 with lysozyme on ice for 15 min followed by 2 min at 37°. The cellular debris was removed by centrifugation for 40 min at 27,000 x g at 4°.

Purification of UvrB*

The cell lysate was diluted to 50 mM NaCl and loaded onto a 150 ml Affigel-Blue (Bio-Rad) column. After extensive washing with 50 mM NaCl, 100 mM Tris, pH 8.0, the column was washed with 50 mM KCl, 0.1 M potassium phosphate, pH 7.6, 2 mM EDTA, 2 mM DTT, 15% glycerol, and then eluted with a 50 to 300 mM KCl gradient in phosphate buffer. The UvrB* peak was identified by Coomassie blue staining of SDS-polyacrylamide gels. The peak fractions were pooled, dialyzed against 0.1 M phosphate buffer to 50 mM KCl, and loaded onto a DEAE column as described and eluted with a KCl gradient (15). The UvrB* eluted at approximately 0.2 M KCl. The pool from the column was adjusted to 0.5 M KCl and passed through a phenyl agarose column equilibrated with 0.5 M KCl in phosphate buffer. This column removed any contaminating UvrB, which was bound to the column, while UvrB* flowed through. The UvrB* was stored at -70° in 50 µl aliquots which were thawed once and then discarded.

Nucleoside Triphosphatase Activity

ATP hydrolysis was followed by the generation of Norit-nonadsorbable radioactive material from ATP-γ-[^{32}P] (ICN) essentially as described (16). ATP was exclusively hydrolyzed to ADP and P_i as confirmed by polyethylene imine thin layer chromatography in 1 M LiCl, formate (12). The hydrolysis of GTP was followed by thin layer chromatography using [^{3}H]-GTP (ICN) as a substrate. Nucleotides were included at a concentration of 2 mM in all cases except in

determinations of kinetic parameters. Unlabeled nucleotides were obtained from Pharmacia, nucleotide analogues were obtained from Boehringer Mannheim and were stored at -70°. The S_p isomer of ATP-ß-S was synthesized using ADP-ß-S (Boehringer Mannheim), phosphoenol pyruvate, and pyruvate kinase as described (17). All reactions were carried out in a 50 µl volume of 40 mM potassium MOPS, pH 7.6, 85 mM potassium chloride, 15 mM magnesium chloride, 2 mM DTT, 2 mM EDTA, unless otherwise specified.

Oligonucleotide displacement

The M13 universal primer (17-mer) was labeled with ^{32}P using ATP-γ-[^{32}P] and polynucleotide kinase, then hybridized to M13mp19 single-strand DNA (18) and purified by gel filtration. The displacement of this oligonucleotide was followed by scintillation counting of the substrate and product after agarose gel electrophoresis.

