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Genetics

An *Escherichia coli* mutation preventing degradation of abnormal periplasmic proteins

(protease/protein breakdown/gene fusions/hybrid proteins)

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ABSTRACT **A fusion between *tsr* (encoding the inner membrane protein Tsr) and *phoA* (encoding the periplasmic protein alkaline phosphatase, AP) generates a membrane-bound hybrid protein (Tsr-AP 2) with AP enzymatic activity. The hybrid protein is proteolytically unstable and is broken down to yield a smaller, soluble species with AP activity. We devised a genetic screen to distinguish between cells containing only membrane-bound AP and those containing soluble AP. The screen depends on diffusion of soluble AP away from cells with a leaky outer membrane to produce a halo of AP activity around colonies on solid growth medium. Several mutants lacking this halo show reduced degradation of Tsr-AP 2. One mutant is also defective in breakdown of five other abnormal periplasmic proteins but not of two cytoplasmic proteins. The mutation in this strain, *degP4*::Tn*5*, defines a locus distinct from previously identified loci that affect protein stability or protease activities. This strain may be useful for preventing the breakdown of unstable foreign proteins in *Escherichia coli*.**

Proteolysis plays an important role in the physiology of *Escherichia coli*. Abnormal proteins are rapidly degraded (1) and normally stable proteins are degraded during starvation for various nutrients (2). In addition, several important regulatory proteins are quite unstable, among them the *sulA* gene product of *E. coli* (3) and the cII and *N* gene products of bacteriophage λ (4, 5). For SulA and cII, the rapid turnover is important for the regulatory roles these proteins play (3–5). The LexA protein and λ repressor are normally stable in *E. coli*, but following DNA damage, rapid cleavage of these proteins by the RecA protease induces DNA-repair mechanisms and bacteriophage production (6). Proteolysis is also a major component of the heat shock response (7, 8).

A number of *E. coli* proteases have been identified and purified. These enzymes are found in the cytoplasm (9), the periplasm (9), the inner membrane (10), and the outer membrane (10). The isolation of mutants defective in a particular protease is essential in determining the *in vivo* substrates and physiological role of the enzymes. However, in only one case has a genetic selection been devised for mutants defective in general proteolysis. A selection for stabilization of proteolytically sensitive amber fragments of proteins yielded *lon* mutants (11, 12). Protease III mutants were obtained by enzymatically assaying mutagenized colonies for loss of protease activity (13), and the isolation of *recA* mutants was not based on knowledge of the function of RecA as a protease (6).

In this paper we describe a general approach to the isolation of mutants defective in proteolysis in the *E. coli* cell envelope. To identify proteases in the periplasm or membranes of *E. coli*, we have isolated and characterized mutations that stabilize hybrid proteins in these compartments. These hybrid proteins were made with Tn*phoA*, a transposon that can generate enzymatically active protein fusions of alkaline phosphatase (AP) to periplasmic or membrane proteins (14). One such fusion to the inner membrane protein Tsr generates a hybrid protein that is membrane-bound and proteolytically unstable. This hybrid protein is broken down to yield a smaller species, which contains the AP moiety of the fusion and is a soluble periplasmic protein (15). We have exploited the change in location of AP from membrane to periplasm to devise a genetic screen for mutants that fail to break down this unstable protein. Such mutants appear to lack periplasmic or membrane proteases of *E. coli* and will be useful for identifying these enzymes and determining their functions. One of these mutations defines a locus of *E. coli* that we have named *degP*. The *degP* mutant is deficient in proteolysis of several fusion proteins and mutant proteins located in the periplasm or inner membrane.

MATERIALS AND METHODS

Media and Chemicals. Standard growth media have been described (16). Ampicillin (200 μg/ml), kanamycin (40 μg/ml), and tetracycline (20 μg/ml) were used as needed. X-*P* (5-bromo-4-chloro-3-indolyl phosphate) was obtained from Bachem Fine Chemicals (Torrance, CA).

