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Saturation and Specificity of the Lon Protease of *Escherichia coli*†

ETIENNE DERVYN,‡* DANIELLE CANCEILL,‡ AND OLIVIER HUISMAN

Unité de Génie Microbiologique, Département de Biotechnologie, Institut Pasteur, 75724 Paris Cedex 15, France

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Lon is an ATP-dependent protease of *Escherichia coli*. The *lon* mutation has a pleiotropic phenotype: UV sensitivity, mucoidy, deficiency for lysogenization by bacteriophage lambda and P1, and lower efficiency in the degradation of abnormal proteins. All of these phenotypes are correlated with the loss of protease activity. Here we examine the effects of overproduction of one Lon substrate, SulA, and show that it protects two other substrates from degradation. To better understand this protection, we mutagenized the *sulA* gene and selected for mutants that have partially or totally lost their ability to saturate the Lon protease and thus can no longer protect another substrate. Some of the SulA mutants lost their ability to protect RcsA from degradation but could still protect the O thermosensitive mutant protein (Ots). All of the mutants retained their capacity to induce cell division inhibition. It was also found that deletion of the C-terminal end of SulA affected its activity but did not affect its susceptibility to Lon. We propose that Lon may have more than one specificity for peptide cleavage.

The *lon* gene, localized at 10 min on the *Escherichia coli* genetic map, codes for the ATP-dependent Lon protease (5, 6). Cells carrying mutations in this gene were isolated by their property of forming long filaments after UV irradiation (15). Independently, protein degradation mutants were isolated as mutations able to stabilize β-galactosidase nonsense fragments and were localized to the *deg* locus (1, 2). The *lon* and *deg* loci were mapped to the same position and were indistinguishable phenotypically (13, 32). The extremely pleiotropic effects of these mutants have been described: they are hypersensitive to UV, mucoid, defective in bacteriophage λ and P1 lysogenization, and defective in the degradation of abnormal proteins. Each of these features is attributable to the inactivation of Lon. UV hypersensitivity is due to an irreversible stabilization of the cell division inhibitor SulA (SfiA) induced as part of the SOS response by any agent damaging DNA (11, 17, 29). The result is filamentation and cell death. Mucoidy is related to overproduction of capsular mucopolysaccharides due to the stabilization of the transcriptional activator RcsA of the *cps* (capsular polysaccharide synthesis) genes (12, 16, 22, 34, 35). The lysogenization defect in a Lon mutant is due to both the stabilization of the lambda antiterminator N and (an unexplained) increased degradation of the positive regulator of lysogeny CII (10, 36, 38); the lysogenization defect is probably also due to the increased mucoidy. In vivo radioactive labeling experiments provide evidence that Lon can degrade SulA (20, 29), RcsA (34), and lambda N protein (10). Lon inactivation strongly stabilizes some abnormal proteins such as nonsense or missense fragments, foreign proteins, and mutated proteins (8, 27).

The *lon* gene has been cloned (40), and the Lon protease has been purified and characterized (5, 6). Transposon insertions have been introduced in the gene, leading to complete loss of protease activity in the cell, showing that *lon* is dispensable (24).

In vitro, in the presence of ATP and Mg^{2+}, Lon degrades many substrates such as casein, globin, and denatured albumin. Two molecules of ATP are hydrolyzed for each peptide bond cleavage (39). Lon also degrades some small peptides in the presence of nonhydrolyzable ATP analogs. The binding of ATP may play a role in the allosteric modification of the proteolytic site (25). Recently, in vitro proteolytic activity of Lon has been demonstrated on physiological substrates, i.e., the N protein of λ phage (23) and the SulA protein (M. R. Maurizi and D. Canceill, unpublished data).

In an attempt to establish the basis of the specificity of Lon for its substrates, we examined more precisely the interaction between Lon and SulA. This choice was motivated by several criteria. (i) SulA is a small (18-kDa) natural protein of *E. coli*, with a known sequence, and which is known in vitro to be a Lon substrate. (ii) The mode of action of SulA is well known: after SOS induction, it interacts with its target SulB (FtsZ), an essential protein of cellular division apparatus, and transitorily blocks septation to permit repair of DNA lesions (14, 18, 19). (iii) In *lon*$^+$ cells, division is restored by degradation of the SulA protein. (iv) SulA activity is easy to test since in *lon* mutants its accumulation after exposure to DNA-damaging agents causes filamentation and cell death.