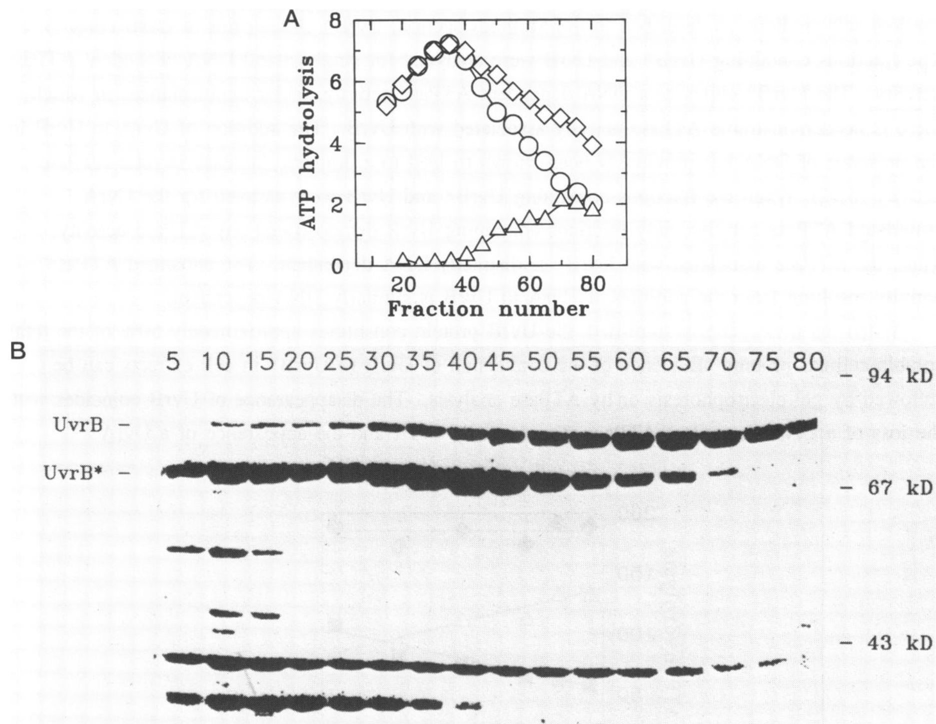

Figure 1. A. Nucleoside triphosphatase activity of fractions containing UvrB and UvrB* from Affigel-Blue. *E. coli* cells overexpressing UvrB to approximately 50% of total protein were lysed as described in Methods and fractionated on an Affigel-Blue column using a 50 mM to 300 mM KCl gradient. Equal aliquots of each fraction (1/10th of what is shown in B) were analyzed for single-strand DNA (200 µg/ml heat-denatured calf thymus DNA) ATPase activity (O) and for single-strand DNA plus UvrA (100 nM) dependent ATPase activity (◇). The amount of UvrA added was sufficient to detect approximately 0.5 µg of UvrB protein. The net increase in activity due to addition of UvrA is indicated by Δ. B. The composition of every fifth fraction (5-80) is shown after Coomassie blue staining of a SDS-polyacrylamide gel.

DNA binding and incision

Damaged DNA binding and incision reactions were measured by nitrocellulose filter binding as previously described (4). Stable UvrAB-DNA binding was measured after dilution with cold 2X SSC (300 mM sodium chloride, 30 mM sodium citrate, pH 7.5). UvrABC incision was followed by conversion of duplex supercoiled DNA to nicked circular DNA which upon denaturation at pH 12.1 and rapid renaturation remains single-stranded and binds to nitrocellulose.

RESULTS

The UvrB protein, from *Escherichia coli* containing a plasmid which expressed the *uvrB* gene under control of the λP_l promoter, is specifically proteolyzed from a 76 kD to a 70 kD protein. The major product of this cleavage is referred to as UvrB* which can be separated from UvrB as a consequence of its altered properties in several chromatographic and electrophoretic systems.

When an *E. coli* cleared cell lysate is loaded onto an Affigel-Blue column and washed with a shallow salt gradient, as described in the Methods section, UvrB* elutes prior to UvrB (Fig. 1). The fractions containing UvrB* and UvrB were analyzed for single-strand DNA dependent ATPase activity. The UvrB* elutes as a DNA dependent ATPase activity, under circumstances in which there is no demonstrable ATPase activity associated with UvrB. The addition of UvrA to UvrB in the presence of DNA has previously been shown to lead to a dramatic increase in total ATPase activity (5,12). When the fractions containing UvrB* and UvrB were assayed for the UvrA stimulated ATPase activity, only fractions containing UvrB demonstrated any ATPase activity. Thus, the ATPase associated with UvrB* is distinctly DNA dependent. The enhanced ATPase activity resulting from the addition of UvrA to UvrB is similarly DNA dependent.

When the *uvrB* gene is amplified, the UvrB protein constitutes approximately 50% of the total soluble cellular protein. Evidence of the conversion of UvrB to UvrB* in cell extracts can be followed by gel electrophoresis or by ATPase analysis. The disappearance of UvrB coincides with the loss of a DNA dependent ATPase stimulated by UvrA and the appearance of a UvrA

