Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3987–3991, June 1987
Biochemistry

Lysine-156 and serine-119 are required for LexA repressor cleavage: A possible mechanism

(site-directed mutagenesis/λ *cI* repressor/RecA protein/autodigestion/intramolecular protease)

STEVE N. SLILATY* AND JOHN W. LITTLE*†‡

Departments of *Biochemistry and †Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721

Communicated by Martin Gellert, February 6, 1987 (received for review December 22, 1986)

ABSTRACT **LexA repressor of *Escherichia coli* is inactivated *in vivo* by a specific cleavage reaction requiring activated RecA protein. *In vitro*, cleavage requires activated RecA at neutral pH and proceeds spontaneously at alkaline pH. These two cleavage reactions have similar specificities, suggesting that RecA acts indirectly to stimulate self-cleavage, rather than directly as a protease. We have studied the chemical mechanism of cleavage by using site-directed mutagenesis to change selected amino acid residues in LexA, chosen on the basis of kinetic data, homology to other cleavable repressors, and potential similarity of the mechanism to that of proteases. Serine-119 and lysine-156 were changed to alanine, a residue with an unreactive side chain, resulting in two mutant proteins that had normal repressor function and apparently normal structure, but were completely deficient in both types of cleavage reaction. Serine-119 was also changed to cysteine, another residue with a nucleophilic side chain, resulting in a protein that was cleaved at a significant rate. These and other observations suggest that hydrolysis of the scissile peptide bond proceeds by a mechanism similar to that of serine proteases, with serine-119 being a nucleophile and lysine-156 being an activator. Possible roles for RecA are discussed.**

Escherichia coli LexA and λ *cI* repressors are inactivated *in vivo* by a specific cleavage reaction that cuts a conserved Ala-Gly bond near the center of the polypeptide chain (1, 2). Cleavage occurs following treatments that damage DNA or inhibit replication (1–3). Inactivation of LexA is rapid and results in derepression of a set of genes, the SOS regulon, largely responsible for DNA repair. Cleavage of the λ repressor is much slower than that of LexA, and more severe treatments are required to induce the prophage. Repressor cleavage *in vivo* requires RecA function; RecA protein is quiescent during normal cell growth but is activated by the inducing treatment to a form that participates in cleavage.

Early *in vitro* studies showed that the role of RecA protein in cleavage is relatively direct (4). At physiological pH, cleavage is dependent on the presence of RecA protein and two cofactors—a nucleoside triphosphate and single-stranded DNA—that activate RecA by forming a ternary complex. For a time, it was tacitly assumed that activated RecA was a conventional protease with a highly specific active site. This view was challenged by the finding (5) that, under different conditions, cleavage of LexA and the λ repressor proceeds spontaneously at the same Ala-Gly bond in an intramolecular reaction, termed "autodigestion," that is stimulated by alkaline pH and does not require RecA. Both RecA-dependent cleavage and autodigestion of LexA are inhibited in a mutant protein, LexA3, that also cannot be cleaved *in vivo*.

It was, therefore, proposed that RecA protein is not itself a protease, but rather that it acts indirectly by stimulating the autodigestion reaction. According to this hypothesis, activated RecA is a positive effector, but the site specificity and the chemistry of bond rearrangement are functions of residues present on the repressors themselves rather than on RecA. These residues would form an active site, similar to that of a protease; the cleavage site could be considered to be analogous to the substrate in an enzyme–substrate reaction. The repressors also would contain a RecA interaction site, where RecA binds to play its effector role in RecA-dependent cleavage.

Studies with proteolytic fragments of LexA and the λ repressor have shown that all of the sites involved in cleavage lie in the COOH-terminal two-thirds of both proteins (5–7). In this region, LexA and three cleavable phage repressors share significant amino acid homology, clustered mainly in three regions that are around the cleavage site and in two blocks near residues 119 and 156 of LexA (8). Genetic and biochemical evidence (refs. 9 and 10; L. Lin and J.W.L., unpublished data) suggests that these conserved residues are important in cleavage, but does not rule out other roles as well.

Major clues to the chemistry of cleavage have emerged from analysis of the reaction kinetics of autodigestion for both LexA and λ repressors (7). The reaction proceeds in the absence of divalent cations, and solvent components do not participate as acid–base catalysts or nucleophiles in the chemistry of the reaction, suggesting that side chains in the protein play direct roles in the chemistry of bond-making and bond-breaking events. In addition, the pH-rate profiles suggest that a group on the protein with a pK near 10 must be deprotonated for autodigestion to proceed efficiently (7). This apparent pK has the same value for both repressors, suggesting that the titrating group might be one of the conserved residues.

