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Biochemistry

UmuD mutagenesis protein of *Escherichia coli*: Overproduction, purification, and cleavage by RecA

(SOS response/LexA cleavage/fidelity of DNA replication/protein self-cleavage)

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ABSTRACT **The mutation rate of *Escherichia coli* increases approximately 100-fold after treatment with replication-inhibiting agents such as UV light. This enhanced mutation rate requires the action of the UmuD and UmuC proteins, which are induced as part of the SOS response to DNA damage. To initiate a biochemical characterization of the role of these proteins, we have developed a plasmid system that gives efficient expression of the *umuD* and *umuC* genes. The *umuD* and *umuC* genes were placed under the control of a regulated phage λ P_L promoter and a synthetic ribosome-binding site, and the distance to the UmuD start was adjusted to maximize gene expression. Starting from this overproduction system, we have purified the UmuD protein and studied its interaction with RecA. The SOS response is turned on by the capacity of RecA protein to mediate cleavage of the LexA repressor for SOS-controlled operons. Others have shown that UmuD exhibits sequence homology to LexA around the cleavage site, suggesting a possible cleavage reaction for UmuD. We show that RecA mediates cleavage of UmuD, probably at this site. As with LexA, UmuD also undergoes a self-cleavage reaction. We infer that RecA-mediated cleavage of UmuD is another role for RecA in SOS mutagenesis, probably activating UmuD for its mutagenic function.**

The introduction of a replication-inhibiting lesion into the DNA of *Escherichia coli* results in a marked increase in mutation rate (1–3). This mutagenesis is one consequence of an induced, multigene response to DNA damage termed the SOS pathway (2–5). Most SOS-induced mutations are targeted to the sites of the DNA lesions [e.g., the pyrimidine-pyrimidone (6–4) photoproduct or cyclobutane pyrimidine dimer for UV mutagenesis] (6–9). Targeted mutagenesis requires the RecA, UmuD, and UmuC proteins (3–5, 10–15). The mutagenic events have been inferred to result from a functional (and possibly a physical) interaction between DNA polymerase III, RecA, UmuD, and UmuC that allows replication across the site of the DNA lesion (16–18). The direct requirement for RecA in mutagenesis is an additional activity to its regulatory role, cleavage of the LexA repressor protein, which induces SOS-controlled operons (4, 5).

The biochemical analysis of SOS mutagenesis has been limited by the lack of the purified UmuD and UmuC proteins. The *umuDC* operon has been cloned into a plasmid vector, and the DNA sequence has been determined (19, 20); however, the cloned operon exhibits limited gene expression (19, 20). One interesting property of UmuD, noted from the DNA sequence, is homology with LexA in the region surrounding the site of RecA-mediated cleavage (19). This observation indicated a possible role in SOS mutagenesis for RecA-mediated cleavage of UmuD (19).

To initiate a biochemical characterization of the role of UmuD and UmuC, we have developed a plasmid system that gives efficient expression of the *umuD* and *umuC* genes. Starting from this overproduction system, we have purified the UmuD protein. We have found that RecA mediates cleavage of UmuD. The accompanying papers present complementary experiments demonstrating that UmuD is cleaved *in vivo* (21) and that the cleavage product is critical for mutagenesis (22). Thus, cleavage of UmuD appears to be another role for RecA in SOS mutagenesis, activating UmuD for its mutagenic function.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The *E. coli* strains used were MC1000 (23), JM101 (24), TK603 (25), TK610 (*umuC36*) (25), and TK612 (*umuD44*) (25). The plasmids used were pRK248cIts2 [encoding the temperature-sensitive (ts) cI repressor for the phage λ P_L promoter] (26), pNS3 (27), pRC23 (contains phage λ P_L) (28), pSE117 (29), and phage M13mp9 vector (24).