In this work we examined the effect of overproduction of the SulA protein on Lon activity with respect to its other substrates. We observe that Lon can be saturated by an excess of SulA, thus protecting the other substrates from degradation. We also generated mutants of *sulA* which retain SulA activity but do not saturate Lon.

MATERIALS AND METHODS

Strains and plasmids. Strains used for this study are summarized in Table 1. P1 transductions were done as described previously (28). The F′ plasmid, carrying the lactose repressor (*lacI*^{q} *lacZ*::Tn*5*), was used for conjugation; F′ was selected by using kanamycin (50 μg/ml) and the recipient was selected by using streptomycin (200 μg/ml). The *sulB** mutation is a double mutation in the *sulB* gene that allows the strain to support the *sulA* gene on a multicopy plasmid (18); the SulB* product has normal activity in the cell division apparatus but is insensitive to SulA-dependent

* Corresponding author.

† Olivier Huisman directed our group for 2 years with enthusiasm, competence, and modesty. He died on 24 March 1988.

‡ Present address: Laboratoire de Génétique Microbienne, Institut des Biotechnologie, Institut National de la Recherche Agronomique, Domaine de Vilvert, 78-352 Jouy-en-Josas Cedex, France.

TABLE 1. Bacterial strains used

Escherichia coli strain	Relevant genotype	Reference, source, or construction
JM101	Δ(*lac pro*) *thi supE* F′ *traD36 proAB lacI*^{q} *lacZ*Δ*M15*	26
JM101 Lon$^-$	Δ*lon-510 gal*	S. Gottesman
SG20250	F$^-$ *araD139* Δ*lacU169 flbB relA rpsL deoC*	12
SG20582	*cpsB10*::*lacZ* (*imm*$^\lambda$)	4
JT4000	Δ*lon-510*	S. Gottesman
OHP3	Δ*lon-510 leu*::Tn*10 sulB**	JT4000 + P1 GC2550
OHP7	Δ*lon-510 leu*::Tn*10 sulB** F′ *lacI*^{q} *lacZ*::Tn*5*	OHP3 + GC2438
OHP8	*cpsB10*::*lacZ* (*imm*λ) *leu*::Tn*10 sulB**	SG20582 + P1 GC2550
OHP14	*leu*::Tn*10 sulB**	SG20250 + P1 GC2550
OHP17	*leu*::Tn*10 sulB** F′ *lacI*^{q} *lacZ*::Tn*5*	OHP14 + GC2438
GC2438	Δ(*lac pro*)*XIII thi rpsE Val*^{r} F′ *lacI*^{q} *lacZ*::Tn*5 pro*$^+$	R. D'Ari
GC2550	*thr pro his arg lon str leu*::Tn*10 sulB** pTU302(*sulA*$^+$ Ap)	R. D'Ari

division inhibition. This mutation has been transferred into the OHP strains (Table 1) by cotransduction with the *leu* gene in which Tn*10* is inserted and was selected by using selecting 10 μg of tetracycline per ml.

The plasmids used in this work are described in Table 2 and Fig. 1.

Media. All of the media used were described previously by Miller (28). Strains were grown in LB medium. The Lac$^+$ phenotype was determined on MacConkey agar medium. TB medium and gelose H have been used for λ phage experiments. M9 minimal medium supplemented with 0.01% thiamine, 0.4% glycerol, and 0.01% of appropriate amino acids (aa) was used for strain constructions and the pulse-chase experiments with [^{35}S]methionine.

Measurement of the saturation of RcsA degradation. RcsA degradation was evaluated in the strain containing the gene fusion *cpsB*::*lacZ* (OHP8). When RcsA accumulates, the *cps*::*lac* gene fusion is induced and a Lac$^+$ phenotype on MacConkey agar plates is detectable.

Measurement of Ots protein stability. The stability of the O thermosensitive mutant protein of λ phage (Ots) was measured (13) as follows. The *lon*$^+$ (OHP14) and *lon* (OHP3) strains, with or without the SulA-overproducing plasmid, were grown in TB medium. The Ots phage was adsorbed for 30 min at room temperature, spread in gelose H on TB plates, and incubated at the nonpermissive temperature (39°C). In a *lon*$^+$ strain, the protein was degraded and no plaques formed; in the *lon* strain, Ots was stabilized and plaques formed.