In this work, we have used site-directed mutagenesis to change amino acid residues implicated in cleavage. Since no crystallographic structure of the COOH-terminal domain for any of the cleavable repressors is available, we used the above information as well as knowledge of the mechanism of peptide bond hydrolysis by well-characterized proteases to identify residues potentially involved in the chemistry of cleavage.

MATERIALS AND METHODS

Site-Directed Mutagenesis and Plasmid Construction. Site-directed mutagenesis was performed as described (11) using M13mp8-42sal, a recombinant phage constructed by cloning an *Eco*RI–*Hin*dIII fragment containing the wild-type *lexA* gene from pJWL42 (12) into M13mp8 (13) followed by introduction of a silent change (14) in codon 101, GAT (Asp) to GAC (Asp), to generate a *Sal* I site. The oligodeoxyribonucleotides 5′ T CAG GCG CGC AAC GGT A 3′, 5′ C TTT CAT CGC CAT CCC GCT G 3′, and 5′ ATC TTT CAT GCA

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

‡To whom reprint requests should be addressed.

CAT CCC GC 3′ (altered nucleotides are underlined, and spaces indicate the reading frame) were used to change codon 156 from AAG (lysine or K) to GCG (alanine or A) and codon 119 from TCG (serine or S) to GCG (alanine or A) or TGC (cysteine or C) to generate the mutants *lexA277*, *lexA278*, and *lexA279*, respectively, which we shall term KA156, SA119, and SC119 for the sake of clarity. The mutants were initially detected by restriction analysis, since each created or abolished a restriction site. Sequence verification was performed between the closest restriction enzyme sites flanking each mutagenized region, *Sna*BI and *Aha* III for position 156 and *Sal* I and *Eco*RV for position 119; the intended changes were the only ones found. Restriction fragments generated by cleavage with these enzymes were subcloned into the wild-type *lexA* gene carried on pSNS111, a plasmid similar to pJWL59 (7) except that it carried the *Sal* I site at amino acid 101 (see above) and the *lac*UV5 promoter instead of the *tac* promoter; the promoter was changed by replacing the promoter-containing *Eco*RI–*Mlu* I fragment in pJWL59 with the analogous fragment from pRB192 (15). Promoter change was necessary because the uninduced level of mutant proteins expressed from the *tac* promoter was stressful or lethal to the host cell. Names of the resulting plasmids are given in Table 1.

Other Materials and Methods. Bacterial strains used for the analysis of LexA repressor function and cleavability *in vivo* were JL859 (*recA730*), JL932 (*recA*$^+$), and JL1246 (*recA*$^-$). Except for their *recA* alleles, these strains were identical; the relevant genotype was (λ *att*$^+$ *recA-lacZ* c*I ind*$^-$) *lexA71*::Tn*5* *sulA211*/F′*lacI*^{q}*I*^{s} *lacZ*$^+$. The prophage (17) and *lexA71*::Tn*5* (18) have been described; details of the constructions will be described elsewhere. Strain JL468 was AB1157/F′ *lacI*^{q} (5). Conditions for the RecA-dependent cleavage reaction, autodigestion, and thermolysin treatments are described in the appropriate figure legends. Wild-type and the mutant LexA proteins were purified from strains JL652 (= JL468/pJWL59), JL1233 (= JL468/pSNS113), JL1174 (= JL468/pSNS123), or JL1205 (= JL468/pSNS125) as described (5) except that the methyl mercury column was omitted. Radiolabeling and purification of wild-type LexA protein and the Ind^s mutant of λ *c*I repressor were performed as described (7). The dideoxy DNA sequencing method was employed using Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) and alkali-denatured supercoiled plasmid DNA or single-stranded M13 DNA as template (19). RecA protein, purified as described (20), was the generous gift of Stephen West (Yale University).

RESULTS

Choice of Targeted Residues and Construction of Mutants. We examined the homology among the cleavable repressors (8) for two types of residues, the first having a titratable group with a pK near 10, and the second carrying a side chain that might plausibly be involved in a nucleophilic attack on the carbonyl carbon of a peptide bond by analogy with known protease mechanisms (21). The only conserved residue with a pK near 10 was a single lysine, lysine-156 in LexA. Among the well-characterized types of proteases, we discounted acid proteases, because they display pKs below 7, and metalloproteases, because LexA autodigestion proceeds in the complete absence of divalent cations (7), which are required by these enzymes. The mechanism cannot be that of a thiol protease, since LexA has no cysteine residues. The final major class of proteases, the serine proteases, therefore, seemed the most likely (despite preliminary evidence that serine protease inhibitors did not inhibit autodigestion—see *Discussion*). One serine, serine-119 in LexA, is conserved in LexA and the phage repressors.

