The (A)BC excinuclease of *Escherichia coli* has only the UvrB and UvrC subunits in the incision complex

(Damage recognition)

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Communicated by Mary Ellen Jones, April 6, 1989 (received for review March 2, 1989)

ABSTRACT The uvrA, uvrB, and uvrC genes control excision repair in *Escherichia coli*. Cells with mutations in any of these three genes cannot repair DNA by nucleotide excision. When the purified gene products—the UvrA, UvrB, and UvrC proteins—are mixed together, an excision complex is formed that incises on both sides of the damaged nucleotide in an ATP-dependent reaction; it has been presumed that the excision complex was an ABC complex containing all three Uvr proteins. To determine the stoichiometry of the subunits in the enzyme, we conducted hydrodynamic studies with mixtures of the subunits with or without DNA substrate. We found that without DNA the UvrA subunit is a dimer and that when UvrB protein is also present, a (UvrA)2(UvrB)1 complex forms. Without DNA no detectable interaction of either the UvrA or UvrB subunits or the (UvrA)2(UvrB)1 complex with the UvrC subunit occurs. Unexpectedly, with UV-irradiated DNA, the UvrA/UvrB ratio in isolated DNA–protein complexes is variable, and the ratio becomes infinitesimally low as the UvrA concentration in the reaction mixture decreases. Under conditions of saturating UvrB protein approximately one UvrB molecule binds to DNA per damaged site in a reaction that requires catalytic amounts of UvrA subunit. Addition of UvrC protein to purified UvrB–DNA complexes results in rapid incision of the DNA, presumably catalyzed by an excision nuclease containing only UvrB and UvrC subunits.

The UvrA, UvrB, and UvrC proteins, referred to collectively as “ABC excinuclease,” initiate nucleotide excision repair in *Escherichia coli* by incising seven bases 5' and four bases 3' to nucleotide mono- and diaducts in DNA (1). The Uvr proteins have been purified individually and characterized in some detail (see refs. 2 and 3). UvrA is an ATPase and a DNA-binding protein. UvrA has higher affinity to damaged DNA than non-damaged DNA, and this discrimination is further enhanced by the presence of ATP, even though there is no increase in ATPase activity when DNA, damaged or otherwise, is present. Thus, UvrA has been considered the damage recognition subunit of the excision complex. UvrB does not hydrolyze ATP, nor does it bind to DNA. However, mixing UvrA, UvrB, and ATP with damaged DNA increases the total ATPase activity (4, 5) and leads to formation of a DNA–protein complex of unknown stoichiometry, but higher stability, as judged by filter binding (6) and DNase protection (“footprinting”) (7) experiments, compared with the UvrA–damaged DNA complex formed in the presence of ATP. Surprisingly, the DNase I footprint of the complex formed in the presence of both UvrA and UvrB is considerably smaller [19 base pairs (bp)] than that observed with UvrA alone (33 bp) (7). UvrC has no ATPase activity and no specific affinity for damaged DNA; however, addition of UvrC to a mixture containing UvrA, UvrB, ATP, and a damaged DNA substrate results in the dual incisions of DNA V, characteristic of excision repair in *E. coli*.

We have conducted physical studies to determine the stoichiometry of the subunits in precission complexes of the Uvr proteins in the absence and presence of DNA. We found that UvrA dimerizes and, in an ATP-dependent reaction, associates with UvrB to form a (UvrA)2(UvrB)1 complex in the absence of DNA. More importantly, we have determined that UvrA, possibly as the (UvrA)2(UvrB)1 complex, delivers UvrB onto damaged DNA and then dissociates from the UvrB–DNA complex. Upon addition of UvrC, the DNA in the UvrB–DNA complex is incised in a manner typical of the so-called ABC excinuclease. Thus, our data indicate that UvrA is essential for the formation of the nucleotide excision nuclease complex, but it is not involved in the incision reactions.