Bacterial Strains and Plasmids. Bacterial strains are described in Table 1. All strains with KS prefixes are derivatives of MC1000 [F⁻ Δ(*ara-leu*)*7697 galE galK* Δ*lacX74 rpsL*(Str^r)]. Genetic nomenclature (18) and standard genetic techniques (16, 19) have been described. The *lpp5508* allele (17) was introduced into KS272 by transduction to Tet^r Aro⁻ with a bacteriophage P1 lysate grown on a donor containing a Tn*10* insertion 95% linked to *aroD6* (this Tn*10* was isolated in AT1360 by standard methods). The resulting *aroD* strain was transduced to Aro⁺ with a P1 lysate grown on strain JE5513 and transductants were screened for Tet^s and for ribonuclease leakage (*lpp* phenotype) by the method of Lopes *et al.* (20); one such transductant was saved as KS303. KS330 and KS332 were constructed by transducing KS303 to kanamycin resistance (Kan^r) with P1 lysates grown on KS325 and KS329, respectively. After transformation with pKS3, KS330/pKS3 and KS332/pKS3 were phenotypically indistinguishable from KS325 and KS329, respectively, with respect to proteolytic breakdown of Tsr-AP 2.

Plasmids pCM204 and pCM203 (15) carry *tsr*::Tn*phoA* insertions leading to production of Tsr-AP fusion proteins 1 and 2, respectively (Fig. 1) and confer ampicillin resistance (Amp^r) and Kan^r. The determinant for Kan^r was removed by *Xho* I and *Sal* I digestion, ligation, and screening Amp^r transformants for Kan^s. The resulting plasmids were pKS1 (encoding Tsr-AP fusion 1) and pKS3 (encoding Tsr-AP fusion 2). Plasmid p*malE*, encoding Amp^r and maltose-

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Abbreviations: AP, alkaline phosphatase; EtMes, ethyl methanesulfonate; MBP, maltose-binding protein; X-*P*, 5-bromo-4-chloro-3-indolyl phosphate.

Table 1. Bacterial strains

Strain	Genotype	Reference
KS272	F⁻ Δ*lac-X74 galE galK rpsL*(Str^r) Δ*phoA*(*Pvu* II)	This study
KS303	KS272 *lpp-5508*	This study
KS323	KS303 pKS1	This study
KS324	KS303 pKS3	This study
KS325	KS324 *degP4*::Tn*5*	This study
KS329	KS324 *deg-K16*::Tn*5*	This study
KS330	KS303 *degP4*::Tn*5*	This study
KS332	KS303 *deg-K16*::Tn*5*	This study
JE5513	Hfr Cavalli *lpp-5508 man-1 pps*	17
AT1360	*proA2 aroD6 his-4 argE3 thi-1 lacY1 galK2 xyl-5 mtl-1 tsx-29 supE44*	B. Bachmann*

*Yale University, New Haven, CT.

binding protein (MBP), was provided by Catherine Lee (Harvard Medical School). This plasmid contains an *Eco*RI–*Stu* I fragment from the *malB* region of *E. coli* (21) ligated to the *Pvu* II–*Eco*RI fragment of pBR322. Tn*phoA* insertions into p*malE* that generate MBP-AP protein fusions were isolated as described (14). Plasmids p*malE*::Tn*phoA15-1* and p*malE*::Tn*phoA18-1* encode MBP-AP fusion proteins of M_r 70,000 and 77,000, respectively. Plasmids pDNC116, pDNC135, pDNC154, and pDNC155 (obtained from David Collier and Philip Bassford, University of North Carolina at Chapel Hill) all encode Amp^r and carry a *malE* gene under the control of the P_{lacUV5} promoter. pDNC116 (*malEΔ116 malE18-1*) encodes MBPΔ116 (in which amino acids 142–150 are deleted; D. Collier, V. A. Bankaitis, J. B. Weiss, and P. J. Bassford, Jr., personal communication) with a defective signal sequence due to the *malE18-1* mutation (22, 23). pDNC135 (*malEΔ116*) encodes MBPΔ116. pDNC154 (*malEΔ57–145*) encodes MBPΔ57–145 (in which amino acids 57–145 are deleted; D. Collier, V. A. Bankaitis, J. B. Weiss, and P. J. Bassford, Jr., personal communication). pDNC155 (*malEΔ57–145 malE19-1*) encodes MBPΔ57–145 with a defective signal sequence due to the *malE19-1* mutation (22, 23).