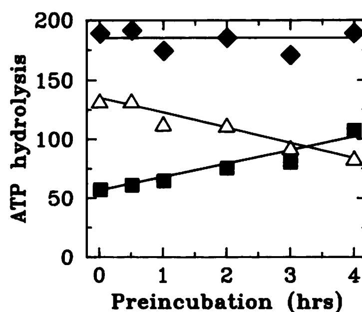

Figure 2. Conversion of UvrA dependent to UvrA independent ATPase activity. Lysed *E. coli* cells overexpressing UvrB were incubated at 37° for various times and then the ATPase activities in the crude extracts were measured with (◆) and without (■) the addition of UvrA. The net effect of UvrA addition is shown as △. The conversion of UvrB to UvrB* followed kinetics similar to the appearance of UvrA independent ATPase activity (data not shown).

independent, DNA dependent ATPase. The total ATPase levels in the extract are relatively constant (Fig. 2). This suggests that the cryptic ATPase associated with UvrB is a DNA dependent activity normally active in the presence of UvrA, whereas that ATPase associated with the specific proteolysis product, UvrB*, is UvrA independent. However, because these experiments were performed with crude preparations other possibilities such as an unrelated protein which is differentially activated by UvrB and UvrB* cannot be ruled out.

That UvrB possesses an associated silent ATPase activity is demonstrable by proteolysis of purified UvrB, lacking ATPase activity, to UvrB*, which exhibits a DNA dependent ATPase. UvrB protein is very sensitive to several proteases in a region which is close to the natural cleavage site (T. Seeley, P. Caron, and L. Grossman, unpublished observations). Incubation of UvrB with trypsin generates two relatively stable intermediate products (71 Kd, and 70 kD) possessing DNA dependent ATPase activity (Fig. 3). The ATPase activity observed in preparations of UvrB* represents an activity inherent to the UvrB protein but masked under normal assay conditions.

The UvrB* produced in *E. coli* was purified to apparent homogeneity (see Methods) and was used in all further experiments. The DNA dependency of the ATPase activity of UvrB* was analyzed using heat denatured calf thymus DNA, sheared double-strand calf thymus DNA, or double-strand supercoiled plasmid DNA (Fig. 4). The ATPase is single-strand DNA dependent

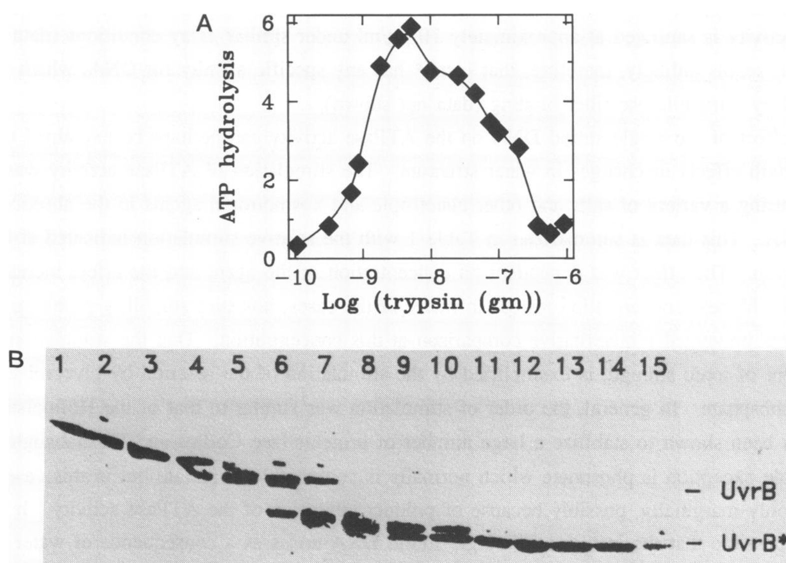

Figure 3. A. Activation of nucleoside triphosphatase activity by trypsin catalyzed proteolysis of UvrB. UvrB (20 μg) was incubated with various concentrations of trypsin at 37° for 1 hour followed by addition of 15 μg of bovine trypsin inhibitor. The ATPase activity of 1 μg of cleaved UvrB was analyzed in the presence of single-strand calf thymus DNA. B. The proteolyzed UvrB fractions above were analyzed by polyacrylamide gel electrophoresis. Lanes 1-15 represent 2-fold increases in the amount of trypsin starting with 100 pg trypsin in lane 1.