MATERIALS AND METHODS

Enzymes and Substrates. The UvrA, UvrB, and UvrC proteins were purified to >90% homogeneity as described (4). Phage T4 endonuclease V was purchased from Applied Genetics (Freeport, NY). Protein standards for hydrodynamic analyses were from Bio-Rad and M13mp19RF DNA was from Boehringer Mannheim. 3H-labeled pBR322 DNA (1.1 × 106 cpn/μg) was purified from *E. coli* AB2487/pBR322 by Sarkosyl lysis followed by two cesium chloride/ethidium bromide density centrifugations. When indicated, DNA was damaged by UV irradiation (25–375 J/m2) by using a Sylvania germicidal lamp (254 nm). The number of pyrimidine dimers generated in pBR322 by UV was obtained from the average number of nicks introduced into irradiated DNA by the dimer-specific T4 endonuclease V. The total number of photoproducts (recognizable by excision repair) was calculated assuming that pyrimidine dimers and 6-4 photoproducts constitute 85% and 15% of the UV photoproducts, respectively (8). Hydrodynamic Analysis. The Uvr proteins were incubated (individually or in combination) for 30 min at 4°C in buffer (200–500 μl) containing 50 mM Tris·HCl (pH 7.5), 300 or 500 mM KCl, 10 mM MgCl2, 10 mM β-mercaptoethanol, 20% (vol/vol) (gel filtration) or 3% (vol/vol) (velocity sedimentation) glycerol, and 2 mM ATP, ADP, or ATP[yS] when indicated. Molecular weight standards (thyroglobulin, 650,000; IgG, 158,000; ovalbumin, 45,000; and myoglobin, 17,000) were added just before the sample was loaded onto either an AcA34 (LKB) column (1.0 × 30 cm) equilibrated with the incubation buffer at 25°C or 4°C or a 5–20% glycereol gradient (8 ml) made in the same buffer. The column was run at 4 ml/hr, and the gradients were centrifuged at 40,000 rpm for 18 hr at 4°C in a Beckman SW41 rotor. Fractions collected from either the column or the gradients were analyzed by SDS/PAGE and then stained with Coomassie blue. The abbreviations UvrA, UvrB, and UvrC are also used to refer to the proteins of the corresponding genes; UV-DNA, UV-irradiated DNA.

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proteins in individual bands were quantified by using a Hoefer GS300 scanning densitometer. From the elution and sedimentation profiles the Stokes radii and sedimentation coefficients were calculated by the methods of Laurent and Killander (9) and of Martin and Ames (10), respectively. The fractions were also assayed for one or more Uvr protein activity by using the incision assay.

Isolation of DNA–Protein Complexes. UvrA (60–1250 nM) and/or UvrB (30–625 nM) were added to nonirradiated or UV-irradiated, 3H-labeled pBR322 (9 or 18 nM) in binding buffer (160 μl) containing 50 mM Tris-HCl (pH 7.5), 50 mM KCl, 10 mM MgCl2, 2 mM ATP, 5 mM dithiothreitol, and 20% glycerol, incubated for 15 min at 37°C and loaded onto an AcA22 (LKB) column (1.0 × 15 cm) equilibrated in binding buffer with 300 mM KCl at 25°C. The column was run at 4 ml/hr, and fractions (700 μl) were collected. The DNA in the fractions was quantified by scintillation counting of 25 μl of each fraction. For protein determination 200 μl of each fraction was analyzed by SDS/PAGE followed by silver staining and quantitative densitometry of protein bands using internal UvrA and/or UvrB standards in each gel. For measuring the incision activity of the proteins complexed with DNA, UvrC was added to a part of each fraction to 110 nM and incubated at 37°C for 25 min, and the nicks introduced into DNA were determined by the incision assay.