In vitro mutagenesis of the *sulA* gene on multicopy plasmid. Hydroxylamine mutagenesis was performed on the pOHP241 plasmid carrying the *sulA* gene under the control of the *lacZp* promoter. To 1 volume of DNA (1 μg/ml) were added 5 volumes of 100 mM Na_2PO_4, 1 mM EDTA (pH 6), 2, 4, or 6 volumes of 1 mM hydroxylamine, in 1 mM EDTA (pH 6). The tubes were then completed to 12 volumes with 1 mM EDTA (pH 6). The samples were incubated for 45 min at 0°C and 30 min at 60°C and then dialyzed against 2 liters of 10 mM $CaCl_2$ for 4 h at 4°C. The mutagenized DNA was used to transform strain JM101. Plasmid DNA was extracted in batch by the alkaline method (3) and used to retransform OHP8. Mutants were screened and analyzed in strain OHP8.

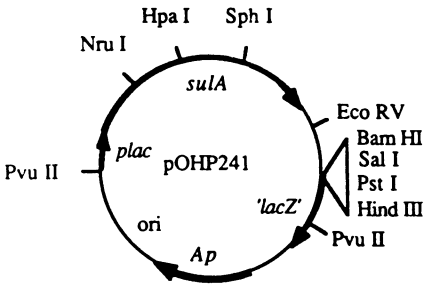
C-terminal deletions in the *sulA* gene. The C-terminal deletions in the *sulA* gene were generated by treating plasmid pOHP293 (containing *sulA*) with *Eco*RV followed by BAL 31 "fast" enzyme (New England BioLabs). The ends were filled in with T4 DNA polymerase (New England BioLabs), and *Xba*I linkers containing a stop codon in all three reading frames (New England BioLabs) were inserted. Constructions were checked by sequencing with the dideoxy method (31).

Filamentation test. Plasmids containing the native or mutated *sulA* gene were introduced in the JM101 *lon*$^+$ and *lon* strains. At the beginning of exponential growth (optical density at 600 nm, 0.2), the culture was diluted twofold and isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 1 mM to induce SulA synthesis. The size of the cells was followed by microscopic observation.

SulA half-life measurement. Measurements of SulA half-life were performed on strains with or without the lactose repressor, containing a cloned copy of *sulA* under control of the *lac* promoter. An overnight culture was diluted 20-fold

TABLE 2. Plasmids used

Plasmid	Relevant properties	Reference or source
Cloning vectors		
pBR322	Ap^r, Tc^r	
pUC8	Ap^r; pBR322 derivative carrying the p_{lac}	37
pRS414	Ap^r; pBR422 derivative carrying a strong terminator (T1)	33
pACYC184	Cm^r, Tc^r; compatible with pBR322	4
Other plasmids		
p*lon*$^+$500	Ap^r; *lon* gene (*Eco*RI-*Sph*I fragment) cloned in pBR322	24
pGC165 *sulA*$^+$	Ap^r; p_{lac}-*sulA* operon fusion constructed on the plasmid pUC8	18
pOHP241	Ap^r; pUC8 derivative (Fig. 1)	This work
pOHP280	Ap^r; p_{lac}-*sulA* from pOHP241 (*Pvu*II-*Bam*HI fragment) cloned in pRS414 (*Sma*I-*Bam*HI digested) downstream of T1	This work
pOHP293	Ap^r; pBR322 derivative (Fig. 1)	This work
pOHP469	Tc^r; *lon* gene (*Eco*RI digestion after insertion of an *Eco*RI linker in the *Eco*RV site of *plon*$^+$500) cloned in the *Eco*RI site of pACYC184	This work



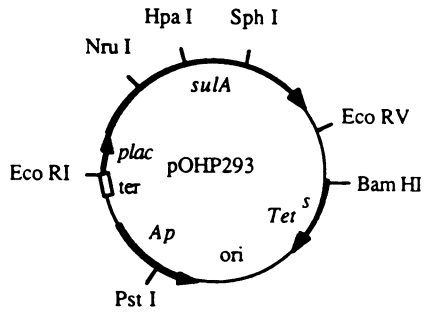


FIG. 1. Map of the plasmids pOHP241 (pUC8-derived plasmid) and pOHP293 (pBR322-derived plasmid). To construct the plasmid pOHP241, the plasmid pGC165 *sulA*$^+$ was cut by *Eco*RI (between p_{lac} and *sulA*) and then digested by the BAL 31 enzyme to delete the SOS box (binding site of the SOS repressor, LexA) to obtain better expression of *sulA*. To construct the pOHP293 plasmid, the p_{lac}-*sulA* fusion was cloned into the pBR322 plasmid after subcloning into the pRS414 plasmid to introduce upstream of the promoter a strong terminator. The *Pvu*II-*Bam*HI fragment of the pOHP241 plasmid was cloned in the pRS414 plasmid digested by *Sma*I-*Bam*HI; the plasmid obtained, pOHP280, was digested by *Pst*I-*Bam*HI and the fragment with the p_{lac}-*sulA* fusion was cloned into the pBR322 plasmid, also digested by *Pst*I-*Bam*HI.