Materials. M9 minimal medium and LB medium were the standard recipes (30). M9 was supplemented with 0.2% glucose, 50 μM thiamine, and 2% (wt/vol) Casamino acids. For ^{35}S labeling, an M9 medium with limited sulfur was used with 2 mM $MgCl_2$, 30 μM $MgSO_4$, 5 μM thiamine, 0.4% glucose, and 18 amino acids at 20 μM each (all except methionine and cysteine). Tetracycline (Sigma) at 25 μg/ml, ampicillin (Sigma) at 50 μg/ml, and 5-bromo-4-chloro-3-indolyl β-D-galactoside (Bethesda Research Laboratories) at 40 μg/ml were added as needed. The 18-mer oligonucleotide was synthesized by Bruce Malcolm (Department of Biochemistry, University of California, Berkeley). $Na_2{}^{35}SO_4$ was from ICN; [γ-^{32}P]ATP, from Amersham; Polymin P, from BASF Wyandotte (Parsippany, NJ); DEAE-Sephacel, from Pharmacia; AcA54, from LKB; hydroxylapatite (Bio-Gel HTP), from Bio-Rad; methyl methanesulfonate, from Aldrich; and dNTPs, from Pharmacia.

Enzymes. All restriction enzymes and phage T4 DNA ligase were purchased from New England Biolabs; *E. coli* polymerase I Klenow fragment, from Pharmacia; and phage T4 polynucleotide kinase and nuclease S1, from Boehringer Mannheim.

Plasmid DNA Preparation. Large-scale preparation of plasmid DNA was performed as described by Clewell and Helinsky (31). Small amounts of plasmid DNA were isolated by the method of Ish-Horowicz and Burke (32).

Overproduction of the UmuD,C Proteins. A *Hpa* I-fragment from pSE117 containing the entire *umuDC* operon was cloned into the single *Sma* I site of M13mp9. A new *Eco*RI site was created 11 base pairs (bp) upstream from the start codon of *umuD* by oligonucleotide-directed mutagenesis (33). An 18-mer oligonucleotide with 89% homology to

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Abbreviation: ATP[S], adenosine 5'-[γ-thio]triphosphate.

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the DNA sequence upstream of *umuD* was end-labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase. This oligonucleotide was hybridized to single-stranded M13mp9 DNA carrying the *umuDC* operon and extended by the addition of deoxyribonucleotide triphosphates and *E. coli* DNA polymerase I Klenow fragment. The newly synthesized DNA was circularized with T4 DNA ligase. Completely synthesized strands were purified on an alkaline sucrose gradient and used to transform MC1000. Clones with the newly constructed *Eco*RI site were selected, as judged by colony hybridization with the ^{32}P-labeled oligonucleotide and by cleavage patterns with restriction enzymes. A 2-kilobase (kb) *Eco*RI fragment, from the newly constructed site to the *Eco*RI site in the vector, was cloned into the single *Eco*RI site of pRC23. The new plasmid, pNS403, contained the *umuD,C* genes under the control of phage λ P_L promoter. This plasmid allowed the strains to make sufficient UmuD to be seen in a pulse-labeling experiment.

To increase further the expression of the *umuD,C* genes, the distance between the ribosome binding site and the *umuD* start codon was shortened. To assay for overproduction, a *lacZ* fusion gene was constructed. A purified 613-bp *Eco*RI-*Bgl* II fragment from pNS403, containing the entire *umuD* gene and the first 59 amino acids of the *umuC* gene, was inserted between the *Eco*RI and *Bam*HI sites of pNS3 to generate a *umuC-lacZ* fusion (27). The desired transformants in strain JM101 had the expected restriction fragment patterns and showed a low β-galactosidase production at 37°C on M9 plates supplemented with ampicillin, tetracycline, and 5-bromo-4-chloro-3-indolyl β-D-galactoside. The DNA of the plasmid containing the phage λ P_L promoter, *umuD*, and *umuC-lacZ* was purified, cut with *Eco*RI, treated with nuclease S1, religated with T4 DNA ligase, and transformed into strain JM101 (pRK248cIts). The highest overproducer was selected by the criterion of high β-galactosidase production at 37°C. The *umuC* gene was then reconstructed. For this purpose, a *Pst* I–*Cla* I fragment from the overproducing plasmid, containing the modified phage λ P_L–*umuD* interval, was cloned into pNS403, replacing the original *Pst* I–*Cla* I fragment. The new plasmid, pSB13, was transformed into strain MC1000 (pRK248cIts) to generate the strain used to overproduce UmuD and UmuC. As a qualitative test for functional UmuD and UmuC, the plasmid was used to complement the *umuC36* and *umuD44* strains, TK610 and TK612. The plasmid-carried *umuD* and *umuC* genes restored reversion from His$^-$ to His$^+$ induced by methyl methanesulfonate in the *umuC36* and *umuD44* strains.

