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Excision Repair of Ultraviolet-Irradiated Deoxyribonucleic Acid in Plasmolyzed Cells of *Escherichia coli*

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A system of cells made permeable by treatment with high concentrations of sucrose (plasmolysis) has been exploited to study the excision repair of ultraviolet-irradiated deoxyribonucleic acid in *Escherichia coli*. It is demonstrated that adenosine 5'-triphosphate is required for incision breaks to be made in the bacterial chromosome as well as in covalently closed bacteriophage λ deoxyribonucleic acid. After plasmolysis, *uvrC* mutant strains appear as defective in the incision step as the *uvrA*-mutated strains. This is in contrast to the situation in intact cells where *uvrC* mutants accumulate single-strand breaks during postirradiation incubation. These observations have led to the proposal of a model for excision repair, in which the ultraviolet-specific endonuclease, coded for by the *uvrA* and *uvrB* genes, exists in a complex with the *uvrC* gene product. The complex is responsible for the incision and possibly also the excision steps of repair. The dark-repair inhibitors acriflavine and caffeine are both shown to interfere with the action of the adenosine 5'-triphosphate-dependent enzyme.

Among several gene functions known to participate in the excision repair of ultraviolet (UV)-irradiated deoxyribonucleic acid (DNA) in *Escherichia coli*, the *uvrA*, *uvrB*, and *uvrC* gene products seem indispensable as judged from the high UV sensitivity of mutants defective in these genes (6, 9). Originally, *uvrA*, *uvrB*, and *uvrC* mutants were characterized as excision defective, being unable to remove the primary photoproduct, pyrimidine dimers, from the DNA (6). The isolation of a UV-specific endonuclease from wild-type *E. coli* that is absent from *uvrA*- and *uvrB*-mutated cells (here termed the *uvrAB* endonuclease) clearly implicates the *uvrA* and *uvrB* gene functions in the incision step of the repair process (3). The observation that this enzyme is present in normal amounts in *uvrC*-mutated cells indicates that the deficiency in these cells lies elsewhere (3). In vivo studies of *uvrC* mutants reveal that UV-induced endonucleolytic incision does take place in these strains (7), although breaks are introduced more slowly than in wild-type cells (11). It has been suggested from studies of *uvrC* mutants lacking either DNA polymerase I or polynucleotide ligase activities that the slow rate of break formation in the *uvrC* mutant may be partially explained by ligase-catalyzed reversal of the incision reaction in the mutant (12). This proposal is consistent with the finding that breaks produced by the purified *uvrAB* endonuclease in vitro are susceptible to ligase sealing (A. Braun and L. Grossman, Fed. Proc. 33:1599, 1974). However, it appears that the difference between the wild-type and *uvrC* cells cannot be entirely accounted for in this way since the dose-dependent increase in the rate of incision terminates at a lower dose in *uvrC* strains, even in the *uvrC lig* mutant (Seeberg, unpublished data).

From studies of UV-induced DNA degradation in permeable cells, Strike and Emmerson (13) proposed that adenosine 5'-triphosphate (ATP) may be a requirement for UV-induced endonucleolytic incision. Recently, Waldstein et al. (14) demonstrated that ATP does stimulate this reaction in *E. coli* cells made permeable to small molecules by treatment with toluene. To characterize the incision reaction more closely, particularly in *uvrC*-mutated cells, we have adopted a system of cells made permeable by treatment with high concentrations of sucrose (15). In permeable cells the activities of DNA polymerase and polynucleotide ligase can be regulated simply by the addition or omission of the relevant cofactors. The incision reaction after UV irradiation can then be studied under conditions in which both ligase and DNA polymerase activities are effectively inhibited, minimizing the influence of break repair.

Experiments are described in which UV-promoted, single-strand breakage of both chromosomal and episomal DNA in plasmolyzed cells and the effect of the dark-repair inhibitors caf-

feine and acriflavine on the incision reaction were investigated.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. All of the strains used are listed in Table 1.

Media, growth, and labeling of bacteria. M9 buffer contained (per liter of water): NH_4Cl, 1 g; KH_2PO_4, 3 g; $Na_2HPO_4 \cdot 2H_2O$, 7.5 g; NaCl, 5 g; $MgSO_4 \cdot 7H_2O$, 0.246 g; and $CaCl_2$, 0.011 g. K medium was M9 buffer supplemented with 1% glucose, 1% Casamino Acids, and 0.0001% thiamine.