Isolation of Proteolysis-Deficient Strains. Strain KS324 was mutagenized with ethyl methanesulfonate (EtMes) or transposon Tn*5*. Mutagenized cells were plated on TYE agar plus ampicillin at 37°C to give approximately 200 colonies per plate. After overnight incubation the colonies were replica-plated to TYE agar plus ampicillin containing X-*P* at 100–200 μg/ml (150 μg/ml was eventually chosen as an optimal concentration) and were incubated overnight at 37°C. Strains that showed a reduced halo of AP around the patch of replica-plated cells were purified for further characterization.

Cell Fractionation. Periplasmic proteins were separated from the cell by osmotic shock as described (15) except that cells were not radioactively labeled and the osmotically shocked cells were not subjected to further fractionation.

AP Assay. AP activity was assayed by measuring the rate of *p*-nitrophenyl phosphate (obtained from Sigma) hydrolysis by osmotic-shock fluids or osmotically shocked cells, as described (24).

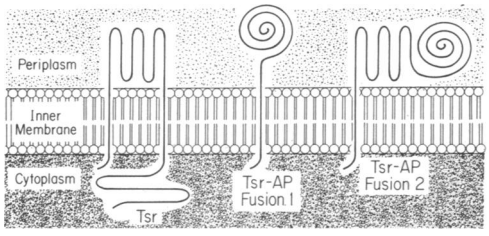

FIG. 1. Structure of Tsr and Tsr-AP hybrid proteins (15). The spiral line represents the AP moiety of the hybrid proteins. This AP moiety is the C-terminal portion of the fusion proteins.

Cell Growth and Radioactive Labeling. Proteins were radioactively labeled in cultures growing exponentially at 37°C in M63 medium supplemented with thiamine (1 μg/ml), each of the common amino acids (50 μg/ml) except methionine and cysteine, and carbon sources as stated in each experiment, by exposing them to [^{35}S]methionine at 50–100 μCi/ml (1 μCi = 37 kBq) for 2 min. Chase was initiated by addition of unlabeled methionine to 100 μg/ml.

Antibody Precipitation. Radioactively labeled proteins were precipitated with trichloroacetic acid, dissolved with $NaDodSO_4$, and immunoprecipitated with polyclonal antisera (to AP, MBP, or ribose-binding protein) as described (25). Radioactivity in labeled proteins was quantitated by digestion of gel slices with a tissue-solubilizing reagent and liquid scintillation counting as described (14).

RESULTS

Properties of Tsr-AP Protein Fusions. The properties of two fusion proteins, in which AP is fused to Tsr, suggested a genetic screen for protease-deficient mutants. Both proteins contain an N-terminal moiety derived from Tsr, an inner membrane protein, and a C-terminal moiety derived from AP, a periplasmic protein (Fig. 1). Both Tsr-AP 1 and Tsr-AP 2 are found in the membrane fraction of cells (15). However, Tsr-AP 1 is proteolytically stable, whereas Tsr-AP 2 is unstable (ref. 15 and Fig. 2). As Tsr-AP 2 is degraded, a smaller product with the approximate molecular weight of the AP moiety accumulates ('AP in Fig. 2). This species is found in the periplasmic fraction of cells (15). In cells expressing Tsr-AP 2, AP enzymatic activity can be found in both the membrane and periplasmic fractions (Table 2 and ref. 15), indicating that both the full-length hybrid protein and the proteolytic breakdown product are enzymatically active. Thus Tsr-AP 2 is a membrane-bound AP that is converted to a soluble AP by proteolysis.