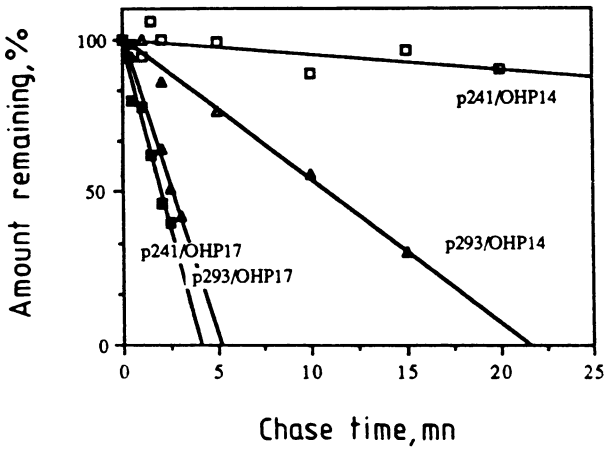


FIG. 2. Measurement of the stability of SulA produced by the *lacZp-sulA* fusion cloned on a multicopy plasmid. The half-lives were measured as described in Materials and Methods. The strains either contained (OHP17; closed symbols) or did not contain (OHP14; open symbols) the lactose repressor. The plasmids carrying the *lacZp-sulA* fusion were either a pBR322-derived plasmid (pOHP293; triangles) or a pUC8-derived plasmid (pOHP241; squares).

and incubated with shaking at 30°C. At an optical density at 600 nm of 0.6, cells were diluted twofold with isopropyl-β-D-thiogalactopyranoside added to a concentration of 5mM and incubated for 30 min at 30°C. The cells were then labeled with 100 μCi of [^{35}S]methionine (Amersham; 1,000 Ci/mmol) at 30°C for 30 s for the *lon*$^+$ strain (OHP17) or for 2 min for the *lon* strain (OHP7). The label was chased by the addition of nonradioactive methionine (final concentration, 0.025%). At given time points, 100-μl samples were removed and prepared by the Laemmli method (21) for loading onto a 15% sodium dodecyl sulfatepolyacrylamide gel. After electrophoresis, the labeled SulA protein bands were identified by autoradiography and the corresponding bands were cut out, solubilized in NCS (Amersham), and evaluated for radioactivity in a scintillation counter.

RESULTS

The overproduction of SulA can saturate the Lon protease. The effect of overproduction of SulA protein on its sensitivity to degradation by Lon was measured by determining its half-life. The SulA gene was cloned under control of the lactose promoter on two multicopy plasmids, pBR322 and pUC8; the copy number of the pUC8 derivative is 5 to 10 times higher than that of pBR322 derivative (30). The half-life of SulA was measured in strains containing the *sulA* gene in multicopy with or without the lactose repressor. In a strain containing either of the *sulA* clones, in the presence of the lactose repressor (F′ *lacI*^{q}), the half-life of SulA is 2 min. In strains without the repressor, i.e., SulA is overproduced, the half-lives are 10 min (pOHP293: pBR322-derived plasmid) and about 100 min (pOHP241: pUC8-derived plasmid) (Fig. 2). The higher stability of SulA overproduced by pUC18 is probably not due to the formation of inclusion bodies because a biphase stability curve was not observed.

As SulA overproduction seems to saturate Lon and thus limit its own degradation, we tested its effect on other Lon substrates. The RcsA protein is the transcriptional activator of the *cps* (capsular mucopolysaccharide synthesis) gene. A *lon*$^+$ strain which contains a *cps*::*lac* operon fusion on its chromosome (OHP8) is phenotypically Lac$^-$ because the activator is degraded by the Lon protease and the *cps* genes are not transcribed. If the plasmids overproducing SulA (pOHP241 or pOHP293) are introduced in this strain (OHP8), the cells have a Lac$^+$ phenotype; we interpret this result as an accumulation of RcsA caused by the saturation of the Lon protease by the excess of SulA. Thus, the RcsA protein seems to be protected from Lon degradation by SulA overproduction.