Thy^- strains were labeled by growth for several generations in K medium to which was added ^3H-labeled thymidine (specific activity, 2 μCi/μg) to a final concentration of 5 μg/ml. Thy^+ strains were labeled in K medium containing 200 μg of deoxyadenosine per ml and 5 μCi of radioactive thymidine (specific activity, ~1 mCi/0.05 mg) per ml. While still in the exponential growth phase, cells were washed in M9 buffer and suspended in K medium containing 10 μg of nonradioactive thymidine per ml, and growth continued for an additional 45 min to allow incorporation of label remaining in the intracellular pool. Strains carrying the temperature-dependent *polA12* mutation (8) were grown at 33 C; the others were grown at 37 C.

Lysogenic bacteria used in the study of bacteriophage λ DNA were grown in K medium at 33 C.

Irradiation. Cells were irradiated with 254-nm light from a low-pressure mercury lamp at a concentration of approximately 10^8 cells/ml in a 0.2-mm layer in a glass petri dish at ice temperature. Doses given are corrected for absorption in the suspension. Under our irradiation conditions, 10 J/m^2 of UV converts 0.041% of the radioactive thymine in the DNA into thymine dimers (Seeberg, manuscript in preparation).

Plasmolysis treatment. Cells were made permeable by treatment with high concentrations of sucrose as described by Wickner and Hurwitz (15) and slightly modified by Strike and Emmerson (13). Approximately 10^9 labeled cells were washed in M9 buffer by centrifugation, resuspended in M9, irradiated, washed in a solution of 0.04 M tris(hydroxymethyl)aminomethane (Tris; pH 8.0) and 0.01 M ethyleneglycol-bis-(β-aminoethyl ether)*N,N'*-tetraacetic acid (EGTA), and resuspended in 50 μl of the same buffer. Sucrose (84% in 0.04 M Tris [pH 8.0], 0.01 M EGTA) was gradually added to yield a final concentration of 2.0 M. After 5 min at ice temperature, cells were gently diluted (1:7.5) into reaction buffer [0.04 M morpholinopropane sulfonic acid (pH 7.5), 0.1 M KCl, 0.02 M $MgSO_4$, 10^{-4} M ethylenediaminetetraacetic acid, 0.01 M $NH_4(SO_4)_2$, 5×10^{-4} M dithiothreitol] supplemented with suitable cofactors, precursors, and inhibitors as indicated in the text. Cells were kept strictly at ice temperature throughout.

Cell lysis and analysis of DNA strand breaks. For studies on chromosomal DNA, cells were lysed by mixing equal volumes of cell suspension and 1% Sarkosyl NL-97–0.01 M EDTA and immediately layering on top of alkaline sucrose gradients. Gradients were spun at 30,000 rpm in an SW50.1 rotor, and 0.2-ml fractions were collected from the bottom of the tubes by means of a peristaltic pump. This method assures collection of fractions of uniform size and avoids the problem of nonuniform drop size when the detergent is present in the lysing solution. Fractions were collected directly into vials for scintillation counting. UV-induced DNA breakdown, as reflected by radioactive material in the top fractions, was less than 5% of the total DNA for all strains studied under the conditions used in these experiments.

The molecular weight, *Mi*, of the DNA in each

TABLE 1. *Bacterial and bacteriophage strains*

Strain	Genotype	Source or reference
AB1157	F^- *thi thr leu pro arg his lac gal xyl mtl ara* str^R *sup-37*	P. Howard-Flanders
AB1886	AB1157 *uvrA6*	P. Howard-Flanders
AB1884	AB1157 *uvrC34*	P. Howard-Flanders
MM383	F^- *polA12 thy lac rha* str^R	B. Low (8)
AB5025	MM383 *uvr C34 polA12* thy^+	E. Seeberg
AB5026	MM383 *uvrA6 polA12* thy^+	E. Seeberg
AB1157 (λ*cI857*)	AB1157 lysogenic for λ*cI857*	E. Seeberg (11)
AB1886 (λ*cI857*)	AB1886 lysogenic for λ*cI857*	E. Seeberg (11)
AB1884 (λ*cI857*)	AB1884 lysogenic for λ*cI857*	E. Seeberg (11)
R694 (λ*cI857S7*)	λ^R, *mal, gal, lac* lysogenic for λ*cI857S7*	D. Rupp

fraction was calculated with reference to a cosedimenting ^{32}P-labeled λ DNA marker (single-strand molecular weight, 1.55×10^7) using the Abelson and Thomas relation (1). The weight average molecular weight, *Mw*, was obtained from the equation $Mw = \Sigma Mi \cdot Ci / \Sigma Ci$, where *Ci* represents the amount of radioactivity in each fraction (10). Fractions at the top and the bottom of the gradient, clearly separated from the main radioactive peak, were omitted from the calculations. A value for the number of breaks per 10^8 atomic mass units of DNA ($10^8/Mn$) was obtained by calculating the reciprocal value of the number average molecular weight, *Mn*. It was assumed that $Mn = Mw/2$, as if breaks were randomly distributed. This assumption appears to be correct for molecular weight, *Mw*, below 10^8 ($10^8/Mn$ larger than 2), as judged from sedimentation profiles. For DNA of higher molecular weight, a high-speed centrifugation effect occurs. Centrifugation of DNA from unirradiated cells at lower speed (15,000 rpm) showed that errors in the parameter $10^8/Mn$ for this DNA due to this effect were less than 0.3 U.