Accordingly, we used site-directed mutagenesis to change lysine-156 and serine-119 to alanine, which bears an unreactive side chain, generating mutants we term KA156 and SA119, respectively. In addition, we made mutant SC119 by changing serine-119 to cysteine; the sulfhydryl moiety of this amino acid is expected to react similarly to that of the serine hydroxyl and is known to be the active group in thiol proteases. The resulting mutant genes were then fused with the *lac*UV5 promoter on plasmids.

Repressor Activity and Cleavage of Mutant Proteins *in Vivo*. Plasmids carrying the wild-type *lexA* gene, the classical noncleavable (Ind$^-$) mutant *lexA3*, or one of the new mutants were examined for LexA function in three indicator strains. These strains had an identical background, including a defective chromosomal *lexA* gene and a fusion of the LexA-controlled *recA* promoter to *lacZ*, and differed in their *recA* alleles, bearing either wild-type (*recA*$^+$), constitutively activated (*recA730*), or defective (*recA*$^-$) function. In these strains, the level of β-galactosidase activity was an inverse measure of LexA repressor function (see ref. 17). Repressor function in the absence of cleavage was assessed in the *recA*$^-$ strain; any decrease in function due to cleavage was then detected in the *recA*$^+$ and *recA730* strains as an increase in the level of β-galactosidase expressed from the LexA-controlled promoter.

In the *recA*$^-$ strain, the mutant proteins provided about as much repressor function as did the wild type and the noncleavable control allele *lexA3* (Table 1) . In the strain carrying the constitutively activated RecA730 protein, the mutants KA156 and SA119 were unaffected by the state of RecA, as was the noncleavable *lexA3* control, whereas the wild-type protein and the SC119 mutant showed elevated levels of β-galactosidase activity, presumably as a result of susceptibility to cleavage. Smaller increases were seen in the strain carrying the wild-type *recA*$^+$ allele, suggesting that a low steady-state level of activated RecA is present under normal growth conditions (see also refs. 3 and 17), yielding low levels of repressor cleavage and, hence, elevated β-galactosidase activity for the wild-type and SC119 repressors.

We conclude that repressor function of all three mutants was essentially unaltered, suggesting that the changes do not disrupt their structure. We also conclude that the KA156 and SA119 mutant proteins are resistant to RecA-mediated *in vivo*

Table 1. Repressor activity and cleavability of the wild-type and mutant LexA proteins *in vivo*

lexA allele	Plasmid	β-Galactosidase activity in recipient strain		
		recA$^+$	*recA*$^-$	*recA730*
None	pBR322	32,000	29,000	29,000
lexA$^+$	pSNS111	6,000	900	11,000
lexA3	pSNS121	1,000	800	1,000
KA156	pSNS113	700	700	700
SA119	pSNS123	800	800	800
SC119	pSNS125	3,500	1,400	6,600

Plasmids carrying the indicated *lexA* allele driven by the *lac*UV5 promoter were introduced into strains JL932 (*recA*$^+$), JL1246 (*recA*$^-$), and JL859 (*recA730*). The amount of β-galactosidase activity produced at the level of LexA expression from the uninduced *lac*UV5 promoter was assayed as described (16). The specific activity values given are an average of at least four experiments and contain a standard deviation of ≈40%. These results are consistent with those obtained using MacConkey indicator plates. The reasons for the present specific activity values being ≈2-fold higher than those of ref. 17 are unclear. This system differs from the natural one with the chromosomal *lexA* gene, since LexA partially represses its own expression (autoregulation) (1). The disparity seen here and in ref. 17 between efficiency of repressor function of wild-type and noncleavable proteins would be much less marked in cells with the chromosomal *lexA* gene, since autoregulation would largely counteract small decreases in repressor function.

cleavage, whereas the SC119 mutant protein is cleaved at a slow rate. We cannot readily deduce the relative rates of cleavage from the relative levels of β-galactosidase, however, since the relationship between these two parameters is complex and not understood.

Cleavage of Mutant Proteins *in Vitro*. Purified mutant proteins were analyzed for cleavage activity *in vitro* by incubation under either autodigestion or RecA-dependent cleavage conditions. As shown in Fig. 1, KA156 and SA119 mutant proteins showed no detectable cleavage products under either condition. Incubations for up to 100 hr still resulted in no detectable cleavage of either protein (data not shown), strongly suggesting that they are completely noncleavable.