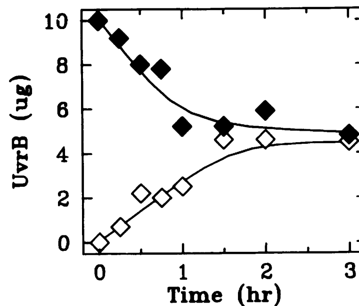

Figure 4. DNA titration of UvrB* nucleoside triphosphatase activity. ATP hydrolysis by UvrB* (20 nM) after 10 min. at 37° with various concentrations of DNA. ◇ - heat denatured calf thymus DNA; ○ - sheared calf thymus double strand DNA; △ - supercoiled pPYC3 plasmid DNA. No difference was seen between heat denatured calf thymus DNA and single strand phage DNA (data not shown).

with very little activation due to supercoiled plasmid. Digestion of the supercoiled plasmid with DNAse led to stimulation of ATPase activity only after significant DNA hydrolysis had occurred (data not shown). The single-strand dependency of the ATPase activity is unusual in that saturation, even at concentrations of 500 µg/ml of DNA, was not achieved. At this concentration there are approximately 60,000 nucleotides per UvrB* molecule. In contrast the UvrAB associated ATPase activity is saturated at approximately 10 µg/ml under similar assay conditions (data not shown). It seems unlikely, therefore, that UvrB* has any specific affinity for DNA, which was confirmed by nitrocellulose filter binding (data not shown).

The effect of the single strand DNA on the ATPase activity can perhaps be explained through ionic strength effects or changes in water structure. The stimulation of ATPase activity was analyzed using a variety of salts and other chaotropic and kosmotropic agents in the absence of added DNA. This data is summarized in Table 1 with the relative stimulation indicated at 0.5 M concentration. The effect was dependent on concentration of the agent and the effect increased up to 2 M (the highest concentration tested) in many of the cases, however solubility problems with some agents prevented a quantitative comparison at this concentration. That the stimulation was independent of ionic strength is exemplified by the stimulation of the reaction by glycerol and glycerol phosphate. In general, the order of stimulation was similar to that of the Hofmeister series which has been shown to stabilize a large number of proteins (see Collins and Washabaugh (19). One notable exception is phosphate which normally is comparable to sulfate but in this case activates only marginally, possibly because of product inhibition of the ATPase activity. It is therefore possible that the influence of single-strand DNA arises as a consequence of water structure changes allowing the UvrB* to adopt a conformation suitable for ATP hydrolysis. Activation of the single-strand DNA dependent RecA ATPase activity by high salt concentrations has also been reported (20).

When UvrA and UvrB are combined in the presence of DNA there is an accompanying large increase in ATPase activity. This activity was examined in detail to determine the nature of the

Table 1. Relative stimulation of UvrB* ATPase activity by various agents

Agent	Relative stimulation
Cyanate (^-SCN)	1.0
Sucrose	1.5
Polyvinyl alcohol	1.6
Inositol	1.6
Bromide	1.7
Dithionite ($S_2O_4^=$)	1.7
Bisulfite ($S_2O_5^=$)	1.8
Citrate	2.0
Nitrite (NO_2^-)	2.0
Chloride	2.1
Phosphate	2.2
Propionate	2.6
Pyruvate	2.7
Thiosulfate ($SSO_3^=$)	2.8
Formate	3.1
Glycerol	3.7
Tartrate	4.0
Glycerol Phosphate	4.0
Bicarbonate	4.4
Ascorbate	4.6
Aspartate	4.7
Glutamate	5.1
Cacodylate	5.4
Acetate	5.5
Sulfate ($SO_4^=$)	5.5
Thioglycolate	6.0
Sulfite ($SO_3^=$)	7.4
Succinate	8.2

Table 1. Relative stimulation of UvrB* ATPase activity by various agents. ATP hydrolysis by UvrB* after 10 min. was examined in the presence of 0.5 M concentrations of the various agents listed. Numbers in the table are normalized to the amount of ATPase observed in the absence of any agent. The sodium form of all salts were used.