For studies on superinfecting λ DNA, cells were lysed, and the fraction of covalently closed DNA was measured by centrifugation as described previously (11).

Preparation of ^{3}H-labeled λcI857S7. R694 (λ*cI857S7*) was grown for many generations in K medium at 33 C. While the cells were still in the exponential growth phase, deoxyadenosine and ^{3}H-labeled thymidine (specific activity, 60 to 80 mCi/mg) were added to final concentrations of 100 μg/ml and 20 μCi/ml, respectively, and incubation continued for 30 min at 33 C before the culture was transferred to 42 C to induce phage propagation. After 2.5 h at 42 C, cells were washed by centrifugation, concentrated 20-fold in λ suspension medium, and lysed by adding 3 drops of chloroform and vortexing for 15 s. Host DNA was degraded by treatment with pancreatic deoxyribonuclease (Calbiochem, 50 μg/ml) for 20 min at 37 C. Cell debris was removed by centrifugation at 12,000 × *g* for 10 min. The phage suspension was purified by CsCl density centrifugation and dialyzed against λ suspension buffer as described previously (11).

Superinfection of lysogens. Exponentially growing lysogenic cells were washed, concentrated 20-fold in 10^{-2} $MgSO_4$ supplemented with 50 μg of thymidine per ml, and superinfected with ^{3}H-labeled λcI857S7 at a multiplicity of 10. After 20 min at 33 C, cells were diluted 20-fold into K medium supplemented with 50 μg of thymidine per ml. After incubation for 45 min at 33 C with aeration, cells were washed twice in M9 buffer, resuspended at a concentration of about 10^8 cells/ml, irradiated, and plasmolyzed as described above.

RESULTS

Formation of UV-induced single-strand breaks in DNA of plasmolyzed cells. Experiments were first performed to investigate the effect of ATP on UV-induced break formation in the chromosomal DNA of plasmolyzed cells (Fig. 1 and 2). To avoid break rejoining, DNA

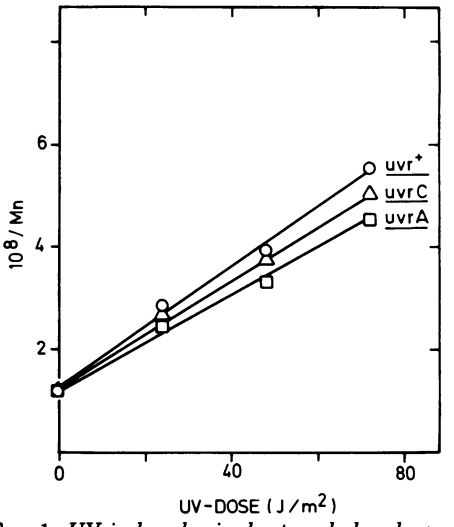

FIG. 1. *UV-induced single-strand breakage in chromosomal DNA of plasmolyzed cells. Cells of each strain, prelabeled with [^{3}H]thymidine, were UV-irradiated, plasmolyzed, and resuspended in reaction buffer containing 5 mM NMN. After incubation for 10 min at 42 C, cells were lysed on top of alkaline sucrose gradients (5 to 20%) and centrifuged for 90 min at 30,000 rpm at 20 C in an SW50.1 rotor. The reciprocal value of the number average molecular weight was calculated from the sedimentation data and plotted versus UV dose. Strains used were: MM383 uvr$^+$ polA12 (○), AB5025 uvrC polA12 (△), and AB5026 uvrA polA12 (□).*

polymerase I-deficient cells were used and the ligase inhibitor nicotinamide mononucleotide (NMN) was included in the reaction buffer. In the absence of exogeneous ATP, breaks are introduced to the same extent in cells having *uvr$^+$*, *uvrA*, and *uvrC* genotypes (Fig. 1). This breakage is probably caused by an endonuclease not involved in the normal excision repair pathway. A similar, small amount of UV-induced breakage has also been observed in intact *uvrA polA12* cells (unpublished data).