Genetic Screen for Changes in Proteolysis of Tsr-AP 2. We expected that this difference in cellular location between the intact Tsr-AP 2 protein and its breakdown product could be

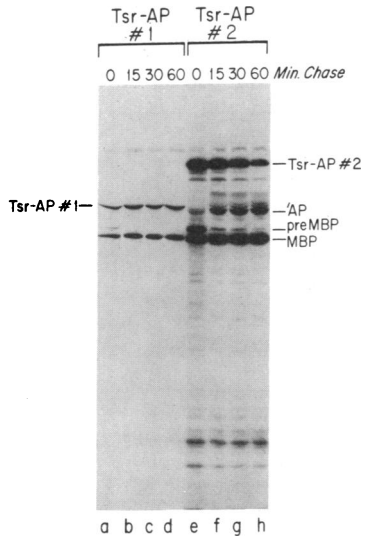

FIG. 2. Stability of Tsr-AP hybrid proteins. Cells were grown in M63 medium containing maltose (0.2%), ampicillin, and other supplements and were radioactively labeled as described in *Materials and Methods*. Lanes a–d, KS323; lanes e–h, KS324. Chase times are indicated above the gel lanes. Labeled proteins were immunoprecipitated with antisera to AP and MBP. 'AP, AP breakdown product derived from Tsr-AP 2 hybrid protein; preMBP, precursor to MBP.

Table 2. Subcellular distribution of AP in mutants

Strain	Tsr-AP hybrid present	AP distribution, %	
		Membranes + cytoplasm	Periplasm
KS323	1	93	7
KS324	2	22	78
1-1*	2	65	35
4-1*	2	65	35
K4 (KS325)†	2	95	5
K16 (KS329)†	2	83	17

Cells were grown in LB medium to mid-exponential phase, harvested by centrifugation, and washed to remove inorganic phosphate from the culture. The cells were then subjected to osmotic shock, and the osmotically shocked cells (membranes plus cytoplasm) or the osmotic-shock fluid (periplasm) were assayed for AP.
*Derived from KS324 by EtMes mutagenesis.
†Derived from KS324 by Tn*5* mutagenesis.

visualized in bacterial colonies on AP indicator medium if the soluble AP species were free to diffuse through the outer membrane and away from cells (Fig. 3). The presence of AP enzymatic activity within colonies or surrounding colonies on agar plates can be detected with X-*P*, which is hydrolyzed by AP to yield an insoluble blue product. In a strain with an outer membrane leaky to AP, the soluble species could diffuse away from cells and hydrolyze X-*P* in the surrounding medium, thus generating a blue halo around the colony. The membrane-bound AP species could only hydrolyze X-*P* within the colony and, because the hydrolyzed X-*P* is insoluble, the resulting blue color would be localized within the colony. Therefore, in a strain with only membrane-bound AP, the colony should be blue but there should be no halo of blue color surrounding the colony. The outer membrane was made permeable to AP with a lipoprotein-deficient mutation, *lpp-5508* (17), which allows periplasmic proteins to be released to the medium; 25% of wild-type AP is released to the culture medium by strains carrying this mutation (26).

Strains expressing Tsr-AP 1 (KS323) and Tsr-AP 2 (KS324) were tested on medium containing the AP indicator X-*P*. An appropriate concentration of X-*P* (≥100 μg/ml) was found at which KS324 had a distinct blue halo around replica-plated patches of cells, whereas KS323 lacked such a halo. We expected that protease-deficient mutants, expressing fusion 2 but unable to break down the hybrid protein, would resemble KS323 and lack a colored halo on these indicator plates.