The Incision Assay. This assay measures the conversion of superhelical 3H-labeled pBR322 to open circular form as a result of nicking by Uvr proteins (11). Fractions from gel filtration columns or velocity sedimentation gradients were tested for incision activity by adding UV-irradiated (125 J/m2), 3H-labeled pBR322, and the appropriate complementing Uvr proteins to the fractions and incubating at 37°C for 25 min unless indicated otherwise. Similarly, the appropriate complementing subunits were added to DNA–Uvr protein complexes isolated by gel exclusion chromatography, and these mixtures were incubated at 37°C for 0–25 min before analysis. The reactions were stopped by adding SDS to 0.5%. The superhelical and nicked DNA were separated on 1% agarose gels, and the radioactivity in each band was quantified by scintillation counting. The average number of nicks per plasmid was calculated assuming Poisson distribution of incisions.

RESULTS

Hydrodynamic Properties of the Uvr Proteins. Before investigating interactions between different Uvr subunits, it was necessary to characterize the hydrodynamic behavior of each subunit in isolation. Because ATP is required for at least one step in the incision reaction, the effect of nucleotide cofactors on the hydrodynamic behavior of each subunit was also examined. Preliminary results showed that, at micromolar concentrations, UvrA and UvrC form high-molecular weight aggregates in low glycerol (<20%) buffers of moderate ionic strength (220–400 mM KCl) strength. Subsequently, experiments involving UvrA or UvrC were carried out in buffers containing 300 mM or 500 mM KCl, respectively.

The hydrodynamic properties of the Uvr proteins are summarized in Table 1. Gel filtration experiments show that, in the presence or absence of ATP, the Stokes radius of UvrA is 59 Å, a size consistent with that of a UvrA dimer, assuming globular shape. In contrast, the sedimentation coefficient of UvrA is strongly influenced by ATP. In the absence of ATP, the sedimentation coefficient is 5.7 S, consistent with a monomeric UvrA of Mr = 100,000. Addition of ATP to both the UvrA solution and the gradient caused an increase in the S value, the magnitude of which depended on the initial UvrA concentration (see ref. 13). At 12 μM, the UvrA protein has an S value of 7.4, corresponding to Mr = 187,500. These experiments suggest that, under sedimentation conditions, a UvrA monomer–dimer equilibrium is established with dimerization being favored by ATP and by high protein concentration. ATP hydrolysis was not required for this effect as both ADP and ATPγS had the same effect on sedimentation behavior of UvrA (data not shown). The UvrB and UvrC subunits behaved as monomers during both gel filtration and sedimentation experiments, and their hydrodynamic properties were not influenced by ATP.

Association of UvrA and UvrB. To determine whether the Uvr subunits assemble into protein complexes in the absence of DNA, we conducted hydrodynamic experiments with pairwise combinations or mixtures of all three subunits with or without nucleotide cofactors. The UvrC protein had the same Stokes radius and sedimentation coefficient when analyzed in the presence or absence of UvrA or UvrB and, thus, apparently UvrC does not interact with the other subunits in the absence of DNA (Table 1). In contrast, we found that UvrA and UvrB coelute and co sediment in the present of ATP. Neither ATPγS nor ADP promote this interaction (data not shown), suggesting that ATP hydrolysis is necessary for complex formation. The hydrodynamic parameters of the UvrA–UvrB complex formed in the presence of 2 mM ATP, 1.0 μM UvrA, and 0.5 μM UvrB were Stokes radius = 60.1 ± 4.8 Å and S = 7.8 ± 0.3, from which Mr = 201,400 is calculated, a size inconsistent with any integral combination of UvrA and UvrB subunits. We attribute this inconsistency to overlap of the UvrA dimer peak with that of the UvrA–UvrB complex. To obtain the true stoichiometry of the subunits in the complex, we conducted velocity sedimentation experiments with excess amounts of UvrB to favor the formation of UvrA–UvrB complex while minimizing the amount of free UvrA. The fractions from such an experiment were analyzed by SDS/PAGE, and the amounts of UvrA and UvrB in each fraction were quantified by densitometry (Fig. 1). By integrating the peaks corresponding to each protein