Labeling Experiments. To measure protein production by ^{35}S labeling, bacteria were grown overnight in L broth (supplemented with ampicillin and tetracycline) and diluted 1:100 into M9 low-sulfur medium. The bacteria were grown to a density of $\approx 2 \times 10^8$ cells per ml at 30°C. $Na_2{}^{35}SO_4$ was added to a final concentration of 100 μCi/ml (1 Ci = 37 GBq). Part of each culture was left at 30°C (uninduced), and the remainder was induced by derepression at 42°C (inactivating the temperature-sensitive cI repressor for phage λ P_L). After 50 min, the reaction was stopped by rapidly freezing the bacteria, and proteins were analyzed by NaDodSO$_4$/PAGE followed by autoradiography.

UmuD Purification. MC1000 (pRK248cIts, pSB13) was grown at 28°C to $\approx 2 \times 10^8$ cells per ml in LB medium supplemented with 0.2% glucose, ampicillin, and tetracycline. The temperature was increased to 42°C for 1 hr to derepress the P_L promoter, and the bacteria were harvested by centrifugation. The wet paste was suspended and lysed as described (34). The subsequent steps were carried out at 4°C. The suspension was centrifuged at 105,000 $\times$ *g* for 2 hr. Polymin P [10% (vol/vol), pH 7.9] was added to a final concentration of 1.2% and stirred for 1 hr. The suspension was centrifuged at 105,000 $\times$ *g* for 30 min. The pellet was resuspended in buffer R (10% glycerol/20 mM Tris, pH 7.5/0.1 mM EDTA/1 mM dithiothreitol) containing 100 mM ammonium sulfate, stirred for 30 min, and centrifuged at 105,000 $\times$ *g* for 30 min. Ammonium sulfate was added to the supernatant to a final concentration of 85% (wt/vol), and the mixture was stirred for 1 hr and centrifuged at 105,000 $\times$ *g* for 1 hr. The pellet was resuspended in buffer R containing 60 mM NaCl and dialyzed against this buffer. A precipitate formed during the dialysis, which was separated from the dissolved fraction by centrifugation at 105,000 $\times$ *g* for 30 min. The pellet was dissolved in buffer R containing 100 mM ammonium sulfate, and the mixture was stirred for 1 hr. The undissolved fraction was separated by centrifugation at 18,000 $\times$ *g* for 30 min. The supernatant was applied to an AcA54 sizing column. The proteins were eluted with buffer R containing 100 mM ammonium sulfate. Fractions with the highest purity of UmuD, as judged by NaDodSO$_4$/PAGE, were pooled and dialyzed against buffer R containing 60 mM NaCl. This solution was applied to a DEAE-Sephacel column. The column was washed with 1 column volume of buffer R containing 60 mM NaCl and developed with 8 column volumes of a linear gradient of 60–200 mM NaCl in buffer R. The fractions eluted at about 160 mM NaCl contained UmuD as judged by NaDodSO$_4$/PAGE; these fractions were pooled and dialyzed against buffer N (10% glycerol/10 mM Na_2HPO_4/NaH_2PO_4, pH 6.8/1 mM dithiothreitol). The dialyzed solution was applied to a hydroxylapatite column. This column was washed with 1 column volume of buffer N and developed with 8 column volumes of a linear gradient of 0–290 mM Na_2HPO_4/NaH_2PO_4 in buffer N. UmuD appeared in the flow-through fractions.