By contrast, the SC119 mutant repressor was cleaved at roughly 10–30% the rate shown by the wild-type protein in both types of cleavage reaction (Fig. 1 and data not shown). This comparison is complicated, however, both by the finding that cleavage of the SC119 protein did not usually go to completion, presumably due to oxidation of the thiol side chain, and by preliminary evidence that the pH-rate profile had a pK lower than that of wild type.

Interaction of Mutant Repressors with RecA Protein. Of itself, an observed defect in RecA-mediated cleavage does not distinguish between effects on the chemistry of cleavage and on the initial binding of RecA to the repressor. We reasoned that, if the mutant proteins could still bind to activated RecA, they would inhibit RecA-mediated cleavage reactions by competing for the RecA. We tested the effects of KA156 or SA119 protein on the rates at which radiolabeled wild-type LexA or λ repressor substrates were cleaved. Each mutant protein inhibited cleavage of both substrates (Fig. 2).

Addition of unlabeled wild-type LexA inhibited cleavage of radiolabeled LexA protein, presumably due to an isotope-dilution effect, and had no effect on the rate of λ repressor cleavage (Fig. 2). In the latter reaction, LexA was cleaved promptly on this time scale (data not shown), suggesting that the cleavage products do not inhibit the reaction. LexA autodigestion products also did not inhibit the RecA-dependent cleavage of radiolabeled LexA (data not shown).

These results are consistent with the mutant proteins acting as competitive inhibitors, but they do not rule out the possibility that inhibition results from some other effect. It is unlikely that the mutant proteins competed with RecA for the single-stranded DNA cofactor needed to activate RecA, because inhibition was still observed in the presence of excess single-stranded DNA (data not shown). Moreover, electron microscopy shows that addition of either mutant protein to activated RecA changes the structure of the spiral filaments (C. Chang and S.N.S., unpublished data). Finally, the fact that cleavage activity continues with time in the

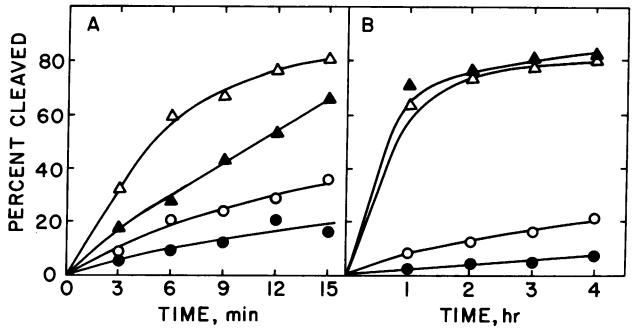

FIG. 2. Effect of added mutant LexA proteins on the rate of RecA-dependent cleavage of wild-type LexA repressor or λ repressor. (*A*) RecA-dependent cleavage of 0.15 μM [^{3}H]methionine-labeled wild-type LexA in the absence (open triangles) or presence (closed triangles) of 1.5 μM of added unlabeled wild-type LexA or of the mutant proteins KA156 (closed circles) or SA119 (open circles). Incubation was at 37°C in a solution containing 10 mM Tris·HCl (pH 7.6), 100 mM NaCl, 0.5 mM EDTA, 2 mM dithiothreitol, 5 mM $MgCl_2$, 0.5 mM adenosine-5′-[γ-thio]-triphosphate (ATP[S]), 2.5% (vol/vol) glycerol, bovine serum albumin at 50 μg/ml, single-stranded M13mp8-42Sal DNA at 0.4 μg/ml, and 0.3 μM RecA protein. (*B*) RecA-dependent cleavage of 1.2 μM of [^{3}H]methionine-labeled Ind^s mutant λ repressor in the absence (open triangles) or presence (closed triangles) of added 1.5 μM unlabeled wild-type LexA protein or the mutant proteins KA156 (closed circles) or SA119 (open circles). Reactions were performed at 37°C in a solution containing 6 mM Tris·HCl (pH 7.6), 25 mM NaCl, 0.1 mM EDTA, 0.6 mM dithiothreitol, 3 mM $MgCl_2$, 1 mM ATP[S], 0.6% glycerol, single-stranded M13mp8-42Sal DNA at 1.1 μg/ml, and 1 μM RecA protein. The extent of cleavage at each time point was assayed by counting the radioactivity in gel slices (7). The Ind^s form of λ repressor was used since it is cleaved at a much higher rate at high concentrations (22, 23) than is wild-type repressor.