A second substrate of the protease, the λ phage protein Ots, was tested. At 39°C in the *lon*$^+$ strain (OHP14), the phage cannot grow (because of degradation of Ots) and no plaques form on the plates, whereas in the *lon* strain the phage grow and plaques form at normal frequency (13). In a *lon*$^+$ strain containing the SulA-overproducing plasmid pOHP241 or pOHP293, plaques are present at 39°C; this stabilization of Ots protein is another indication of the saturation of the Lon protease. If the explanation for the above results is that Lon is present in limiting amounts and can be saturated by an abundance of substrate, the introduction of a Lon overproducer should reverse this saturation. A multicopy plasmid containing the *lon* gene (pOHP469; Table 2) was introduced into strains OHP8 or OHP14 together with SulA-overproducing plasmids (pOHP293 or pOHP241). As

TABLE 3. Characteristics of the SulA mutants obtained from hydroxylamine mutagenesis

Plasmid	Filamentation[a]		Competition[b]		SulA half-life (min)	
	lon$^+$	*lon*	Ots	RcsA	*lon*$^+$	*lon*
pUC8	−	−	−	−		
pOHP241 (wt)	+	+++	++	++	2	55
Mutant						
A	−	+++	−	−	2	60
B	−	+++	−	−	2	50
C	−	+++	+	−	5	60
D	+	+++	+++	−	5	55
E	+	+++	+	−	2	60

[a] Symbols: −, no filaments observed; +, small filaments observed; +++, large filaments observed.
[b] Symbols: −, no competition; +, low levels of competition; ++, wild-type levels of competition; +++, higher levels of competition.

TABLE 4. Characteristics of the SulA C-terminus deletions

Plasmid	No. of aa in protein	Filamentation[a]		Competition[b]		SulA half-life (min)	
		lon$^+$	*lon*	Ots	RcsA	*lon*$^+$	*lon*
pBR322		−	−	−	−		
pOHP293 wild type	169	+	+++	++	++	2.5	46
Deletion							
1	71	−	−	−	−	ND[c]	ND
2	113	−	−	−	−	2	40
3	127	−	−	−	−	2.5	48
4	149	−	−	−	−	2	35
5	169	+	+++	++	++	2	44
6	169	+	+++	++	++	2	42

[a] Symbols: −, no filaments observed; +, small filaments observed; +++, large filaments observed.
[b] Symbols: −, no competition; ++, wild-type levels of competition.
[c] ND, Not done.

above, Lon activity was measured by the Lac phenotype of the *cps*::*lac* fusion (in strain OHP8) or by the ability of the λ phage Ots to grow at 39°C (in strain OHP14). The Lac$^+$ phenotype was reversed, and no growth of the λ phage Ots at the nonpermissive temperature was observed in the presence of pOHP469 in the appropriate strains tested. Thus, protection of RcsA or the abnormal protein Ots permitted by the overproduction of SulA was abolished by the introduction of the Lon-overproducing plasmid. These results indicate that the Lon protease is the limiting element in the degradation of proteins.

In vitro mutagenesis of the *sulA* gene on a multicopy plasmid. We have demonstrated that, in a *lon*$^+$*cpsB*::*lacZ* strain, the introduction of the plasmid overproducing SulA conferred a Lac$^+$ phenotype, probably by stabilizing RcsA. We asked if SulA mutants could be generated so that the second "natural" substrate, RcsA, is not protected from degradation by Lon and the strain would be Lac$^-$. Such SulA mutants may not be recognized by Lon, i.e., are Lon insensitive. To test this, plasmid pOHP241 was mutagenized with hydroxylamine and used to transform strain OHP8. Transformants were examined on MacConkey lactose plates for white Lac$^-$ colonies among red Lac$^+$ colonies. Plasmid DNA was prepared from 73 white colonies and used to retransform the strain to be sure that the mutation responsible for the Lac$^-$ phenotype was plasmid associated. Restriction enzyme patterns were analyzed to eliminate the plasmids in which DNA rearrangements had occurred. For the 60 remaining clones, we carried out a 30-s [^{35}S]methionine pulse-label after isopropyl-β-D-thiogalactopyranoside induction in *lon*$^+$ strains and analyzed the SulA product on polyacrylamide gels. Bands corresponding to SulA in size were excised and counted in a scintillation counter. All clones producing an abnormal amount of protein (promoter mutations) or a protein of abnormal size (nonsense mutations) were discarded. Five mutants were retained after this test.