Isolation of Protease Mutants. To identify protease-deficient mutants, KS324 was mutagenized and derivatives that had a reduced AP halo around colonies were isolated. From approximately 5000 EtMes-mutagenized colonies and 4000 Tn*5*-mutagenized colonies, 28 EtMes-induced mutants and 17 Tn*5*-induced mutants were chosen for further analysis. The cellular location of AP in these strains was measured by separating the periplasmic fraction from the cytoplasm and membranes by osmotic shock and assaying AP activity. Four strains were identified that had a lower percentage of AP in the periplasmic fraction than did the parent strain (Table 2). This behavior indicates reduced proteolysis of Tsr-AP 2. The proteolytic stability of Tsr-AP 2 in these strains was assayed directly by labeling with [^{35}S]methionine and immunoprecipitating the fusion protein with antiserum to AP (data not shown). In mutants 1-1 and 4-1 (both induced by EtMes) the half-life of the Tsr-AP 2 was approximately 30 min compared to 15 min in the parent strain. In mutants K4 and K16 (both induced by Tn*5*) no detectable breakdown of Tsr-AP 2 occurred during the course of the experiment (60 min).

Tn*10* insertions cotransducible with the mutations in strains 4-1, K4, and K16 were isolated and used to determine whether any of these mutations occurred at the same locus. We were not successful in identifying a Tn*10* insertion linked to the mutation in strain 1-1. P1 transductions showed that the mutations in 4-1, K4, and K16 were not cotransducible and therefore define three different loci (data not shown).

The two mutants with the strongest effect on the breakdown of Tsr-AP 2, K4 and K16, were tested to see if they reduced breakdown of other unstable fusion proteins. Two MBP-AP fusion proteins (MBP-AP 15-1 and MBP-AP 18-1) and a β-lactamase-AP fusion protein (14) were tested in both mutants. Mutant K4 stabilized all three of these proteins, whereas mutant K16 did not reduce breakdown of any of them (Fig. 4 and data not shown). Thus, the K4 mutation has pleiotropic effects on the stability of proteins, but the K16 mutation seems to be specific for Tsr-AP 2.

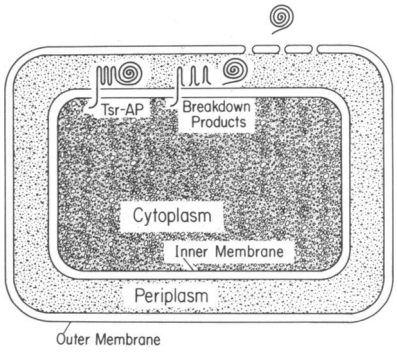

FIG. 3. Release of AP breakdown product of Tsr-AP 2 to growth medium from a strain with a leaky outer membrane.

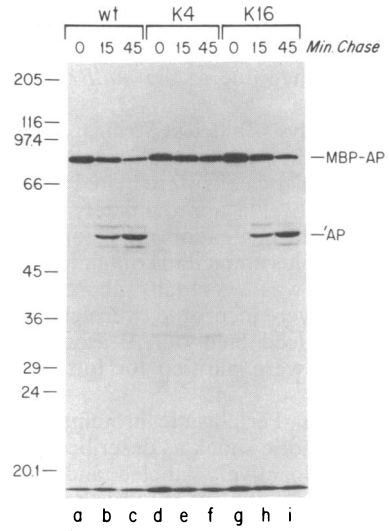

FIG. 4. Stability of an MBP-AP hybrid protein in mutant strains. Cells were grown in M63 medium containing glycerol (0.2%), ampicillin, and other supplements, as described in *Materials and Methods*. Maltose (0.2%) was added 90 min before radioactive labeling of the cultures. Lanes a–c, KS303/p*malE*::Tn*phoA18-1* (wild type, wt); lanes d–f, KS330/p*malE*::Tn*phoA18-1*; lanes g–i, KS332/p*malE*::Tn*phoA18-1*. Chase times are indicated above the gel lanes. Labeled proteins were immunoprecipitated with antiserum to AP. MBP-AP, MBP-AP hybrid protein 18-1. 'AP, AP breakdown product of MBP-AP hybrid protein. Positions of molecular weight markers ($M_r \times 10^{-3}$) are indicated at left. Quantitation of radioactivity in labeled protein bands showed that MBP-AP in lane f contained at least 84% as many cpm as MBP-AP in lane d. Lanes c and i contained 24% as many cpm in MBP-AP as lanes a and g, respectively.