Table 1. Hydrodynamic properties of the Uvr proteins

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Stokes radius, Å</th>
<th>S</th>
<th>Hydrodynamic</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>UvrA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−ATP</td>
<td>59.2 ± 2.9</td>
<td>—</td>
<td>210,000*</td>
<td>103,874</td>
</tr>
<tr>
<td>+ATP</td>
<td>59.0 ± 0.4</td>
<td>7.4</td>
<td>187,500</td>
<td></td>
</tr>
<tr>
<td>UvrB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−ATP</td>
<td>42.4 ± 2.3</td>
<td>5.2± 0.2</td>
<td>91,000</td>
<td>78,116</td>
</tr>
<tr>
<td>+ATP</td>
<td>39.9 ± 0.5</td>
<td>5.9± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UvrC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−ATP</td>
<td>38.7 ± 1.6</td>
<td>4.9± 0.1</td>
<td>86,000</td>
<td>66,038</td>
</tr>
<tr>
<td>+ATP</td>
<td>40.8 ± 2.3</td>
<td>4.9± 0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All hydrodynamic Mr values, except those otherwise noted, were calculated from the formula Mr = RTS/(1 − v) and D = kT/6πηS, where a = Stokes radius (12). For UvrB and UvrC, the Mr values were calculated from the mean values of a and S obtained with and without ATP. The UvrB and UvrC concentrations used in these experiments ranged from 0.5 to 5.0 μM. There was no effect of concentration on the hydrodynamic values of UvrB and UvrC. Sequence Mr is the calculated Mr based on sequence of each subunit (2).

†This value was calculated solely from gel filtration, assuming a globular shape for the protein.

‡This value was calculated solely from the sedimentation coefficient by using the formula (8), Mr = MrγS/(1 + 1.0/S)2/3.

§The sedimentation coefficient of UvrA depends on the concentration of the protein (13). This value was obtained with 12 μM initial concentration. Concentrations ranging from 0.1 to 12 μM UvrA gave the same Stokes radius.

The a and S values obtained in the presence of UvrA and/or UvrB were not significantly different from those obtained with UvrC alone and have been included in calculating the mean.
the complex we obtain UvrA/UvrB = 1.8 and, therefore, conclude that, in the presence of ATP, UvrA and UvrB form a \((UvrA)_2(UvrB)_2\) complex. UvrC does not interact with the complex under these conditions (Table 1).

**UvrA Delivers UvrB to UV-Irradiated DNA.** UvrA binds to damaged DNA with high affinity (1, 2, 7, 14), whereas UvrB has no affinity for DNA, but it apparently binds to damaged DNA in the presence of UvrA and ATP (6, 7, 15). Because of our finding that these subunits form a \((UvrA)_2(UvrB)_2\) complex in the absence of DNA, we wished to determine whether this stoichiometry was maintained in complexes with damaged DNA.

UvrA and UvrB were incubated with UV-irradiated or nonirradiated DNA in the presence of ATP; then DNA–protein complexes were isolated by size-exclusion chromatography and analyzed for protein content by SDS/PAGE with silver staining followed by quantitative densitometry. As expected, UvrA did bind to both UV- and nonirradiated DNA (Table 2), but UvrB bound to DNA only when the DNA was UV-irradiated, when UvrA was present in the reaction mixture, and when ATP was present in both the reaction mixture and the column buffer (data not shown). When the ratio of UvrA to UvrB in the reaction mixture was varied, the ratio of UvrA to UvrB bound to UV-irradiated DNA changed dramatically. At the highest UvrA-to-UvrB ratio, ~5-fold more UvrA than UvrB was bound to DNA. Considering the affinity of UvrA for DNA, even in the absence of UvrB, this result was not surprising. However, as the UvrA-to-UvrB ratio in the reaction mixture decreased, the ratio in the DNA–protein complex also decreased, eventually becoming infinitesimally low (Table 2). This result indicates that UvrA promotes the binding of UvrB to UV-irradiated DNA catalytically and not stoichiometrically.