The addition of ATP to the reaction buffer causes a large increase in the number of UV-induced breaks in plasmolyzed *uvr$^+$* cells (Fig. 2). In contrast, ATP has only a minor effect on break formation in *uvrC*- and *uvrA*-mutated cells. Kinetic measurements show that in the presence of ATP most breaks in *uvr$^+$* cells are introduced during the first 5 min of incubation (Fig. 3). This corresponds to the kinetics of break formation observed in intact irradiated cells (Seeberg, manuscript in preparation). We infer that the *uvrA$^+$*-dependent break formation observed in plasmolyzed cells in the presence of ATP reflects the initial incision step of the excision repair process in vivo.

In the experiments described above, we failed

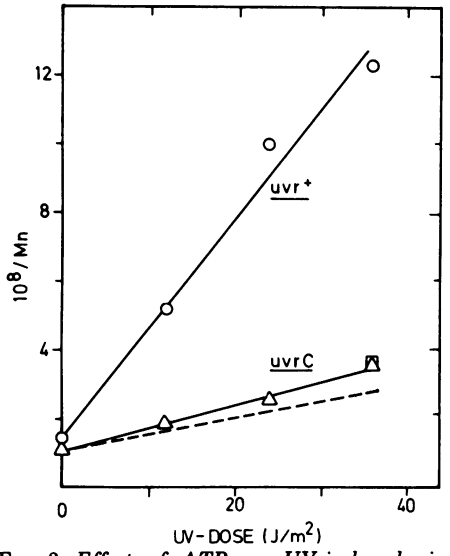

FIG. 2. *Effect of ATP on UV-induced single-strand breakage in chromosomal DNA of plasmolyzed cells. Experiments were done and results were obtained as described in the legend to Fig. 1, except that 1.5 mM ATP was included in the reaction buffer. Irradiated samples of uvr⁺ cells were centrifuged for 135 min and the others were centrifuged for 90 min at 30,000 rpm at 20 C in an SW50.1 rotor. Strains used were: MM383 uvr⁺ polA12 (○), AB5025 uvrC polA12 (△), and AB5026 uvrA polA12 (□). The dotted line shows the response without ATP in the reaction buffer (data shown for strain AB5025, taken from Fig. 1).*

to detect any activity of the *uvrAB* endonuclease in plasmolyzed *uvrC* cells (Fig. 1 and 2). This was also the case when *polA*⁺ cells were examined and when the incubation time was prolonged (Fig. 4). Irrespective of the presence of ATP, no significant difference was observed between sedimentation profiles of DNA from *uvrA* cells, which lack the *uvrAB* endonuclease, and *uvrC* cells, which contain this enzyme (3). In this respect plasmolyzed cells behave quite differently from intact cells. It has been clearly demonstrated that many more breaks appear in the chromosome of intact *uvrC* cells than *uvrA* cells during postirradiation incubation (7).

It has been suggested that one consequence of the *uvrC* gene mutation is that breaks produced by the *uvrAB* endonuclease in the mutant are susceptible to direct ligase closure (12). The possibility was investigated that the lack of incision breaks in plasmolyzed *uvrC* cells might reflect rapid, ligase-catalyzed reversal of the incision reaction, despite the presence of the ligase inhibitor NMN in the reaction buffer. However, NMN was found to be very effective in preventing ligase activity in plasmolyzed cells as judged from the inhibition of rapid strand rejoining after X-irradiation (data not shown). It appears unlikely, therefore, that ligase reversal can account for the observed behavior of *uvrC* mutant strains.

Waldstein et al. (14) recently reported that ATP stimulates the UV-dependent introduction of single-strand breaks in the DNA of toluene-treated cells. They suggested that ATP may be specifically needed for incisions to be made in the highly folded bacterial chromosome since ATP does not stimulate the action of the purified *uvrAB* endonuclease on irradiated, covalently closed φX-174 RF1 DNA. To determine whether ATP is needed only for the repair of folded chromosomes or is generally required for excision repair in *E. coli*, an investigation was made of UV-induced break formation in intracellular λ DNA (Fig. 5). The results appear analogous to those obtained for chromosomal DNA. Again, *uvrA*⁺-independent nicking is observed to the same extent in all strains tested, irrespective of ATP. *uvrA*⁺-dependent nicking is observed only in *uvr*⁺ strains and only in the presence of ATP. These data pre-