The K4 mutation is able to stabilize AP fusion proteins located in the periplasm or inner membrane. We wished to determine (*i*) whether the K4 mutation could stabilize a protein unrelated to AP and (*ii*) whether the protease(s) affected by this mutation is found only in the periplasm and/or membrane or is located in the cytoplasm as well. Mutations in the gene (*malE*) that encodes MBP were used for this purpose. Native MBP is a stable periplasmic protein of 370 amino acids. However, two internal, in-frame deletions in the *malE* gene, *malEΔ116* and *malEΔ57–145*, render MBP unstable in the periplasm (ref. 27 and D. Collier, V. A. Bankaitis, J. B. Weiss, and P. J. Bassford, Jr., personal communication). The K4 mutation significantly reduced the degradation of both mutant MBP species (Fig. 5).

In addition, it was possible to examine the stability of these same mutant MBPs when they were localized to the cytoplasm. This aberrant localization was achieved by incorporation of a signal-sequence mutation into the already mutant *malE* genes. These proteins are also unstable when localized to the cytoplasm (D. Collier, V. A. Bankaitis, J. B. Weiss, and P. J. Bassford, Jr., personal communication). When the K4 mutation was introduced into strains producing these mutant MBPs in the cytoplasm, there was no increase in stability, in contrast to the results with the periplasmic forms of the proteins (Fig. 5).

We tested for linkage of the K4 mutation to other known loci that affect proteolysis in *E. coli*. We detected no linkage by P1 transduction between this mutation and a Tn*10* insertion near *lon* (obtained from S. Gottesman, National Institutes of Health, Bethesda, MD), a Tn*10* insertion (isolated in this laboratory) near *rpoH* (formerly designated *htpR*), and a Tn*10* insertion in *argA* (obtained from N. Kleckner, Harvard University), which is linked to *ptr*, the gene for protease III. Thus, K4 thus appears to define a new locus affecting proteolysis. We have chosen to designate this locus *degP* and the K4 mutation, *degP4*::Tn*5*.

The *degP4*::Tn*5* mutation is clearly not lethal to the cell, but it does affect cell growth. On agar plates, *degP* strains grow comparably to wild-type strains at 30°C, but at 37°C or 42°C *degP* colonies are somewhat smaller than the wild type. The growth abnormality is accentuated in *degP* strains with plasmids carrying the genes for some of the unstable proteins used in this work. For example, expression of Tsr-AP 2, MBP-AP 15-1, MBP-AP 18-1, or MBPΔ57-145 results in extremely poor growth of the *degP* mutant at 37°C or 42°C, although growth at 30°C appears to be normal. In contrast, expression of the β-lactamase-AP fusion or MBPΔ116 does not cause the *degP* mutant to grow poorly. (These proteins are less unstable than the other proteins mentioned above.) The growth of a wild-type strain is not dramatically affected by the synthesis of any of these six proteins.

DISCUSSION

We have described a genetic screen that distinguishes between colonies that release AP into the medium and those