substrate specificity and kinetic parameters of UvrAB as well as UvrA and UvrB* (Table 2). Contrary to the data reported by Seeberg (11), UvrA was found to hydrolyze both GTP and ATP. The K_i for GTP, in the presence of DNA, was 0.2 mM which was similar to the K_m for ATP and GTP. The ATPase and GTPase activities could not be distinguished by DNA binding or ionic strength or various salts. Furthermore, it was possible to detect synthesis of ^{3}H-GTP from ^{3}H-GDP and ATP by UvrA, which suggests that both purines can bind to the same site on UvrA and that a phosphorylated UvrA covalent intermediate exists (data not shown). In contrast to UvrA, UvrB* failed to hydrolyze GTP, and was not inhibited by GTP.

The relative substrate specificities permit discrimination of the separate ATPase properties of the UvrAB complex. The NTPase activity of UvrA can be assayed by analyzing GTP hydrolysis, while the activity associated with UvrB can be assayed by analyzing ATP hydrolysis in the presence of GTP. The K_m and V_{max} of the UvrA associated ATPase and GTPase decrease when

Table 2. Kinetic analysis of nucleotidase activities

A

	ATPase				GTPase			
	-DNA		+DNA		-DNA		+DNA	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
UvrA	0.21	56	0.20	53	0.91	114	0.23	63
UvrB*	0.96	25	0.83	254	ND		ND	
UvrAB	0.21	19	1.50	614	0.19	26	0.07	15

B

	% ATPase activity remaining			
	GTP-γ-S	ATP-γ-S	ADP-ß-S	ATP-ß-S
UvrA	4.6	2.2	43.6	109.2
UvrB*	105.0	11.0	89.0	57.1
UvrAB	102.2	10.8	90.2	47.6

Table 2. A. Kinetic analysis of nucleoside triphosphatase activities. The K_m (mM) and V_{max} (min^{-1}) were measured using 50 nM UvrA, UvrB, UvrB*, and UvrAB. The measurements were repeated for ATP and GTP hydrolysis in the presence and absence of 200 µg/ml single strand DNA. No detectable activity was seen with UvrB alone. ND = not detectable. B. Inhibition of ATPase activity by nucleotide analogues at a concentration of 100 µm analogue and 0.2 mM ATP.

UvrB is added in the absence of DNA. These parameters remain lowered when GTP hydrolysis is assayed in the presence of UvrB and DNA under circumstances in which there is a large stimulation in ATPase activity. This is consistent with the inhibition of the UvrA NTPase upon UvrAB complex formation and stimulation of the UvrB ATPase in this complex in the presence of DNA. The kinetic parameters of UvrB* associated ATP hydrolysis in the presence of DNA are similar to those of the UvrAB complex.

Inhibition of the ATPase activities by nucleotide analogues was consistent with the above observations. For example, GTP-γ-S specifically inhibits the UvrA associated ATPase but has no effect on the UvrB* or the UvrAB associated ATPases, whereas the S_p isomer of ATP-ß-S was found to specifically inhibit UvrB* and UvrAB, but not UvrA (Table 2). UvrA is extremely sensitive to both ATP-γ-S and GTP-γ-S and does not show typical competitive inhibition. Furthermore, base-labile, acid stable, covalent labeling of UvrA with ATP-γ-[^{35}S] can be detected (P. Caron and L. Grossman, unpublished observations). UvrB* and UvrAB are inhibited by ATP-γ-S, but can hydrolyze ATP-γ-S slowly, with a V_{max} of 0.2 min^{-1}.