Biological activity of *sulA* mutants. For the five *sulA* mutants, we tested cell filamentation in *lon* or *lon*$^+$ strains, the half-life of the SulA protein, and competition with an abnormal protein, Ots (Table 3). Every plasmid expressing mutated SulA induced filamentation in the *lon* strain after isopropyl-β-D-thiogalactopyranoside induction, indicating that the mutant proteins were still active in their ability to interact with SulB and inhibit cell division. As the mutants were selected indirectly, i.e., for their inability to protect RcsA from degradation by Lon, we then tested directly whether their own degradation by Lon was altered (Table 3). The half-life measurements show that none of the mutants was significantly stabilized: half-lives were between 2 and 5 min, compared with 2 min for the wild type. The protective effects of the SulA mutant proteins with respect to Ots were not all diminished as they were for RcsA; two of the mutants lost their ability to protect Ots from degradation, while the other three protected Ots. These results suggest that Lon may have several aa sequence specificities, one for native substrates and possibly one for abnormal substrates.

C-terminal deletions. Results of maxicell experiments (20) suggested that SulA cleavage by Lon takes place at a polypeptide end. Since the C-terminal region of SulA contains a sequence similar to the Lon cleavage site of the lambda N protein (4-aa identity in a 9-aa box; 7, 23) and is the conserved region in the family of known SulA-related proteins (the unique N-terminal 30-aa sequence is thought to be the part of SulA interacting with SulB) (7), it was proposed that the C-terminal end is recognized by Lon (7). To test this, we generated C-terminal deletions in the SulA protein. After digestion by BAL 31 enzyme and insertion of *Xba*I linkers at the end of the deletion, the DNAs of the clones obtained were analyzed by restriction enzymes. The C-terminal endpoints of the mutants were precisely determined by sequencing the region adjacent to the inserted *Xba*I linker.

Four deletions were obtained in the *sulA* gene (deletions 5 and 6 are at 22 and 35 nucleotides after the TAA stop codon, respectively). Activities of these mutants were examined (Table 4). In every case, the deleted SulA protein lost all its known characteristics; the mutants did not induce filamentation in a *lon* strain and were unable to protect either RcsA or Ots from Lon degradation. Nevertheless, the proteins were still actively degraded by Lon, with a half-life in *lon*$^+$ strains equal to that of the wild-type protein. We conclude that the N-terminal 113 aa of SulA are recognized by the Lon protease, possibly by a secondary specificity. In addition, the activity of SulA depends at least in part on the integrity of the C-terminal end of the protein, as deletion of the C-terminal 20 aa resulted in inactivation of SulA.

DISCUSSION

In this work we have shown that it is possible to saturate degradation by the Lon system: the overproduction of one substrate of Lon protease is able to protect a second substrate from Lon degradation. This protection is active on

either a natural substrate (RcsA) or a mutant protein (Ots) which may be recognized as an abnormal protein. This observation, together with the fact that degradation is restored by introduction of a Lon-overproducing plasmid, suggests that the limiting element is the protease itself.

By using these observations, we isolated five mutants of SulA which have lost the ability to protect a natural substrate, RcsA, from Lon; these were expected to have lost their Lon recognition sites. All of these SulA mutants retained the capacity to induce filamentation in a *lon*$^+$ strain, indicating that their interaction with SulB is correct. Surprisingly, of five such mutants isolated, all were still recognized by Lon (the half-lives of the mutants obtained were 1- to 2.5-fold that of nonmutated SulA) and they were not degraded in a *lon* strain. However, these mutants had different effects on a second Lon substrate; while two mutants lost the ability to protect the abnormal protein Ots from Lon degradation, one had an even greater ability to protect it. Two mutants showed intermediate phenotypes with respect to Ots. To explain these results, we propose that Lon has two active sites, one with a high affinity for specific substrates such a RcsA and SulA and the second with low affinity for secondary sites exposed on abnormal proteins. Mutants of SulA which have lost the primary recognition site may no longer compete with RcsA for high-affinity sites. However a SulA mutant protein may take on a new conformation which could change the availability of its low-affinity sites for Lon. The competition would be hierarchical: high-affinity active sites compete and are more active than the low-affinity sites. However, the latter are also in competition. Thus, SulA mutants no longer compete with the high-affinity RcsA sites but, having undergone structural changes, will compete with Ots. These proposals are still at a speculative stage; a more quantitative measure of Lon activity and a comparative measure of its affinities are necessary.

The hypothesis that Lon has several specificities has been previously suggested (9). It will be of interest to examine the peptide digestion patterns of SulA and the mutants to see if the latter differs from the parental. A more direct approach to identify Lon protease specificity is to look for SulA mutants which give rise after induction to irreversible filamentation and cell death; this mutant may be Lon resistant. Such mutants could be studied for their effects on the degradation of other Lon substrates.