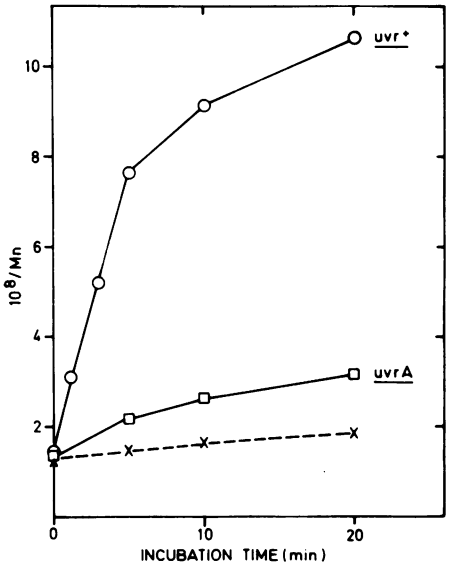

FIG. 3. *Kinetics of UV-induced DNA break formation in plasmolyzed cells. Cells of each strain, prelabeled with [³H]thymidine, were irradiated with UV (24 J/m²), plasmolyzed, and incubated at 42 C in reaction buffer containing 5 mM NMN and 1.5 mM ATP. At intervals, cell samples were lysed and the DNA was analyzed in alkaline sucrose gradients as described in the legend to Fig. 1. Strains used were: MM383 uvr⁺ polA12 (○) and AB5026 uvrA polA12 (□). The dotted line shows the response of unirradiated control cells (strain MM383).*

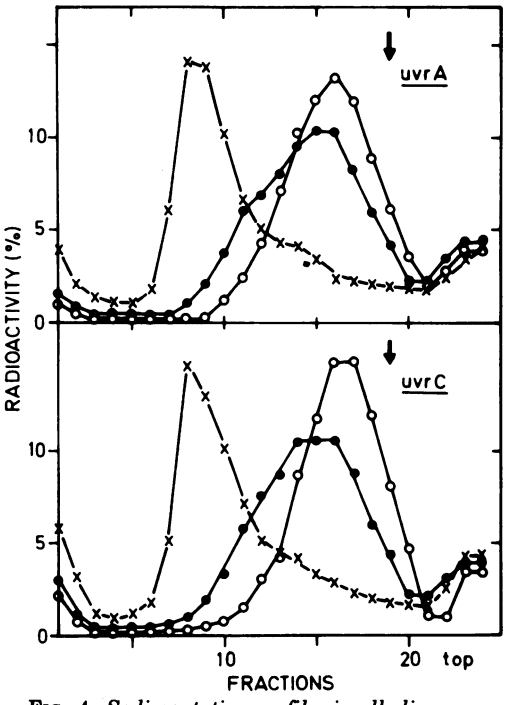

FIG. 4. *Sedimentation profiles in alkaline sucrose of DNA from plasmolyzed cells. Cells of each strain, prelabeled with [^{3}H]thymidine, were irradiated with UV (36 J/m^2), plasmolyzed, and incubated for 20 min at 37 C in reaction buffer containing 5 mM NMN. Cells were lysed on top of alkaline sucrose gradients (5 to 20%) and centrifuged for 90 min at 30,000 rpm at 20 C in an SW50.1 rotor. Fractions (0.2 ml) were collected from the bottom of the tubes by means of a peristaltic pump. Results are plotted as a percentage of radioactivity in each fraction versus total on the gradient. Strains used were AB1886 uvrA (top) and AB1884 uvrC (bottom). Cells were incubated either in the absence (●) or presence of 1.5 mM ATP (○). Unirradiated controls were incubated in the absence of ATP (×). The arrow indicates the position of a cosedimenting ^{32}P-labeled λ DNA marker (molecular weight of single strand, 1.55 × 10^7). Values for 10^8/Mn calculated for each profile were 1.3 (no UV), 2.7 (UV), and 4.0 (UV and ATP) for AB1884, and 1.3, 2.7, 3.7, respectively, for AB1886.*

clude the idea that ATP is involved only in the nicking of folded chromosomes and suggest rather that the ATP requirement is an inherent property of the nicking enzyme.

It is striking that for λ DNA, as for chromosomal DNA, the extent of UV-induced breakage in plasmolyzed cells does not differ in the *uvrC* and *uvrA* mutant. In contrast, *uvrA*$^+$-dependent nicking of irradiated λ circles in intact *uvrC* host cells after 10 min of incubation has been clearly observed (11).

Effect of dark-repair inhibitors. Both caffeine and acriflavine are potent inhibitors of excision repair (16). With this in mind, experiments were performed to determine whether these agents were inhibiting the ATP-dependent incision reaction or possibly another step of the repair process (Fig. 6). The DNA from irradiated, plasmolyzed cells incubated in the presence of ATP sedimented faster when either caffeine or acriflavine was included in the reaction buffer (Fig. 6A). It was calculated from these data that the ATP-dependent break formation was inhibited 50% by caffeine (10 mM, 1.9 mg/ml) and 90% by acriflavine (1 μg/ml).