These studies are consistent with a model in which the UvrAB associated ATPase reflects the

Table 3. Salt stimulation of UvrAB complex nucleotidase activities

	rate (min^{-1}) GTP	ATP	Δ
UvrA	48	50	2
UvrA + salt	63	64	1
UvrAB	36	36	0
UvrAB + salt	38	115	75

Table 3. Salt stimulation of UvrAB complex nucleoside triphosphatase activities. The ability of salt to mimic the DNA requirement for activation of the UvrAB ATPase was analyzed using 0.5 M sodium thioglycolate, 50 nM UvrAB, and 2 mM ATP.

cryptic ATPase associated with UvrB. The interaction of UvrB with UvrA, furthermore, influences the activity of UvrA resulting in a decrease in its NTPase activity. The interaction of UvrB with UvrA additionally influences the properties of UvrB, preparing UvrB for nucleotide hydrolysis. A similar change in properties also results after proteolysis of UvrB: however, there is no evidence that UvrA binding has any effect on the cleavage of UvrB (data not shown). Furthermore, the addition of DNA to UvrAB results in a secondary effect on UvrB (or UvrB*) as expressed in a DNA dependent increase in ATP hydrolysis.

Relative to UvrAB, UvrB* requires an inordinate concentration of single strand DNA for ATPase activation. This discrepancy may be due either to a change in the properties of UvrB when bound to UvrA such that it binds to DNA with a greater and more specific affinity or to the ability of UvrA, through its ability to bind tightly to DNA, to bring UvrB into close proximity to the DNA, providing for a high effective concentration. A possible explanation for the effect of the Hofmeister salts is that they may mimic the environment near DNA. To investigate whether UvrB can be activated with these salts, the NTPase activities of UvrA and UvrAB were analyzed in the presence and absence of 0.5 M sodium thioglycolate (Table 3). The UvrA NTPase activity was only slightly activated by the salt whereas the UvrAB ATPase activity was greatly increased, suggesting that the salt was, similarly, able to mimic the DNA with the UvrAB as well as with UvrB*.

The function of the UvrB ATPase activity in the overall UvrABC incision reaction was analyzed by looking at the nucleotide requirements for DNA incision and for various intermediate steps in the incision pathway. The UvrAB proteins have previously been shown to form a complex with UV damaged DNA which is dependent on ATP hydrolysis. A damage specific complex, similar to that formed using ATP, can be formed with UvrAB and GTP (Table 4). This is consistent with the ability of UvrA to bind to damaged DNA and of UvrB to influence the stability of this binding (1,4,21). The increased binding resulting when GTP is substituted for ATP is reproducible but its significance is unclear. The binding of GTP-γ-S to UvrA resembles the

Table 4. Nucleotide dependence of UvrAB complex formation (fmol DNA bound)

	Nucleotide				
	None	ATP	GTP	ATP-γ-S	GTP-γ-S
UvrA	1.7	3.3	5.1	64.0	76.1
UvrB	0.5	0.0	0.0	0.0	0.6
UvrAB	8.1	47.7	70.7	73.6	74.8

Table 4. Nucleotide dependence of UvrAB complex formation. UvrAB (20 nM) DNA complex formation was assayed using 80 fmol UV-irradiated supercoiled plasmid DNA (6 dimers/circle) in the presence of 2 mM concentrations of ATP or GTP or 100 μM concentrations of the nucleotide analogues. After incubation at 37° for 20 min the reactions were diluted to 5 ml with 2X SSC buffer, 0° and filtered through nitrocellulose. Numbers in the table represent fmol of DNA bound to nitrocellulose. Little binding was seen to nondamaged DNA under these conditions.

effect seen with ATP-γ-S in which the binding affinity of UvrA is increased, but the specificity for damaged DNA is lost (11).

The UvrAB proteins are also able to displace short oligonucleotides from duplex DNA in the presence of ATP (5). This effect was not observed when GTP is substituted for ATP. The ability of GTP-γ-S to specifically inhibit UvrA allows the oligonucleotide displacement to be assayed under conditions where the UvrA NTPase is completely inhibited and the UvrB ATPase is functional and can hydrolyze ATP (Fig. 5). Under these conditions oligonucleotide displacement is functional and is inhibited by GTP-γ-S at a concentration which is over 500 fold greater than its apparent K_i of 0.2 μM for UvrA. In contrast ATP-γ-S, which can inhibit both the UvrA and the UvrB NTPase activities, inhibits the oligonucleotide displacement completely at less than 10 μM.