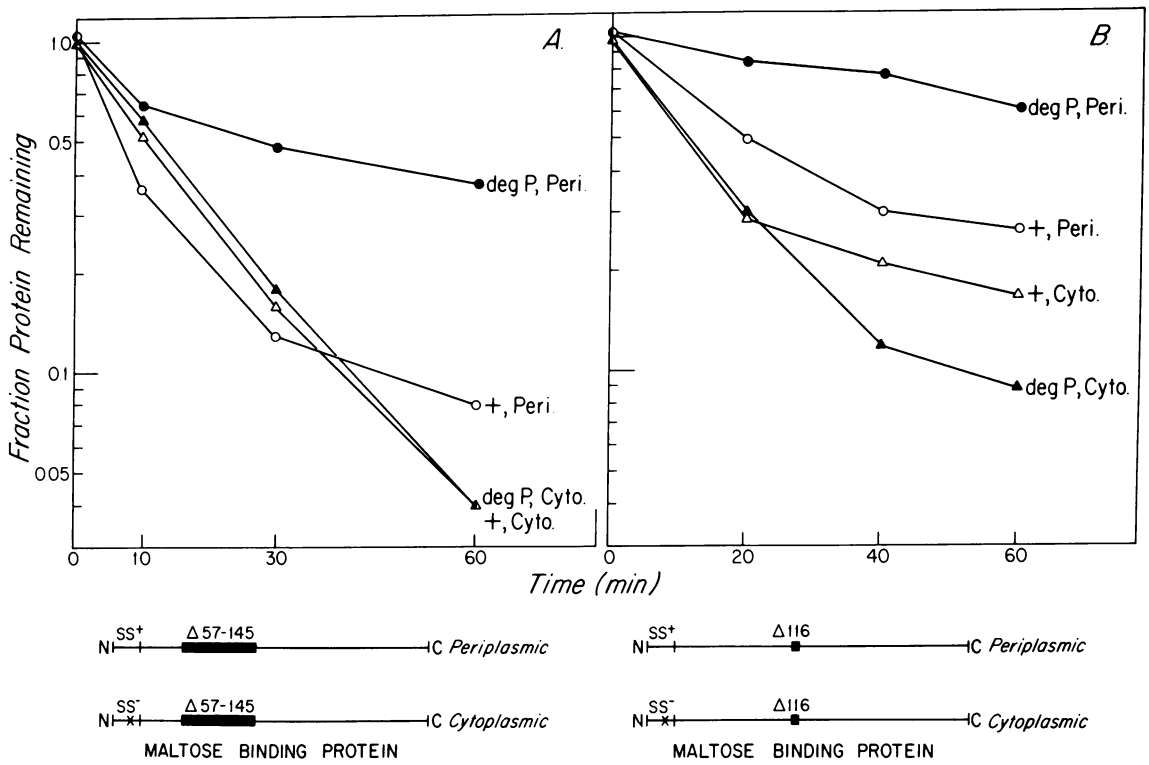

FIG. 5. Stability of MBPΔ57–145 and MBPΔ116 in the *degP* (K4) mutant. The structures of the MBP species tested are shown below the graphs. SS+, wild-type signal sequence; SS−, mutant signal sequence. (*A*) Cells were grown in M63 medium containing maltose (0.2%), isopropyl β-D-thiogalactopyranoside (1 mM), ampicillin, and other supplements, and were radioactively labeled as described in *Materials and Methods*. Wild-type MBP and MBPΔ57–145 were precipitated with antiserum to MBP. After $NaDodSO_4$/polyacrylamide gel electrophoresis the radioactivity in labeled protein bands was quantitated. The fraction of protein remaining is defined as (cpm in MBPΔ57–145/cpm in MBP)/[(cpm in MBPΔ57–145/cpm in MBP) at 0 min of chase]. ○, KS303 F′*lac*$^+$*I*^{q}/pDNC154; △, KS303 F′*lac*$^+$*I*^{q}/pDNC155; ●, KS330 F′*lac*$^+$*I*^{q}/pDNC154; ▲, KS330 F′*lac*$^+$*I*^{q}/pDNC155. (*B*) Cells were grown in M63 medium containing ribose (0.2%), ampicillin, and other supplements as described in *Materials and Methods*. Isopropyl β-D-thiogalactopyranoside (1 mM) was added 60 min before radioactive labeling of cells. Ribose-binding protein (RBP) and MBPΔ116 were precipitated with antisera to RBP and MBP. After $NaDodSO_4$/polyacrylamide gel electrophoresis, the radioactivity in labeled protein bands was quantitated. The fraction of protein remaining is defined as (cpm in MBPΔ116/cpm in RBP)/[(cpm in MBPΔ116/cpm in RBP) at 0 min of chase]. ○, KS303 F′*lac*$^+$*I*^{q}/pDNC135; △, KS303 F′*lac*$^+$*I*^{q}/pDNC116; ●, KS330 F′*lac*$^+$*I*^{q}/pDNC135; ▲, KS330 F′*lac*$^+$*I*^{q}/pDNC116.