We have shown that the elimination of a small part of the C-terminal sequence of the SulA protein is correlated with the loss of both the saturation and filamentation properties but does not affect protein half-life. Even with the shortest deletion (20 out of 169 aa from the C terminus), filamentation is abolished in the *lon* strain, possibly because of an inability of mutant SulA to interact with SulB. This loss of functional activity is concomitant with the loss of the ability to protect the second substrate from degradation by Lon. Nevertheless, these truncated proteins are still substrates for Lon degradation because their half-lives are comparable to that of wild-type SulA and they are stabilized in the *lon* strain (Table 4). These results are consistent neither with the proposal that SulA is cleaved exclusively by Lon at its C-terminal end (7) nor with the idea that interaction with SulB is affected only by the N-terminal end. However, they are consistent with the hypothesis described above, i.e., that Lon has two pathways of degradation; a specific one, whose target may be deleted from the truncated SulA proteins, and a nonspecific pathway, to which the deleted proteins become sensitive. This secondary activity may be due to Lon or controlled by it. The deleted proteins cannot compete with specific substrates of Lon (RcsA) nor with a nonspecific substrate (Ots). Their recognition, like that of mutants A and B (Table 3), may not have sufficient affinity to saturate the Ots degradation.

All of these results show that the three phenotypes, i.e., filamentation, saturation, and stability, are separable: a SulA mutant can induce filamentation but lose its ability to protect RcsA from degradation, but without appreciably changing its own susceptibility to Lon.

Lon proteolysis is more complex than the recognition of a simple signal on the substrate and the degradation of proteins that carry this signal, since even with the deletion of nearly half of the SulA protein we could not obtain a protein resistant to degradation. The obtainment of proteins that are not degradable by Lon could be rendered more difficult in view of our results which suggest that Lon has more than one specificity (9), so that more than one mutation would be necessary to obtain such a mutant. Another difficulty could be that this kind of mutant is toxic to the cell by induction of an irreversible filamentation.

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LITERATURE CITED

1. **Apte, B. N., H. Rhodes, and D. Zipser.** 1975. Mutation blocking the specific degradation of reinitiation polypeptides in *E. coli*. Nature (London) **257**:329–331.
2. **Bukhari, A. I., and D. Zipser.** 1973. Mutants of *Escherichia coli* with a defect in the degradation of nonsense fragment. Nature (London) New Biol. **243**:238–241.
3. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
4. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid J. Bacteriol. **134**: 1141–1156.
5. **Charette, M. F., G. W. Henderson, and A. Markovitz.** 1981. ATP hydrolysis-dependent protease activity of the *lon* (*capR*) protein of *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **78**:4728–4732.
6. **Chung, C. H., and A. L. Golberg.** 1981. The product of the *lon* (*capR*) gene in *Escherichia coli* is the ATP-dependent protease, protease La. Proc. Natl. Acad. Sci. USA **78**:4931–4935.
7. **Freudl, R., G. Braun, N. Honore, and S. T. Cole.** 1987. Evolution of the enterobacterial *sulA* gene: a component of the SOS system encoding an inhibitor of cell division. Gene **52**:31–47.
8. **Gottesman, S.** 1987. Regulation by proteolysis, p. 1308–1312. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
9. **Gottesman, S.** 1989. Genetics of proteolysis in *Escherichia coli*. Annu. Rev. Genet. **23**:163–198.
10. **Gottesman, S., M. E. Gottesman, J. E. Shaw, and L. Pearson.** 1981. Protein degradation in *E. coli*: the *lon* mutation and bacteriophage lambda N and CII protein stability. Cell **24**:225–233.
11. **Gottesman, S., E. Halpern, and P. Trisler.** 1981. Role of *sulA* and *sulB* in filamentation by *lon* mutants of *Escherichia coli* K-12. J. Bacteriol. **148**:265–273.
12. **Gottesman, S., P. Trisler, and A. Torres-Cabassa.** 1985. Regu-