The nucleotide requirements for DNA incision were measured using ATP, GTP and nucleotide

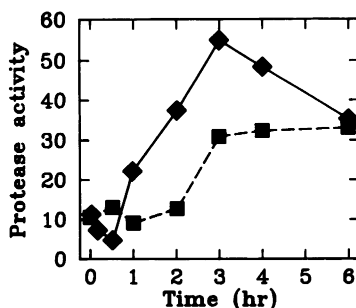

Figure 5. Inhibition of oligonucleotide displacement by nucleotide analogues. The displacement of a 17-mer hybridized to M13 phage DNA was followed in a reaction containing 50 nM UvrA and 50 nM UvrB. The effect of addition of various concentrations of nucleotide analogues in the presence of 0.2 mM ATP was examined. ◆ - ATP-γ-S; ■ - GTP-γ-S.

analogues. DNA incision is not observed in the absence of nucleotide or when GTP was substituted for ATP. Inhibition was seen when ATP-γ-S and GTP-γ-S were tested in the presence of ATP (data not shown). Thus, nucleoside triphosphate hydrolysis seems to be required of both the UvrA and the UvrB proteins in order to carry out DNA repair.

DISCUSSION

The analysis of the properties of the UvrB* protein has uncovered the existence of a cryptic ATPase associated with UvrB. This ATPase is normally activated by UvrA binding, but has not been observed previously because of the interference of the UvrA associated ATPase. The properties of the ATPase activity of UvrB* closely resemble those of the ATPase activity stimulated by DNA after formation of a UvrAB complex (Table 2 and (12)). The UvrA NTPase was found to hydrolyze both GTP and ATP with similar kinetics, whereas the ATPase of UvrB is limited to hydrolysis of ATP. Contrary to earlier models, the UvrA NTPase is inhibited rather than stimulated by binding to UvrB. Nucleotide binding to UvrA in a UvrAB complex appears to be sufficient to allow formation of a damage specific DNA-protein complex which is stable in 2X SSC. Nucleoside triphosphate hydrolysis by UvrB is required, however, in order to catalyze strand displacement reactions, and nucleoside triphosphate hydrolysis by both UvrA and UvrB are required at some step for incision of DNA.

The existence of activities which are expressed in a protein complex and suppressed when the subunits are individually analyzed requires that either the active sites for these activities are at the interface of two subunits or that one subunit can allosterically effect the activity which is inherent in another subunit. Recently a number of protein complexes involved in DNA replication and repair have been found in which the activities of the individual components are effected following complex formation. For example, the dnaB protein is a DNA-dependent ATPase which has DNA helicase activity and it has been proposed to be involved in DNA replication initiation and elongation (22). The binding of dnaC to dnaB (23) increases nucleotide binding by dnaC (24) whereas ATP binding and hydrolysis by dnaB is inhibited (25). The replication of T4 phage DNA requires the action of a number of T4 gene products, many of which are isolated as part of tight protein complexes (26). Some of the individual subunits of these complexes have little or no detectable activity unless assembled into a protein complex. For example, the gene 61 protein has been shown to be a very weak DNA primase, greatly stimulated by the gene 41 protein (27). In addition the gene 41 protein has been shown to be a DNA helicase which is stimulated by the gene 61 protein (28).

The requirement for multiple protein complex formation to actuate activities which are inherent in the individual subunits, allows for the generation of a higher degree of specificity for the individual steps in the reaction and also insures that, once a reaction is initiated, the enzymatic activities required for completion of the reaction will be present. The *E. coli* UvrABC system appears to rely on the ability of UvrA to recognize DNA damage and on protein complex formation to bring the UvrB and UvrC proteins to the damaged site. The binding of UvrB to UvrA leads to the unmasking of an ATPase activity which is essential for completion of the repair reaction, but the exact function of this activity is still not understood.

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ABBREVIATIONS

ADP-ß-S	adenosine-5'-O-(2-thiodiphosphate)
ATP-ß-S	adenosine-5'-O-(2-thiotriphosphate)
ATP-γ-S	adenosine-5'-O-(3-thiotriphosphate)
MOPS	morpholinopropanesulfonate
NTPase	nucleoside triphosphatase

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