that retain it in the cell envelope. This screen is similar to another screen for ribonuclease release to the medium, which was used to isolate leaky outer membrane mutations (20). In this study, the extracellular enzyme resulted from leakage of a periplasmic form of AP that is a proteolytic breakdown product of a membrane-bound fusion protein. Mutants lacking the extracellular AP also lack the periplasmic form and are deficient in proteolysis of the fusion protein. This screen depended on use of a strain with an outer membrane leaky to periplasmic proteins due to absence of lipoprotein. In a strain with a wild-type outer membrane, the halo screen could be used to study true secretion of proteins through the outer membrane. For instance, AP could be fused to a protein of this type. If the fusion protein is also secreted, the strain should exhibit a halo of AP around colonies, thus providing a screen for mutant cells affected in their ability to secrete this protein.

We have used this screen to identify mutants with alterations in the proteolytic degradation of a membrane-bound Tsr-AP fusion protein. The screen allowed us to identify several loci in which mutations stabilizing this protein occur. One of these mutations, *degP4*::Tn*5*, has pleiotropic effects, reducing the degradation of all six unstable periplasmic or inner membrane proteins that we tested. The unstable proteins tested are fusion proteins or proteins with internal deletions. We assume that these proteins are not properly folded and are therefore susceptible to proteolytic attack. Foreign proteins expressed in *E. coli* may fail to assume their native conformation and be similarly unstable. The *degP* mutant may be quite useful in reducing breakdown of such proteins in the periplasm of *E. coli*.

Although the *degP* mutation reduced degradation of MBPΔ116 and MBPΔ57-145, these proteins were still broken down to some extent in the *degP* mutant. This observation indicates that other protease activities remain in the cell. The halo screen used to isolate the *degP* mutant could be used to isolate mutations in these remaining protease activities. To accomplish this, one needs to identify a polypeptide domain that is unstable in a *degP* strain. This unstable domain could be incorporated into a membrane-bound AP fusion (for instance, it could be inserted between the Tsr and AP moieties of Tsr-AP 1). The resulting hybrid (or "tribrid") protein may be unstable in a *degP* strain because of the additional protein moiety, and, if so, it could be used to isolate protease mutants in the same way that Tsr-AP 2 was used.

The inner membrane protein, Tsr-AP 2, stabilized by the *degP* mutation, has a large soluble domain facing the periplasm. The ability of *degP4*::Tn*5* to stabilize Tsr-AP 2 and periplasmic proteins and its inability to stabilize cytoplasmic proteins suggest that the proteolytic activity missing in this mutant is located in the periplasm or perhaps in the inner or outer membrane with its active site facing the periplasm. Development of an *in vitro* assay for the activity deficient in this mutant should help define its location more accurately.

Although we have shown that the protease activity missing in *degP* strains is involved in degradation of several fusion proteins and proteins with internal deletions, we do not know what the normal physiological substrates of this protease are. Since the *degP* mutation is not lethal for the cell, this protease activity is not required for viability. However, expression of *degP* substrates at high levels can make cell growth quite poor. There are at least two possible explanations for this phenomenon. (*i*) The unstable proteins are toxic when not degraded and directly act to inhibit growth. (*ii*) The unstable proteins overwhelm other protease activities still present in the *degP* mutant and compete for degradation with native unstable *E. coli* proteins. The failure to degrade these postulated native unstable proteins then results in poor cell growth. We hope that examination of this poor cell growth and mutations that restore normal growth will give information about the normal substrates of the *degP* protease.

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