Cleavage Assay for UmuD. The RecA$^+$, RecA430, RecA-441, and LexA proteins were purified as described (18, 34, 35). The assay for RecA-mediated cleavage was carried out essentially as described (18, 35). Standard reaction mixtures (20 μl) contained 40 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 30 mM NaCl, 2 mM dithiothreitol, 40 ng of ϕX174 single-stranded DNA, 1 mM adenosine 5′-[γ-thio]triphosphate (ATP[S]), and UmuD, LexA, and RecA as noted. Incubation was at 37°C for the times noted. The products of the reaction were separated by NaDodSO$_4$/PAGE (in 19% polyacrylamide), and the proteins were visualized by staining with Coomassie blue. The self-cleavage assay was done at pH 10 essentially as described (35). Reaction mixtures (100 μl) contained 20 mM glycine (adjusted with NaOH to pH 10.0), 30 mM NaCl, 10 mM $CaCl_2$, 10 mM dithiothreitol, 0.05% bovine serum albumin, and 10 μg of LexA or UmuD. Incubation was at 37°C; at the times noted, 20-μl aliquots were removed and analyzed for cleavage products as for the RecA reaction.

RESULTS

Overproduction and Purification of UmuD. The UmuD and UmuC proteins are produced from a polycistronic RNA in which the termination codon for *umuD* overlaps the initiation codon of *umuC* (19, 20). Lacking an obvious enzyme assay for UmuD and UmuC, we sought to achieve large overproduction of these proteins to allow ready detection by a gel electrophoresis assay. We initially placed the *umuD,C* region downstream from the highly efficient phage λ P_L promoter. However, UmuD production was not clearly discernable; therefore, the rate of synthesis was considerably less than that possible from maximal translation of a P_L transcript. Thus, the expression of the *umuD,C* region is likely to be translationally limited (UmuC production may depend on translation of *umuD* because of the overlapping terminator and initiator codons).

To achieve better translational expression of *umuD*, we attempted to optimize the spacing between the *umuD* coding region and the ribosome-binding site on the phage λ P_L-

containing plasmid, pRC23 (28). For this purpose, we introduced an *Eco*RI site 11 bp upstream from the start codon for UmuD and inserted this fragment into pRC23, yielding a 15-bp spacing between the ribosome-binding site and the initiator AUG codon. The expression of *umuD* was improved to about 0.2% of cellular protein, still far less than the 10% range possible for phage λ P_L (e.g., see refs. 27 and 28). We decided to shorten further the distance between the ribosome-binding site and the AUG. We first fused *umuC* with *lacZ* to assay gene expression. We then recleaved the DNA at the *Eco*RI site, treated with nuclease S1, rejoined, and screened the resultant plasmids for β-galactosidase production. The construction producing the most β-galactosidase (which also overproduced UmuD) was then used to regenerate the *umuDC* operon under the control of phage λ P_L (see *Materials and Methods*). After derepression of the P_L promoter, the resultant plasmid, pSB13, produced about 10% of the total ^{35}S-labeled protein as UmuD, as judged by $NaDodSO_4$/PAGE (Fig. 1, lane I). There was also production of a protein with the molecular mass expected for UmuC (Fig. 1, lane I). The proteins presumed to be UmuD and UmuC were not produced after induction of a strain carrying the vector plasmid pRC23 lacking the *umuD* and *umuC* genes (Fig. 1, lane I_v).

The purification of UmuD was followed by $NaDodSO_4$/PAGE (Fig. 2). The induced lysate (Fig. 2, lane I) was fractionated by precipitation with Polymin P (Fig. 2, lane P), an additional precipitation step, an AcA54 sizing column (Fig. 2, lane A), and chromatography on DEAE-Sephacel (Fig. 2, lane D) and hydroxylapatite (Fig. 2, lane H) columns. The last three lanes are overloaded with UmuD to show bands due to contaminating proteins. We estimate that the final fraction is ≈95% UmuD.

RecA-Mediated Cleavage of UmuD. As noted in the Introduction, an analysis of protein homology indicated that UmuD might be a cleavage target for RecA (19). To examine this possibility, we looked for proteolysis of UmuD in a standard cleavage assay with RecA. We incubated UmuD with RecA, single-stranded DNA as polynucleotide cofactor, and ATP[S] or dATP as mononucleotide cofactor. The products of the reaction were separated by $NaDodSO_4$/PAGE, and proteins were visualized by staining with Coomassie blue (Fig. 3). The migration positions are marked for RecA (38 kDa), UmuD (15 kDa), and a cleavage product of UmuD that we designate UmuD′ (≈12 kDa). UmuD was