lation of capsular polysaccharide synthesis in *Escherichia coli* K-12: characterization of three regulatory genes. J. Bacteriol. **162**:1111–1119.
13. **Gottesman, S., and D. Zipser.** 1978. Deg phenotype of *Escherichia coli lon* mutants. J. Bacteriol. **113**:844–851.
14. **Holland, I. B., and C. Jones.** 1985. The role of the FtsZ protein (SfiB) in UV-induced division inhibition and in the normal *E. coli* cell division cycle. Ann. Microbiol. **136A**:165–171.
15. **Howard-Flanders, P., E. Simson, and L. Theriot.** 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K12. Genetics **49**:237–246.
16. **Hua, S. S., and A. Markovitz.** 1972. Multiple regulatory gene control of the galactose operon in *Escherichia coli* K-12. J. Bacteriol. **110**:1089–1099.
17. **Huisman, O., R. D'Ari, and J. George.** 1980. Further characterization of *sfiA* and *sfiB* mutation in *Escherichia coli*. J. Bacteriol. **144**:185–191.
18. **Huisman, O., R. D'Ari, and S. Gottesman.** 1984. Cell-division control in *Escherichia coli*: specific induction of the SOS function SfiA protein is sufficient to block septation. Proc. Natl. Acad. Sci. USA **81**:4490–4494.
19. **Huisman, O., M. Jacques, R. D'Ari, and L. Caro.** 1983. Role of the *sfiA*-dependent cell division regulation system in *Escherichia coli*. J. Bacteriol. **153**:1072–1074.
20. **Jones, C. A., and I. B. Holland.** 1985. Role of the SulB (FtsZ) protein in division inhibition during the SOS response in *E. coli*: FtsZ stabilizes the inhibitor SulA in maxicells. Proc. Natl. Acad. Sci. USA **82**:6045–6049.
21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
22. **Mackie, G., and D. B. Wilson.** 1972. Regulation of the *gal* operon of *E. coli* by the *capR* gene. J. Biol. Chem. **247**:2973–2978.
23. **Maurizi, M. R.** 1987. Degradation *in vitro* of bacteriophage lambda N protein by Lon protease from *Escherichia coli*. J. Biol. Chem. **262**:2696–2703.
24. **Maurizi, M. R., P. Trisler, and S. Gottesman.** 1985. Insertional mutagenesis of the *lon* gene in *E. coli*: *lon* is dispensable. J. Bacteriol. **164**:1124–1135.
25. **Menon, A. S., L. Waxman, and A. L. Goldberg.** 1987. The energy utilized in protein breakdown by ATP-dependent protease (La) from *Escherichia coli*. J. Biol. Chem. **262**:722–726.
26. **Messing, J., R. Crea, and P. N. Seeburg.** 1981. A system for shotgun DNA sequencing Nucleic Acids Res. **9**:309–321.
27. **Miller, C. G.** 1987. Protein degradation and proteolytic modification, p. 680–691. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
28. **Miller, J. H.** 1972. Experiment in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Mitzusawa, S., and S. Gottesman.** 1983. Protein degradation in Escherichia coli: the *lon* gene controls the stability of SulA protein. Proc. Natl. Acad. Sci. USA **80**:358–362.
30. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Sanger, F., S. Nickler, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitor. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
32. **Shineberg, J. B., and D. Zipser.** 1973. The *lon* gene and degradation of β-galactosidase nonsense fragments. J. Bacteriol. **116**:1469–1473.
33. **Simons, R. W., F. Houman, and N. Kleckner.** 1987. Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. Gene **53**:85–96.
34. **Torres-Cabassa, A. S., and S. Gottesman.** 1987. Capsule synthesis in *Escherichia coli* K-12 is regulated by proteolysis J. Bacteriol. **169**:981–989.
35. **Trisler, P., and S. Gottesman.** 1984. *lon* transcriptional regulation of genes necessary for capsular polysaccharide synthesis in *Escherichia coli* K-12. J. Bacteriol. **160**:184–191.
36. **Truitt, C. L., W. G. Haldenwang, and J. R. Walker.** 1976. Interaction of host and viral regulatory mechanisms: effect of the *lon* cell division defect on regulation of repression by bacteriophage lambda. J. Mol. Biol. **105**:231–244.
37. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
38. **Walker, J. R., C. L. Ussery, and J. S. Allen.** 1973. Bacterial cell division regulation: lysogenization of conditional cell division *lon* mutants of *Escherichia coli* by bacteriophage lambda. J. Bacteriol. **111**:1326–1332.
39. **Waxman, L., and A. L. Goldberg.** 1986. Selectivity of intracellular proteolysis: protein substrates activate the ATP-dependent protease (La). Science **232**:500–503.
40. **Zehnbauer, B. A., and A. Markovitz.** 1980. Cloning of gene *lon* (*capR*) of *Escherichia coli* K-12 and identification of polypeptides specified by the cloned deoxyribonucleic acid fragment. J. Bacteriol. **143**:852–863.