We envisage two mechanisms by which UvrA could load UvrB onto damaged DNA nonstoichiometrically: (i) either UvrA creates a nucleation site on DNA where UvrB molecules bind, or (ii) UvrA delivers UvrB onto damaged sites and then dissociates. We reasoned that if UvrA were delivering UvrB to damaged sites and the amount of UV-damage to DNA is increased (while maintaining constant concentrations of DNA, UvrA, and excess UvrB) in the reaction mixture, the ratio of UvrB to UvrA in the DNA–protein complex should increase as well. On the other hand, if UvrA-dependent nucleation of UvrB is occurring, then the UvrB-to-UvrA ratio should remain relatively constant with increasing UV doses. The results of such an experiment are presented in Fig. 2. The amount of UvrA bound to DNA is UV dose-independent under the conditions of these experiments in the absence (Fig. 2 I) or the presence (Fig. 2 III) of UvrB. The UvrB subunit does not bind to DNA when UvrA is absent in the reaction mixture (Fig. 2 II), but it binds in a dose-dependent manner when the reaction mixture contains UvrA (Fig. 2 III). Thus, we conclude that UvrA delivers UvrB to damaged sites on DNA.

**Stoichiometry of the UvrB–Photoproduct Complex.** To determine the ratio of UvrB to photoproducts, we conducted binding experiments by using constant concentrations of UvrA and UV-irradiated DNA but increasing concentrations of UvrB in the reaction mixtures. The DNA–protein complexes formed were isolated, and the amount of UvrB was quantified. The data from these experiments is plotted in the form of a saturation curve and also analyzed by a Scatchard plot in Fig. 3. The x-axis intercept gives a value of 0.16, from which we conclude that one UvrB molecule is bound per photoproduct. Although a binding constant \(K_a = 10^7 M^{-1}\) is

![Graph](image-url)

**Table 2.** Protein composition in the isolated DNA–protein complexes as a function of UvrA and UvrB concentrations in the binding reaction mixtures

<table>
<thead>
<tr>
<th>Reaction mixture*</th>
<th>DNA–protein complex†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UvrA, nM</td>
</tr>
<tr>
<td>UV-DNA</td>
<td>1250 625 2.0</td>
</tr>
<tr>
<td></td>
<td>625 625 1.0</td>
</tr>
<tr>
<td></td>
<td>210 625 0.33</td>
</tr>
<tr>
<td></td>
<td>63 625 0.1</td>
</tr>
<tr>
<td></td>
<td>0 375 —</td>
</tr>
<tr>
<td>Non-UV-DNA</td>
<td>1250 625 2.0</td>
</tr>
</tbody>
</table>

*Reaction mixtures (160 μl) containing the indicated amount of Uvr proteins and UV (125 J/m²)pBR322 (9 nM) were incubated in binding buffer for 15 min at 37°C and then loaded onto the size-exclusion column; 700-μl fractions were collected.

†Two hundred microliters of the DNA peak fraction was analyzed by SDS/PAGE, silver staining, and quantitative densitometry with known amounts of UvrA and UvrB proteins used as internal standards. Protein bands observed visually but below the sensitivity of the densitometer are denoted by <. ND, not detectable.
FIG. 2. Binding of UvrA and UvrB to UV-DNA as a function of UV fluence. Reaction mixtures in 160 μl of binding buffer containing 60 nM UvrA (I), 375 nM UvrB (II), or 60 nM UvrA and 375 nM UvrB (III) plus 9 nM pBR322 irradiated with the indicated doses were loaded onto a gel exclusion column; 700-μl fractions were then collected, and 200 μl of the peak DNA fraction from each run was analyzed by SDS/PAGE and silver staining. The first lane in each panel contains 0.25 μg each of UvrA and UvrB as internal standards.

calculated from the slope of the Scatchard plot, this number cannot be interpreted as the binding constant of UvrB to damaged DNA. Most likely it reflects all steps leading to the binding of UvrB to photoproducts, including the extent of dimerization of UvrA, the association of UvrA dimer with UvrB, and the binding of the (UvrA)2(UvrB)j to damaged DNA.