When both DNA precursors (deoxynucleoside 5'-triphosphate) and the ligase cofactor (nicotinamide adenine dinucleotide) were present to

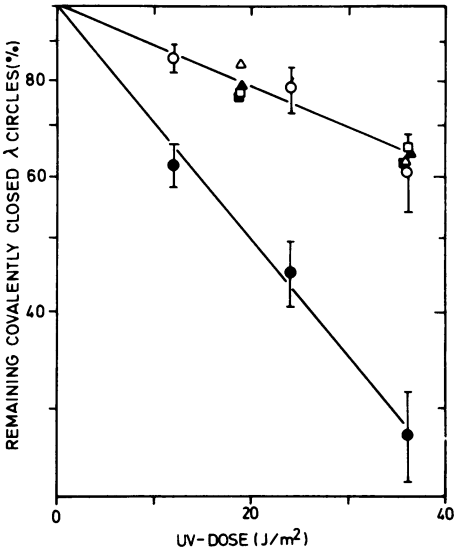

FIG. 5. *Effect of ATP on UV-induced breakage of plasmid DNA in plasmolyzed cells. Cells, lysogenic for λcI857, were superinfected with ^{3}H-labeled λcI857S7, UV-irradiated, plasmolyzed, and incubated for 10 min at 33 C before lysis. Covalently closed circular λ DNA was separated from nicked molecules by sedimentation in alkaline sucrose, and the fraction of closed circular DNA was calculated relative to unirradiated controls normalized to 100% for each individual experiment. The yield of closed circles in controls amounted to 40 to 60% of the total ^{3}H-labeled DNA. All points are the average of three independent experiments. Open symbols refer to DNA from cells incubated in reaction buffer containing 5 mM NMN, and closed symbols refer to cells incubated in reaction buffer containing 5 mM NMN and 1.5 mM ATP. Strains used were AB1157 (λcI857) uvr$^+$ (circles), AB1884 (λcI857) uvrC (triangles), and AB1886 (λcI857) uvrA (rectangles). Bars indicate standard errors and are shown only for AB1157, but were similar for the mutant strains.*

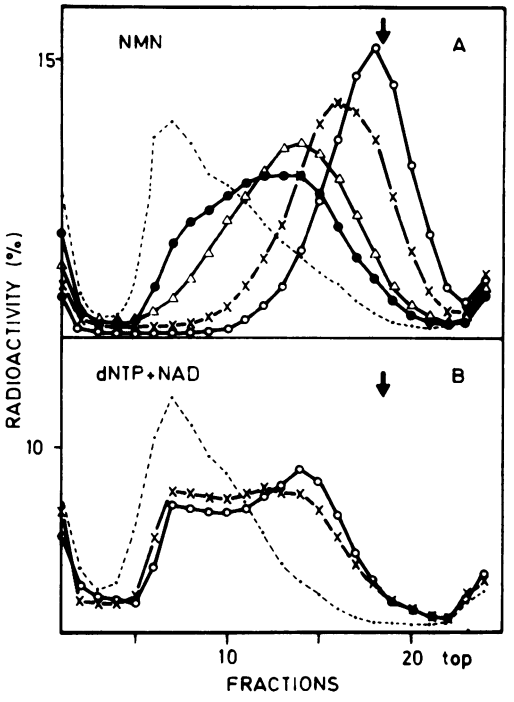

FIG. 6. *Effect of caffeine and acriflavine on formation and rejoining of UV-induced single-strand breaks in plasmolyzed cells. Wild-type cells (strain AB1157), prelabeled with [^{3}H]thymidine, were irradiated with UV (24 J/m^2), plasmolyzed, and incubated for 10 min at 37 C. Cells were lysed and sedimented through alkaline sucrose and fractions were collected as described in the legend to Fig. 4. The reaction buffer contained 1.5 mM ATP and either (A) NMN (5 mM) or (B) deoxynucleoside 5'-triphosphates (TTP, dATP, dCTP, and dGTP, 40 μM each) and nicotinamide adenine dinucleotide (1.3 mM) in addition to other compounds as follows: 10 mM caffeine (×, [A] 4.8, [B] 1.7); 1 μg of acriflavine per ml (△, 2.5); no addition (○, [A] 7.6, [B] 1.9); no ATP (●, 2.0). Numbers in parentheses are the values for 10^8/Mn calculated for each profile. The sedimentation profiles of unirradiated cells are shown by the dotted lines (A, 1.4; B, 1.2). The arrow indicates the position of a cosedimenting ^{32}P-labeled λ DNA marker (molecular weight of single strand, 1.55 × 10^7).*

allow repair completion, the sedimentation profiles of DNA from irradiated, plasmolyzed wild-type cells were not much different from that of unirradiated cells (Fig. 6B). Under these conditions, addition of caffeine (10 mM) did not change the sedimentation pattern of DNA from irradiated cells (Fig. 6B). This DNA sedimented considerably faster than DNA from cells incubated under conditions in which rejoining was inhibited (Fig. 6A). Presumably, once the incision break is made, repair is rapidly completed and the break is rejoined irrespective of the presence of caffeine. Thus, it seems reasonable to conclude that 10 mM caffeine has little effect on the steps involved in rapid repair of incision breaks.