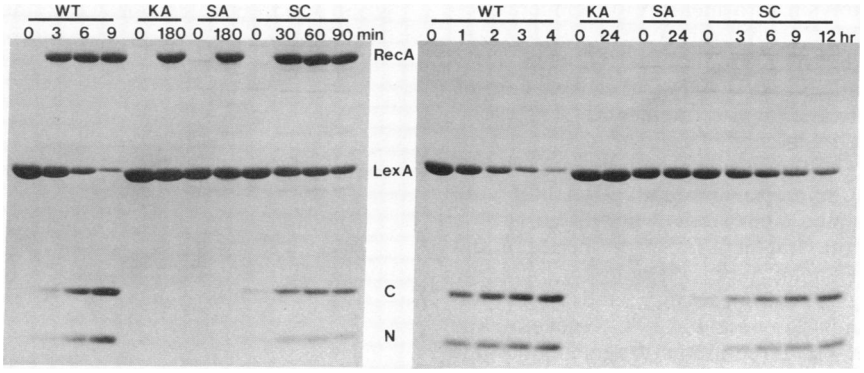

FIG. 1. RecA-dependent cleavage and autodigestion of LexA proteins. Reactions of RecA-dependent cleavage (*Left*) and autodigestion (*Right*) of wild-type LexA protein (WT), and the mutant proteins KA156 (KA), SA119 (SA), and SC119 (SC) were carried out at 37°C for the indicated times. RecA-dependent cleavage was performed at 11.3 μM LexA and 4.5 μM RecA in a reaction mixture containing 20 mM Tris·HCl (pH 7.7 at 25°C), 100 mM NaCl, 2 mM dithiothreitol, 5 mM 2-mercaptoethanol, 2 mM $MgCl_2$, 0.5 mM EDTA, 2.5% (vol/vol) glycerol, 1 mM adenosine-5′-[γ-thio]-triphosphate, and single-stranded M13mp8-42sal DNA at 6 μg/ml. Autodigestion was performed at 22.5 μM LexA in a buffer containing 100 mM Tris·HCl (pH 9.4 at 25°C), 200 mM NaCl, 1 mM EDTA, 12 mM dithiothreitol, and 5% (vol/vol) glycerol. Aliquots (20 μl) were treated and subjected to electrophoresis in 15% $NaDodSO_4$/polyacrylamide gels as described (7). The chosen pH for autodigestion was below optimum because cleavage of the SC119 mutant was inhibited at higher pH, presumably due to the formation of the highly reactive thiolate ion. Under these conditions, the half-life of wild-type LexA is 1 hr. In a separate experiment (data not shown), autodigestion of the mutants KA156 and SA119 at pH 9.4 and 37°C for 100 hr yielded no detectable cleavage products when assayed as above. In our hands, the detection limit of this system is about 50 ng of protein per gel band, which corresponded to ≈1% of input in this experiment. The positions of RecA protein, intact LexA protein, and the COOH-terminal (C) and NH_2-terminal (N) cleavage fragments of LexA are labeled.

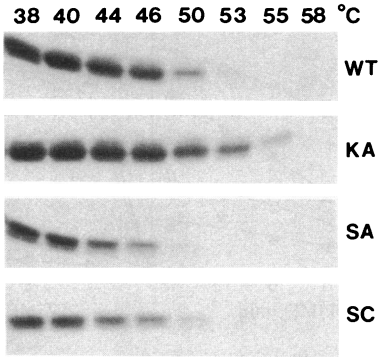

FIG. 3. Analysis of the thermal stability of the COOH-terminal domain and hinge region of LexA proteins. Thermolysin digestions were carried out at 22.5 μM LexA and 1.4 μM thermolysin in a buffer containing 50 mM Tris·HCl (pH 8.0 at 25°C), 200 mM NaCl, 2 mM dithiothreitol, 10 mM $CaCl_2$, 0.5 mM EDTA, and 5% (vol/vol) glycerol. The reactions, performed for all four proteins simultaneously, were preincubated at 37°C for 15 min to generate the TL1 fragment and held at 0°C; then aliquots were shifted to the indicated temperatures for 15 min and subjected to electrophoresis. Abbreviations are as in Fig. 1.

presence of the noncleavable proteins suggests that RecA continues to be active, as would be expected for reversible binding of an inhibitor. If we conclude that the inhibition results from binding of noncleavable mutant proteins to RecA, we may draw several inferences from these data.