Properties of the UvrB–DNA Complex. When UvrC is added to isolated DNA–UvrB complex, the DNA is nicked (Table 3). The molar ratio of nicks to UvrA in these complexes is as high as 10-fold suggesting UvrA simply delivers UvrB to the damage site and UvrA itself is not involved in the reaction per se. However, it is conceivable that the contaminating UvrA in the isolated DNA–UvrB complexes may participate in multiple rounds of incision upon addition of UvrC to produce more than stoichiometric (with regard to UvrA) incisions in DNA. If this were true, addition of UvrA (along with UvrC) to UvrB–DNA complexes would be expected to increase the rate and/or the extent of incision. Fig. 4 shows that addition of UvrA does not increase the rate or the extent of the reaction. In fact, the rate is slightly decreased—perhaps because transient binding of UvrA to UvrB–DNA complex interferes with binding of UvrC to the complex and prevents incision. That UvrA does not participate in incision was further supported by adding competing DNA to the isolated UvrB–DNA complexes. This DNA, which had 50-fold molar excess of photoproducts over those in the complexes, had no significant effect on the incision kinetics, even when preincubated with the complexes up to 50 min before the addition of UvrC (data not shown). Thus, neither supplementing the UvrB–DNA complexes with UvrC nor the “removal” of the contaminating UvrA from these complexes by competing DNA affects the rate or extent of DNA incision. Apparently, UvrA simply delivers UvrB to DNA, and only UvrB and UvrC are involved in incision.

FIG. 3. Scatchard analysis of binding of UvrB to UV-DNA. Reaction mixtures (160 μl) containing UvrA (60 nM), UvrB (30–500 nM), and UV-3H-labeled pBR322 (18 nM, 75 nM photoproducts) in binding buffer were loaded on a size-exclusion column, and the UvrB associated with DNA was quantified by SDS/PAGE followed by silver staining and densitometry. [UvrB]b and [UvrB]i, concentrations of bound and free UvrB, respectively; [PP], total concentration of UV photoproducts. (Inset) The binding curve used to generate the Scatchard plot. [UvrB]i, total concentration of UvrB in the initial binding reaction.

FIG. 4. Incision of DNA in DNA–UvrB complex upon addition of UvrC. UvrB–DNA complexes were isolated from a reaction mixture (160 μl) containing UvrA (60 nM), UvrB (375 nM), UV-pBR322 (9 nM, 125 nM photoproducts) by size-exclusion chromatography. The peak fraction (700 μl) contained UvrA (<0.9 nM), UvrB (6.8 nM), and pBR322 (1.1 nM and 15 nM photoproducts). To 200 μl of this fraction, either UvrC (110 nM) (●), or UvrA (90 nM) plus UvrC (110 nM) (□) was added, and the reaction mixtures (220 μl) were incubated at 37°C. At the indicated time points, aliquots (25 μl) were removed for measuring incisions per plasmid.

### Table 3. Binding of UvrB to UV-DNA in the presence of UvrA as a function of UV dose to DNA

<table>
<thead>
<tr>
<th>UV fluence, J/m²</th>
<th>DNA–protein complex, nM</th>
<th>Incision, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.38</td>
<td>2.6</td>
</tr>
<tr>
<td>50</td>
<td>0.48</td>
<td>5.4</td>
</tr>
<tr>
<td>125</td>
<td>0.28</td>
<td>9.8</td>
</tr>
<tr>
<td>250</td>
<td>0.38</td>
<td>14.4</td>
</tr>
<tr>
<td>375</td>
<td>0.33</td>
<td>13.1</td>
</tr>
</tbody>
</table>

The binding reaction contained UvrA (60 nM), UvrB (375 nM), and UV-pBR322 (9 nM) in 160 μl of binding buffer. The mixture was incubated for 15 min at 37°C before loading onto the size-exclusion column. Protein concentrations in DNA–protein complexes isolated by size exclusion were determined by analysis of 200 μl of peak fraction by SDS/PAGE and silver staining. Values are the means of two experiments.