To investigate whether the inhibition of the incision reaction caused by caffeine was competitive with respect to ATP, experiments were carried out with different concentrations of ATP and caffeine (Fig. 7). The results demonstrate that caffeine at 10 mM inhibits the incision reaction by 50% irrespective of ATP concentration, whereas inhibition appears complete when the caffeine concentration is increased to 25 mM. This excludes the possibility that caffeine and ATP compete for the same site on the enzyme and suggests, rather, that caffeine interferes with the endonucleolytic reaction at the DNA level. The concentration of caffeine needed for complete inhibition corresponds well with the concentration needed for complete inactivation of excision repair in intact cells, judged from survival determinations (16).

DISCUSSION

Cells of *E. coli* made permeable by treatment with high concentrations of sucrose (plasmoly-

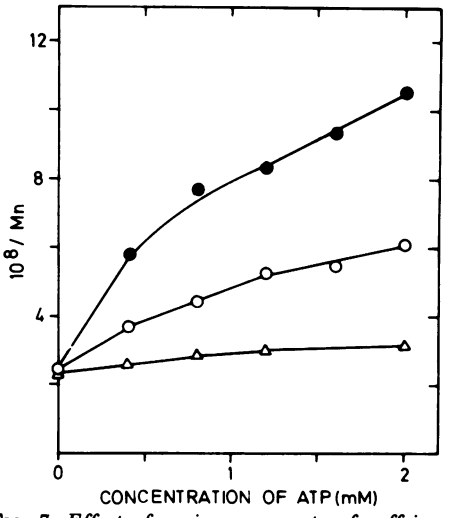

FIG. 7. *Effect of various amounts of caffeine on UV-induced break formation as a function of increasing ATP concentration. Cells of strain MM383 uvr$^+$ polA12, prelabeled with [^{3}H]thymidine, were irradiated with UV (24 J/m^2), plasmolyzed, and incubated for 10 min at 42 C in reaction buffer containing no (●), 10 mM (○), or 25 mM (△) caffeine in addition to various concentrations of ATP as indicated. The cells were lysed and the DNA was sedimented in alkaline sucrose as described in the legend to Fig. 1. Results are plotted as the reciprocal of the number average molecular weight as a function of ATP concentration.*

sis) have proved useful for the study of excision repair of UV-damaged DNA. The results presented above demonstrate that the incision step of excision repair requires ATP both for the highly folded bacterial chromosome and for covalently closed bacteriophage λ DNA. The number of chromosomal DNA strand breaks per dose unit observed in plasmolyzed cells as compared with intact and toluene-treated cells is given in Table 2. We observe one incision break for every three dimers in the DNA of intact cells and one for every five dimers in the DNA of plasmolyzed cells. These figures are derived from the data of Boyle and Setlow (2), and in intact cells the degree of incision corresponds well with the number of dimers excised (2). The lower efficiency of incision in permeable cells has been noted by other workers (4, 14) and may be due to reduced accessibility of the dimers to the repair enzymes after permeabilization. Plasmolyzed cells differ from toluene-treated cells in that virtually no *uvrA*$^+$-dependent breakage is detected in the absence of exogeneous ATP (Fig. 1). In toluene-treated cells this breakage is significant (14). This may be due to different degrees of permeability caused by the toluene and sucrose treatment (15), resulting in significant levels of residual ATP in toluene-treated cells (14). Plasmolysis treatment is particularly useful in the study of *endI*$^+$ strains since it does not seem to induce significant levels of endonuclease I activity. Even after 20 min of incubation the amount of unspecific breakage in plasmolyzed cells is negligible (Fig. 3 and 4). In our experiments this is not the case for toluene- or ether-treated cells. We infer that plasmolyzed cells provide an accurate picture of the events of excision repair in vivo.