First, the defect of the mutant proteins in RecA-mediated cleavage is not due to defects in binding to RecA. Second, KA156 protein may have a greater affinity for RecA than does SA119 protein. Third, the data suggest that the LexA binding site on RecA overlaps, at least partly, with that of λ repressor. Finally, the lack of inhibition by LexA cleavage fragments suggests either that the RecA binding site on LexA is composed of regions lying in both of the cleavage products, or alternatively that the conformation of the binding site changes due to cleavage so that the fragment carrying it can no longer bind efficiently to RecA.

Thermal Stability of Mutant Repressors. Since all three amino acid substitutions are in the COOH-terminal domain, we probed the stability of this portion of the protein by using susceptibility to thermolysin as a measure of temperature-induced protein unfolding (24). The substrate for this test was a partial thermolysin digestion product that we term TL1, which comprises the hinge and COOH-terminal domain of LexA, and is similar to the tryptic fragment TC1 (5). The tryptic fragment, and an analogous fragment of λ repressor (5–7), can undergo both autodigestion and RecA-mediated cleavage; TL1 was also found to autodigest efficiently (data not shown; RecA-mediated cleavage was not tested).

Thermal stability of this fragment for wild-type LexA and the mutant proteins was analyzed by assaying for increased sensitivity to thermolysin with increasing temperatures following a 15-min preincubation period at 37°C to generate the TL1 fragment (Fig. 3). The three mutant fragments became susceptible to further thermolysin digestion at temperatures that were about the same, to within a few degrees, as that seen with the wild type. Larger melting temperature differences (6–14°C) have been observed for mutant forms of λ repressor and T4 lysozyme that retain nearly full biological activity (24, 25). Our findings indicate that the structure of the hinge plus COOH-terminal domain has not been markedly destabilized by the substitutions.

DISCUSSION

Several lines of evidence suggest that the three substitutions did not have a substantial effect on the structure of LexA. The altered proteins retained full repressor function and displayed melting temperatures similar to that of the wild type. Kinetic evidence suggested that they could also interact with activated RecA. We conclude that the SA119 and KA156 mutant proteins bear specific defects in the cleavage activities.

It is more difficult to be certain that the mutations directly affect the chemical events of cleavage, as opposed to less-direct effects such as impairing interaction of the active site with the cleavage site, but several observations are consistent with this notion. First, the mutants KA156 and SA119 are completely defective in cleavage, as would be expected for changes in residues affecting the chemistry of the reaction. Many changes in residues that mold the substrate-binding site would lead to less-severe defects, and our collection of Ind^- mutants lying near alanine-84, serine-119, and lysine-156 includes several examples of this (L. Lin and J.W.L., unpublished data). Second, it seems highly likely that the groups involved in facilitating cleavage are conserved among the four cleavable repressors. Of these conserved residues, only four—asparagine-171, glutamic acid-152, and the two we studied, serine-119 and lysine-156—have side chains that could plausibly be involved in the chemistry of bond rearrangement; the two we targeted have the side chains with chemical properties consistent with the kinetics data and capable of effecting amide bond hydrolysis. Finally, replacement of the essential hydroxyl group of serine-119 with the chemically similar thiol group resulted in retention of appreciable levels of activity. A similar pattern has been observed for the serine hydrolytic enzymes β-lactamase, alkaline phosphatase, trypsin, and subtilisin (26–29). In what follows, we shall assume that both serine-119 and lysine-156 are directly involved in the chemistry of bond rearrangement.

Model for Chemical Mechanism of Cleavage. Our results, as well as kinetic data (7), suggest a model for the mechanism of LexA repressor cleavage (Fig. 4) and by extension that of the phage repressors. According to this model, serine-119 and lysine-156 lie near each other in the folded polypeptide, and the conserved amino acid residues near these two groups make up the elements of the active site that interact specifically with the cleavage site. The hydroxyl group of serine-119 is the nucleophile that attacks the carbonyl carbon of the scissile peptide bond. The unprotonated form of the lysine-156 ε-amino group serves to activate the serine-119 hydroxyl group and to transfer a proton to the incipient amino group. This model is based on the mechanism of peptide bond