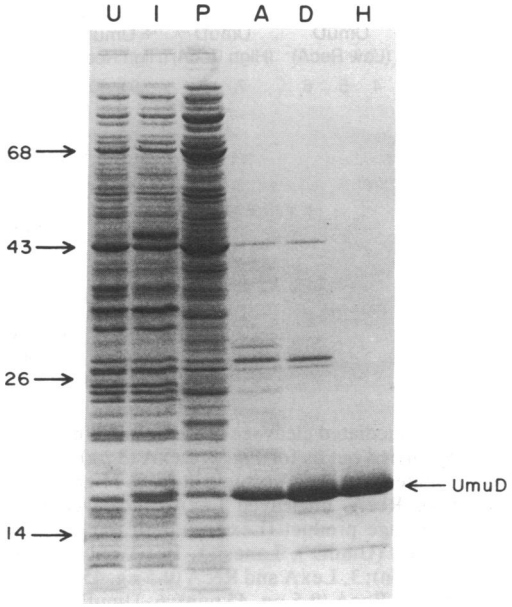

FIG. 2. Purification of UmuD. Proteins were fractionated by $NaDodSO_4$/PAGE and visualized by Coomassie blue. Lanes: U and I, total cellular protein from lysed uninduced or induced cells, respectively; P, pellet after adding Polymin P to the cell lysate supernatant; A, fraction after separation on AcA54 column; D, fraction obtained by chromatography of A on DEAE-Sephacel; H, fraction after development of hydroxylapatite column. The position of UmuD is marked on the right; the positions and apparent molecular masses (in kDa) of marker proteins are given on the left.

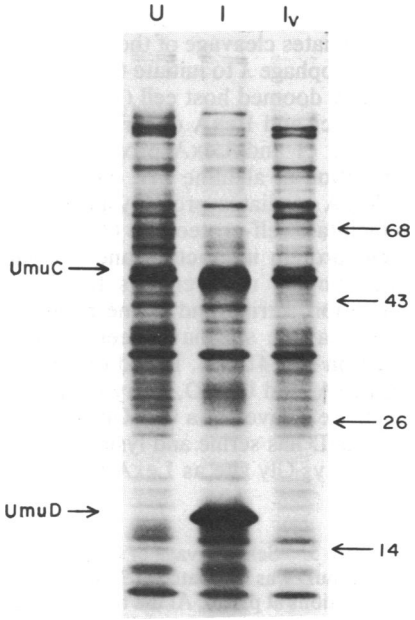

FIG. 1. Induced synthesis of UmuD and UmuC. Proteins were separated on a $NaDodSO_4$/9–19% polyacrylamide gel and visualized by autoradiography. Arrows indicate ^{35}S-labeled proteins with molecular masses (in kDa) similar to those expected for UmuD and UmuC. Lanes: U, MC1000 (pRK248cIts, pSB13) uninduced; I, MC1000 (pRK248cIts, pSB13) induced for 50 min at 42°C; I_v, MC1000 (pRK248cIts, pRC23) induced for 50 min at 42°C.