However, plasmolyzed cells differ from intact cells in one important respect. The *uvrC* gene mutation apparently imposes a complete block on the UV-specific incision reaction in plasmolyzed cells, both for chromosomal and episomal DNA. This is not the case in whole cells, since single-strand breaks are readily detected in the *uvrC* mutant (7, 11) and are presumably caused by the *uvrAB* endonuclease that is known to be present in the mutant strain (3, 7). These findings have led to the model for excision repair outlined in Fig. 8. It is proposed that in *uvr*$^+$ cells the *uvrAB* enzyme exists in a complex with the *uvrC* gene product. This complex acts specifically at the site of UV damage and requires ATP to make single-strand breaks. The function of the *uvrC* gene product in the complex may be to facilitate the excision of dimers (7; Seeberg, manuscript in preparation). Rapid excision, repair synthesis, and rejoining may then be accomplished by another repair complex such as that containing DNA polymerase I/exonuclease V (5), acting in concert with ligase. It is proposed that in *uvrC* strains the *uvrAB* enzyme exists unbound and is responsible for the break formation observed in intact cells. The breaks produced are susceptible to direct ligase closure (Braun and Grossman, Fed. Proc. 33:1599, 1974). One possible explanation for the absence of *uvrA*$^+$-dependent break formation in plasmolyzed *uvrC* cells is that *uvrAB* endonuclease in the unbound state can pass through the permeable cell membrane, becoming so diluted that significant activity can no longer be detected. The *uvrAB* endonuclease has been characterized as a small enzyme with a molecular weight of less than 15,000 atomic mass units (3). Plasmolysis treatment is known to allow pancreatic deoxyribonuclease (molecular weight, 31,000 atomic mass units) to pass across the cell membrane, and causes as much as 10% of the intracellular proteins to leak out of the cells (15). A larger molecule, such as the postulated complex containing the *uvrAB* endonuclease in *uvr*$^+$ cells, would be retained within the cells after plasmolysis. Alternatively, it may simply be that plasmolysis treatment inactivates the unbound enzyme. Clarification of this point awaits further enzymological studies.

There is no obvious need for the energy bound in the ATP molecule for the incision reaction, since nucleolytic reactions in general are endothermic. Rather, ATP may serve to activate or maintain the association of the complex. In an attempt to test the latter possibility, we subjected irradiated, plasmolyzed *uvr*$^+$ cells to incubation in the absence of ATP to allow any unbound *uvrAB* endonuclease to escape

TABLE 2. *Number of UV-induced chromosomal DNA strand breaks per dose unit in plasmolyzed cells compared with reported values for intact and toluene-treated cells*

Cell system	% Thymine in pyrimidine dimers per 10 J/m^2 of UV irradiation	Breaks per 10 J/mm^2 per 10^8 atomic mass units of DNA
Intact cells[a]	0.041	5.3
Toluenized cells[b]	0.036	1.4
Plasmolyzed cells[c]	0.041	3.1

[a] Measured in *polA12*-mutated cells after 10 min of incubation at 42 C (Seeberg, manuscript in preparation).

[b] Measured in *polA*$^+$ cells after 30 min of incubation at 37 C in the presence of 5 mM NMN and optimal concentrations of ATP (14).

[c] Data taken from Fig. 2.

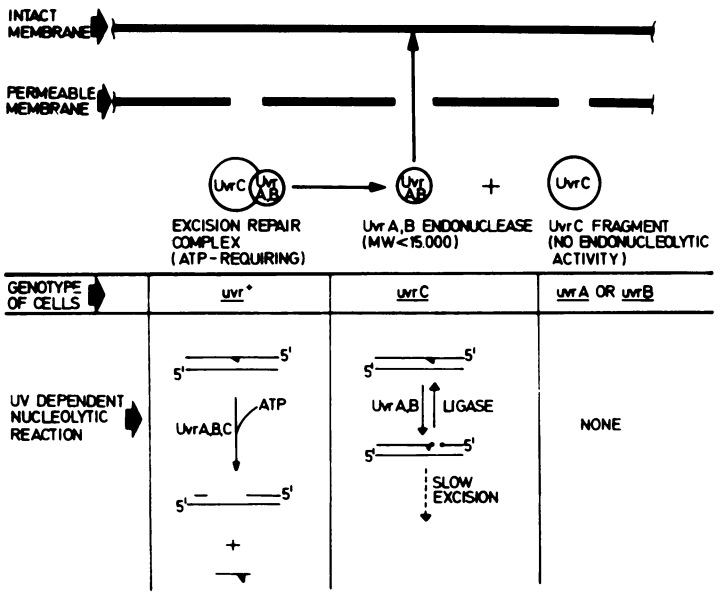

FIG. 8. *Model for excision repair.*

from the cells. However, when ATP was supplied after 5 min of incubation, the incision reaction was activated to the extent observed previously. The idea that ATP is needed to keep the complex together is therefore not suported.

Different branches of excision repair in *E. coli* have been described that require different gene functions (17). However, all of them seem to depend upon the *uvrA*, *uvrB*, and *uvrC* genes, indicating that all excision repair of UV damage in *E. coli* is channeled through the ATP-requiring reaction. The essential role of this reaction is emphasized further by the demonstration that the dark-repair inhibitors acriflavine and caffeine both act at this particular step in the repair process. We have recently detected an endonucleolytic activity in cell extracts that is UV specific and ATP dependent. Purification of this activity should enable us to answer the question as to the existence of a complex coded for by the *uvrA*, *uvrB*, and *uvrC* genes.

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