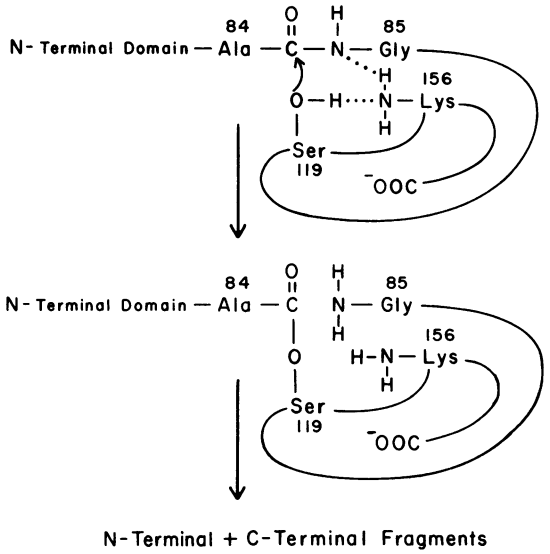

FIG. 4. Proposed mechanism for LexA repressor cleavage.

hydrolysis by serine and thiol proteases, which requires a proton transfer mechanism and proceeds by way of a covalent intermediate (21).

The following several observations support the proposal that serine-119 is the nucleophile: First, changing it to alanine, which replaces a hydroxyl group with a hydrogen, completely inactivates cleavage; we have also found (A. Cegielska, L. Lin, and J.W.L., unpublished data) that changing serine to leucine inactivates cleavage, but in this case repressor function is partially deficient, suggesting that this bulky residue distorts the structure of the protein. Second, reversible oxidation of the nucleophilic thiol group in the active SC119 mutant with 2-mercaptoethanol abolishes cleavage under autodigestion conditions (RecA-dependent cleavage was not tested), but activity is regained following reducing treatments (unpublished data). Third, preliminary analysis of pH-dependence for autodigestion of the mutant SC119 reveals a significantly lower pK than that of the wild type, as would be expected if the cysteine residue were titrated. Finally, the amino acid sequences around this position in LexA, Gly-Met-Ser-Met, and in λ repressor, Gly-Asp-Ser-Met, are somewhat homologous to the consensus sequences around the active serine in mammalian serine proteases, Gly-Asp-Ser-Gly (30), and in microbial proteases such as subtilisin, Gly-Thr-Ser-Met (31).

One prediction of this model is that serine protease inhibitors such as diisopropyl fluorophosphate would inhibit or prevent cleavage; preliminary experiments suggest that they do not (unpublished data). We can rationalize this negative result in the following two ways: First, it is plausible that the active serine is located not on the surface but in a pocket of the protein inaccessible to the reagent; since the reaction is intramolecular, the reagent might not be able to compete very well with the cleavage site, which would be present at very high effective local concentration (21). Second, the rate of cleavage is actually rather low by comparison with hydrolysis of esters by chymotrypsin, suggesting that the serine is not highly activated. A precedent for this is a mutant of trypsin in which aspartic acid-102 is replaced by asparagine (32); relative to wild-type trypsin, this protein has a very low activity, and its activity is far less sensitive to diisopropyl fluorophosphate.

In addition to activation of the serine hydroxyl group, we postulate that lysine-156 plays some other role, such as transfer of a proton to the leaving amino group. This is supported by the properties of the double mutant SC119-KA156. This mutant LexA protein is completely defective in both autodigestion and RecA-mediated cleavage, yet retains full repressor activity and RecA interaction ability (unpublished observations). Since cysteine residues typically have a pK value of ≈8.5, we would not expect cysteine-119 to require activation at moderately alkaline pH; accordingly, the inactivity of the double mutant suggests an additional role for lysine-156.

We cannot rule out an alternative mechanism in which uncharged lysine-156 acts as a general base catalyst, activating a water molecule for attack on the peptide bond. Such a mechanism does not provide a role for serine-119. Certainly, serine-119 could form an essential hydrogen bond, but one would not expect cysteine to be able to perform this function efficiently.

Roles for RecA. Although we chose the residues to be changed from the properties of the autodigestion reaction, the mutant proteins are also completely deficient in RecA-mediated cleavage. This finding further supports our proposal that RecA protein acts indirectly to stimulate autodigestion, rather than being a protease itself. How might it do so?

Whatever the true functions of lysine-156 and serine-119 may prove to be, our data suggest that residues in RecA protein cannot simply substitute for these functions, since the mutants are deficient in RecA-mediated cleavage. In terms of our model (Fig. 4), groups on RecA might titrate the amino group of lysine-156 by direct interaction, or by removal of water from the active site. Alternatively, RecA might activate serine-119 directly; if this is the case, however, it must be deficient in some other step (such as proton transfer to the leaving amino group) for which lysine-156 is needed. A final possibility is that residues on RecA do not interact directly with serine-119 or lysine-156, but that RecA binding alters the conformation of LexA, thereby stimulating autodigestion, for example, by reducing the pK of lysine-156, by stabilizing the binding of the cleavage site to the active site, or by optimizing the distance between the carbonyl group of alanine-84 and the serine hydroxyl, allowing a nucleophilic attack to proceed.