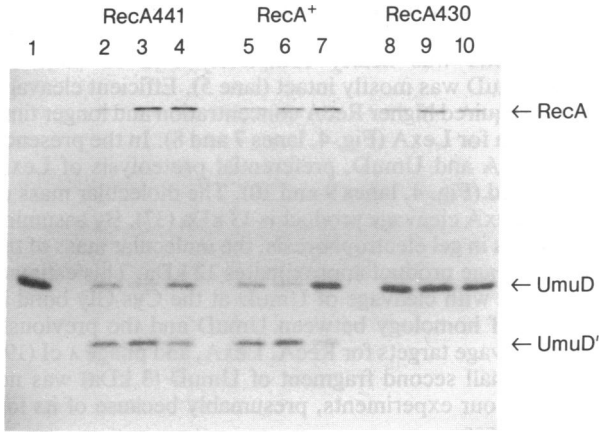

FIG. 3. RecA-mediated cleavage of UmuD. UmuD (2 μg) was incubated with wild-type or mutant RecA at 37°C for 90 min in the presence of single-stranded DNA (40 ng) and ATP[S] (1 mM) or dATP (2 mM). To avoid depletion of the dATP by the dATPase activity of RecA, an additional 2 mM dATP was added after 45 min of incubation. The products of the reaction were separated by $NaDodSO_4$/19% PAGE, and the proteins were visualized by staining with Coomassie blue. Migration positions are indicated for RecA, UmuD, and the largest cleavage fragment of UmuD (UmuD′). Lanes: 1, no RecA; 2, RecA441 (0.5 μg); 3, RecA441 (1 μg); 4, RecA441 (1 μg + dATP); 5, $RecA^+$ (0.5 μg); 6, $RecA^+$ (1 μg); 7, $RecA^+$ (1 μg + dATP); 8, RecA430 (0.5 μg); 9, RecA430 (1 μg); 10, RecA430 (1 μg + dATP).

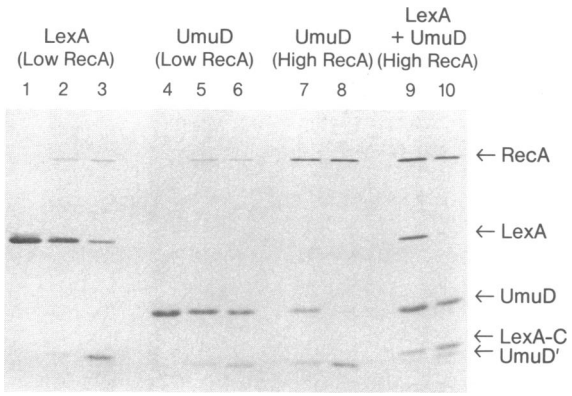

FIG. 4. RecA-mediated cleavage of LexA and UmuD. Cleavage reactions were carried out as for Fig. 3. LexA (3 μg) and/or UmuD (2 μg) was incubated with RecA protein at 37°C. Migration positions are indicated for RecA, uncleaved LexA, uncleaved UmuD, the larger LexA cleavage product (LexA-C), and the larger cleavage fragment of UmuD (UmuD'). Lanes: 1, LexA only; 2, LexA and RecA (0.5 μg, 20 min); 3, LexA and RecA (0.5 μg, 45 min); 4, UmuD only; 5, UmuD and RecA (0.5 μg, 45 min); 6, UmuD and RecA (0.5 μg, 90 min); 7, UmuD and RecA (2 μg, 45 min); 8, UmuD and RecA (2 μg, 90 min); 9, LexA, UmuD, and RecA (2 μg, 45 min); 10, LexA, UmuD, and RecA (2 μg, 90 min).

effectively cleaved by the RecA441 protein, a mutant RecA that is extremely efficient in cleaving the LexA and cI proteins (18, 36) (Fig. 3, lanes 2–4). Either ATP[S] (lanes 2 and 3) or dATP (lane 4) served as mononucleotide cofactor. UmuD was also efficiently cleaved by wild-type RecA, although dATP did not work well as a cofactor (Fig. 3, lanes 5–7). UmuD was not cleaved effectively by the RecA430 protein, a mutant RecA that is defective in mutagenesis (13, 15) (Fig. 3, lanes 8–10). This observation indicates that the RecA-mediated cleavage of UmuD is likely to be important for SOS mutagenesis.

To study relative cleavage efficiencies of LexA and UmuD, we compared LexA and UmuD in the same experiment. LexA was cleaved much more efficiently than UmuD (Fig. 4). The time course of the LexA and UmuD cleavage reactions at low RecA concentration is shown (Fig. 4, lanes 1–6). After 45 min, LexA was nearly completely cleaved (lane 3), whereas UmuD was mostly intact (lane 5). Efficient cleavage of UmuD required higher RecA concentration and longer time (90 min) than for LexA (Fig. 4, lanes 7 and 8). In the presence of both LexA and UmuD, preferential proteolysis of LexA was observed (Fig. 4, lanes 9 and 10). The molecular mass of the larger LexA cleavage product is 13 kDa (37). By assuming no anomalies in gel electrophoresis, the molecular mass of the UmuD cleavage product approximates 12 kDa. This estimate is consistent with cleavage of UmuD at the Cys-Gly bond in the region of homology between UmuD and the previously defined cleavage targets for RecA, LexA, and phage λ cI (19). The very small second fragment of UmuD (3 kDa) was not observed in our experiments, presumably because of its low molecular mass.