*Incisions were measured by adding UvrC (110 nM) to 50 μl of the isolated protein–DNA complex followed by incubation for 25 min at 37°C and analysis on an agarose gel.
Because both genetic and biochemical evidence indicate that all three uvr gene products are required for the incision of damaged DNA (2, 3), it has been generally assumed that all three subunits are present in the actual incision complex. The demonstration that UvrA is the damage recognition subunit (14) and the discovery of two DNA recognition "zinc fingers" in this protein (16) were taken as strong evidence that UvrA must be a component of this damage-specific nucleosome complex (2, 3). However, the data presented in this paper clearly demonstrate that UvrA simply delivers UvrB to the damage site and is not a part of the actual nucleosome complex. Such a function for a DNA-binding protein in a multiprotein pathway has a precedent. Six proteins, including DnaB and DnaC, form a preprimosome at a specific site on the single-stranded φX174 template, but only a subset of these move to the few sites where primers are synthesized by primase (17). Similarly, in an ATP-dependent reaction, the "γ complex" of E. coli DNA polymerase III (Pol III) holoenzyme delivers the β subunit to the Pol III core and then dissociates from the complex (18).

Our current model regarding the events leading up to the incision of damaged DNA may be summarized as follows (Fig. 5). UvrA dimersize in an ATP-stimulated reaction and interacts with UvrB in an ATP-dependent reaction to form a (UvrA)2(UvrB)1 complex. This complex, guided by the affinity of UvrA for damaged DNA, delivers one UvrB molecule to the damaged site, and UvrA dissociates from the complex. Then, UvrC binds to this UvrB–DNA complex to form the active nulease. Alternatively (broken arrow, Fig. 5), (UvrA)2 can bind initially to DNA followed by the ATP-dependent association of UvrB at the damaged site before dissociation of the UvrA dimer. An uninduced E. coli cell contains ∼20 UvrA and 200 UvrB molecules (2). According to our model, the 10 UvrA dimers can deliver 200 UvrB molecules to damaged sites in DNA and thus initiate the removal of a much greater number of DNA adducts than the number of UvrA molecules available.

The data presented in this paper also provide a simple explanation to a puzzling observation made earlier (7): UvrA alone makes a 33-bp DNase I footprint around a psoralen-adducted base, which upon addition of UvrB (in the presence of ATP) shrinks to 19 bp. This "UvrA–UvrB" footprint of 19 bp must now be reinterpreted as the footprint of UvrB in the preincision complex. Similarly, a stable DNA–protein complex (t1/2 = 42 min) identified by filter binding assay and interpreted to be a UvrA–UvrB–DNA complex (6) appears to be a UvrB–DNA complex. Our measurements (data not shown) show that isolated UvrB–DNA complexes are very stable with koff = 1.1 × 10⁻⁴ sec⁻¹, which corresponds to t1/2 = 100 min.

Finally, although we obtain about 1:1 stoichiometry for UvrB:UV photoproducts (Fig. 3), we obtain only ∼30% incision at these complexes upon addition of saturating UvrC (Table 3). We believe that this less than stoichiometric incision is due to the presence of UvrB and UvrC subunits with near-normal binding affinity but diminished incision activity, as we find better correspondence between the amount of UvrB bound and nicks made when we use fresh subunits (data not shown). Clearly, this point, as well as the stoichiometry of UvrC in the complex, need further investigation. However, we believe that our data show unambiguously that the active site of E. coli excision repair enzyme is contained within the UvrB and UvrC subunits and, therefore, propose renaming this enzyme the (A)BC excinuclease.

We thank Drs. M. E. Jones and C. P. Selby for critically reading the manuscript. This work was supported by National Institutes of Health Grant GM32833.