We are grateful to John Rupley, Carol Dieckmann, Don Ennis, Lih-ling Lin, and Ivan Rayment for helpful discussions; to Sylvia Hill for excellent technical assistance; to Bob Zagursky, Carl Bauer, Jeffrey Gardner, Fred Gimble, Bob Sauer, and William Rutter for communication of unpublished results; and to Steve West for a generous gift of RecA protein. This work was supported by Grant GM24178 from the National Institutes of Health (to J.W.L.) and by National Institutes of Health Cancer Biology Training Grant Postdoctoral Fellowship CA09123 (to S.N.S.).

1. Little, J. W. & Mount, D. W. (1982) *Cell* **29,** 11–22.
2. Walker, G. C. (1984) *Microbiol. Rev.* **48,** 60–93.
3. Little, J. W. (1983) *J. Mol. Biol.* **167,** 791–808.
4. Craig, N. L. & Roberts, J. W. (1980) *Nature (London)* **283,** 26–29.
5. Little, J. W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1375–1379.
6. Sauer, R. T., Ross, M. J. & Ptashne, M. (1982) *J. Biol. Chem.* **257,** 4458–4462.
7. Slilaty, S. N., Rupley, J. A. & Little, J. W. (1986) *Biochemistry* **25,** 6866–6875.
8. Sauer, R. T., Yocum, R. R., Doolittle, R. F. & Pabo, C. O. (1982) *Nature (London)* **298,** 447–451.
9. Gimble, F. S. & Sauer, R. T. (1985) *J. Bacteriol.* **162,** 147–154.
10. Gimble, F. S. & Sauer, R. T. (1986) *J. Mol. Biol.* **192,** 39–47.
11. Bauer, C. E., Hesse, S. D., Waechter-Brulla, D. A., Lynn, S. P., Gumport, R. I. & Gardner, J. F. (1984) *Gene* **37,** 73–81.
12. Markham, B. E., Little, J. W. & Mount, D. W. (1981) *Nucleic Acids Res.* **9,** 4149–4161.
13. Messing, J. (1983) *Methods Enzymol.* **101,** 20–78.
14. Little, J. W. & Mount, D. W. (1984) *Gene* **32,** 67–73.
15. Brent, R. & Ptashne, M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4204–4208.
16. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
17. Little, J. W. & Hill, S. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2301–2305.
18. Krueger, J. H., Elledge, S. J. & Walker, G. C. (1983) *J. Bacteriol.* **153,** 1368–1378.
19. Zagursky, R. J., Baumeister, K., Lomax, N. & Berman, M. L. (1985) *Gene Anal. Tech.* **2,** 89–94.
20. Cox, M. M., McEntee, K. & Lehman, I. R. (1981) *J. Biol. Chem.* **256,** 4676–4678.
21. Fersht, A. (1985) *Enzyme Structure and Mechanism* (Freeman, New York).
22. Cohen, S., Knoll, B. J., Little, J. W. & Mount, D. W. (1981) *Nature (London)* **294,** 182–184.
23. Crowl, R. M., Boyce, R. P. & Echols, H. (1981) *J. Mol. Biol.* **152,** 815–820.
24. Hecht, M. H., Sturtevant, J. M. & Sauer, R. T. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5685–5689.
25. Hawkes, R., Grutter, M. G. & Schellman, J. (1984) *J. Mol. Biol.* **175,** 195–212.
26. Sigal, I. S., DeGrado, W. F., Thomas, B. J. & Petteway, S. R., Jr. (1984) *J. Biol. Chem.* **259,** 5327–5332.
27. Ghosh, S. S., Bock, S. C., Rokita, S. E. & Kaiser, E. T. (1986) *Science* **231,** 145–148.
28. Neet, K. E. & Koshland, D. E., Jr. (1966) *Proc. Natl. Acad. Sci. USA* **56,** 1606–1611.
29. Yokosawa, H., Ojima, S. & Ishii, S. (1977) *J. Biochem. (Tokyo)* **82,** 869–876.
30. Neurath, H. (1975) in *Proteases and Biological Control,* eds. Reich, E., Rifkin, D. B. & Shaw, E. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 51–64.
31. Mikeš, O., Turková, J., Toan, N. B. & Šorm, F. (1969) *Biochim. Biophys. Acta* **178,** 112–117.
32. Craik, C. S., Roczniak, S., Largman, C. & Rutter, W. J. 1987) *Science,* in press.