From the data of Fig. 3 and Fig. 4, we conclude that UmuD is subject to RecA-mediated cleavage. There is a substantial difference in the efficiency of proteolysis between the mutagenesis protein UmuD and the repressor protein LexA which might have significance for the biology of the SOS response.

Self-Cleavage of UmuD. The LexA and cI proteins undergo a self-cleavage reaction at alkaline pH in addition to the RecA-mediated proteolysis (35). We have found that UmuD also executes a self-cleavage reaction (Fig. 5). The reaction for UmuD was much less efficient than that of LexA; LexA was nearly completely cleaved by 2 hr (lane 4), whereas substantial degradation of UmuD required 18 hr (lane 8). However, self-cleavage by UmuD was similar to that found for cI under similar conditions (35). Autoproteolysis of UmuD was more efficient at pH 10 than at pH 9 and was not observable at pH 8 (data not shown); these observations indicate that self-cleavage of UmuD proceeds by a mechanism similar to that of LexA and cI (35).

DISCUSSION

Protein Cleavage Reactions Mediated by RecA. We have shown that purified UmuD protein undergoes proteolytic cleavage in a reaction with pure RecA, single-stranded DNA as polynucleotide cofactor, and ATP[S] or dATP as mononucleotide cofactor. In the accompanying papers, Shinagawa *et al.* (21) demonstrate that UmuD is cleaved *in vivo* in a reaction requiring RecA, and Nohmi *et al.* (22) show that the cleavage product is the active agent in mutagenesis.

RecA also mediates cleavage of the LexA protein, a repressor for the genes of the SOS response, including *umuD* and *umuC* (4, 5). The LexA proteolysis provides for induction of the SOS response *in vivo* (4, 5). RecA is presumably activated for the cleavage reaction by a polynucleotide cofactor produced by the inducing DNA damage—either single-stranded DNA (38, 39) or damaged double-stranded DNA (17, 18). The mutant RecA generated by the *recA441* mutations can turn on the SOS response without DNA damage (4, 5), possibly because undamaged double-stranded DNA will serve as a polynucleotide cofactor (18). In an additional proteolytic reaction, RecA mediates cleavage of the phage λ cI repressor protein, allowing prophage λ to initiate the lytic response and escape a potentially doomed host cell (38, 39).

The cleavage of cI and LexA occurs at a single Ala-Gly bond (37, 40). The cI and LexA proteins also undergo a self-cleavage reaction at alkaline pH, which has led to the proposal that RecA mediates proteolysis by serving as an "effector" to facilitate self-proteolysis (35, 41, 42). Thus, an effective protein–protein interaction and a labile bond appear to be the minimal requirements for RecA-mediated cleavage. In addition, serine and lysine residues some distance from the cleavage site have been implicated in the proteolytic reaction (35, 41, 42). Based on sequence homology among cI, LexA, and UmuD, Perry *et al.* (19) suggested that UmuD might be cleaved at a Cys-Gly bond in the homologous region. UmuD has serine and lysine residues the same distance from the Cys-Gly site as LexA has for Ala-Gly. We

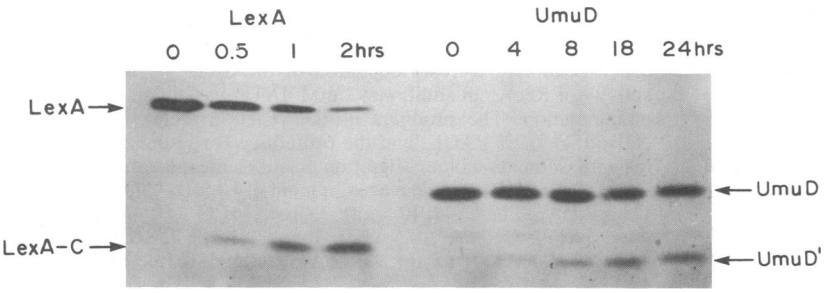

FIG. 5. Self-cleavage of LexA and UmuD. LexA or UmuD was incubated at 37°C under autodigestion conditions at pH 10. At the times indicated at the top of the lanes, samples were subjected to $NaDodSO_4$/PAGE, and the proteins were visualized by staining with Coomassie blue. Migration positions are indicated for LexA, its larger COOH-terminal fragment (LexA-C), UmuD, and its larger cleavage fragment (UmuD'). Lanes: 1–4, LexA at 0, 0.5, 1, and 2 hr, respectively; 5–9, UmuD at 0, 4, 8, 18, and 24 hr, respectively.

have found that the major cleavage product for UmuD has a molecular mass consistent with the predicted Cys-Gly scission. We also have shown that UmuD exhibits a self-cleavage reaction. Thus, LexA, UmuD, and cI appear to undergo a highly similar proteolytic reaction; all three proteins carry a "self-destruct" domain for which the reactivity is greatly accelerated by RecA. One notable functional difference in the three proteolytic reactions is that cleavage probably activates UmuD for its role in mutagenesis in contrast to the inactivation of the LexA and cI repressors.

Possible Biological Design Features of Proteolytic Reactions in the SOS Response. An interesting feature of the three RecA-mediated cleavages is a substantial difference in the rate of the reactions. In our experiments, proteolysis of LexA is considerably more efficient than that of UmuD. Based on other work, proteolysis of LexA is also considerably more efficient than that of cI (4, 35). The inefficient cleavage of UmuD might result from the nonphysiological *in vitro* situation; the UmuD reaction might be more effective in the presence of UmuC or with a special mononucleotide cofactor. However, the qualitative variation in reaction rates does make sense in terms of the probable role of the three proteins. LexA must be cleaved to induce the SOS response, and the reaction must occur at noninduced concentrations of RecA (RecA synthesis is increased by SOS induction) (4, 5). The efficient cleavage of cI needed to induce the λ prophage requires SOS-amplified concentrations of RecA (4, 5, 39); this property probably serves to prevent prophage induction under conditions of mild DNA damage. Since cleavage of UmuD is probably required to activate the protein for its mutagenic role (22), SOS mutagenesis appears to be regulated at two levels: (*i*) cleavage of LexA to induce the *umuD* and *umuC* genes and (*ii*) subsequent cleavage of UmuD at high concentrations of activated RecA. Therefore, the mutagenic response will likely require amplified RecA and many RecA-activating lesions. Extensive mutagenesis might represent primarily a last resort effort at a genetic escape from a major environmental disaster through enhanced genetic variation in the endangered population (43).

Multiple Activities of RecA in the SOS Response. RecA has many roles in the SOS response: (*i*) derepression of LexA-regulated genes, (*ii*) enhanced mutation rate for point mutations, (*iii*) repair by homologous recombination (44), (*iv*) restart of DNA replication following inhibition by DNA lesions (45, 46), and (*v*) enhanced formation of large duplications (J. Dimpfl and H.E., unpublished work). As noted above, the derepression function is cleavage of LexA. The mutagenesis function probably involves cleavage of UmuD because an engineered form of UmuD that is synthesized as the major cleavage product can restore UV mutagenesis to cells carrying a mutant RecA (RecA430) that is refractory to mutagenesis (22). In addition, we have found that UmuD is insensitive to cleavage *in vitro* mediated by RecA430. Based on these observations, the cleavage of UmuD might be the entire "direct" mutagenic function of RecA. However, other experiments have been interpreted in terms of a direct participation of RecA in the mutagenic bypass of UV lesions, possibly defining a role for RecA in addition to UmuD cleavage (16–18). This viewpoint is supported by the failure of the UmuD cleavage product to restore mutagenesis to bacteria with a highly defective RecA protein (22). The replication restart function probably does require the direct participation of RecA because UmuD is not normally needed (45, 46). Induced duplication formation also likely involves the direct participation of RecA.

From the above brief summary, the multiple roles of RecA in the SOS response depend upon two protein cleavage reactions and three or four other activities. All of these activities probably require the association of RecA with single-stranded or damaged double-stranded DNA; however, the diversity of responses to this signal is remarkable.

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