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The Acylated Precursor Form of the Colicin A Lysis Protein Is a Natural Substrate of the DegP Protease

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The acylated precursor form of the colicin A lysis protein ($pCal^m$) is specifically cleaved by the DegP protease into two acylated fragments of 6 and 4.5 kilodaltons (kDa). This cleavage was observed after globomycin treatment, which inhibits the processing of $pCal^m$ into mature colicin A lysis protein (Cal) and the signal peptide. The cleavage took place in *lpp*, *pldA*, and wild-type strains carrying plasmids which express the lysis protein following SOS induction and also in cells containing a plasmid which expresses it under the control of the *tac* promoter. Furthermore, the DegP protease was responsible for the production of two acylated Cal fragments of 3 and 2.5 kDa in cells carrying plasmids which overproduce the Cal protein, without treatment with globomycin. DegP could also cleave the acylated precursor form of a mutant Cal protein containing a substitution in the amino-terminal portion of the protein, but not that of a mutant Cal containing a frameshift mutation in its carboxyl-terminal end. The functions of Cal in causing protein release, quasi-lysis, and lethality were increased in *degP41* cells, suggesting that mature Cal was produced in higher amounts in the mutant than in the wild type. These effects were limited in cells deficient in phospholipase A. Interactions between the DegP protease and phospholipase A were suggested by the characteristics of *degP pldA* double mutants.

The colicin A lysis protein (Cal) is a small lipoprotein essential for colicin A secretion by *Escherichia coli* carrying the plasmid ColA (2, 5). Its structure is very homologous to that of other colicin lysis proteins (6). The *cal* gene coding for Cal is located in the same operon as the *caa* gene, which encodes colicin A (5, 15). Both genes are expressed after induction by SOS agents such as mitomycin but at different rates, due to the presence of a terminator between the *caa* gene and the downstream *cal* gene (15, 16).

The Cal protein (3), like all bacterial lipoproteins (26), is synthesized as a precursor form (pCal) of 51 amino acids, which is converted to a modified precursor form ($pCal^m$) by fixation of a diglyceride on the cysteine residue which will become the +1 amino acid of the mature form. $pCal^m$ is then processed by signal peptidase (SPase) II to yield the signal peptide and mature Cal, which contains 33 amino acids. The maturation of Cal is a slow process in contrast to that of the various lipoproteins that have been described. The Cal signal peptide is stable and accumulates along with mature Cal in induced cells. After globomycin treatment, which specifically blocks SPase II (9), $pCal^m$ does not accumulate as expected but appears to be hydrolyzed into two fragments without release of the signal peptide (3, 4).

In this article, we show that the enzyme involved in the degradation of the acylated precursor form of the colicin A lysis protein after globomycin treatment is the DegP protease. The DegP protease has recently been characterized by Strauch and Beckwith (24) as an enzyme hydrolyzing several fusion proteins and mutant proteins located in the periplasm or inner membrane of the cell. In *degP* bacteria carrying various plasmids encoding Cal, the modified precursor form of Cal, $pCal^m$, accumulates with time after globomycin treatment of induced cells without undergoing the degradation observed in wild-type cells. $pCal^m$ also accumulates to a greater extent in induced *degP* cells even without treatment with globomycin, indicating that a significant amount is usually destroyed by the DegP protease. The various effects of Cal on the cell, which include protein release, quasi-lysis, and cell death, are more pronounced in *degP* than in wild-type cells, but these effects are limited in cells carrying a *pldA* mutation, indicating that the requirement for activation of the outer membrane phospholipase A (22) for Cal function is not bypassed by the *degP* mutation. The *degP* mutation itself may result in activation of phospholipase A, since we observed that *degP* cells were leaky and that this leakiness was corrected in *pldA degP* cells.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* KS272 and its *degP41* derivative KS474 were from J. Beckwith (24). *E. coli* K-12 W3110 (Nal^r), CBM (W3110 *pldA*) (4), and JE5505 (*lpp pps his proA argE thi gal xyl mtl tsx*) (7) were used as the host strains. The *degP41* allele was introduced into these strains by transduction to kanamycin resistance with a bacteriophage P1 lysate grown on KS474.

Plasmid ColA9, which contains a wild-type colicin A operon, and the Cal-overproducing plasmids AT1 and CK4 have been described before (4, 15). The Cal mutant plasmids used were FS2 and A2 (8). Plasmid isolation and transformation were carried out by standard procedures (19).

Media and chemicals. Standard growth media and antibiotic concentrations were used as described before (19). Mitomycin (Sigma Chemical Co.) was used at 300 ng/ml, and IPTG (isopropyl-β-D-thiogalactopyranoside [Sigma]) was used at 1 mM. Globomycin, a gift from Mamoru Arai (Sankyo Co. Ltd., Tokyo), was used at 100 μg/ml.

Radioactive labeling. Cells were grown at 37°C with good aeration in M9 medium supplemented with thiamine (1 μg/ml), lactate (0.4%), required amino acids (50 μg/ml), and Casamino Acids (0.01%; Difco Laboratories). Cultures having an OD_{600} of 1 were induced with mitomycin or IPTG. Globomycin was added 10 min after mitomycin addition and

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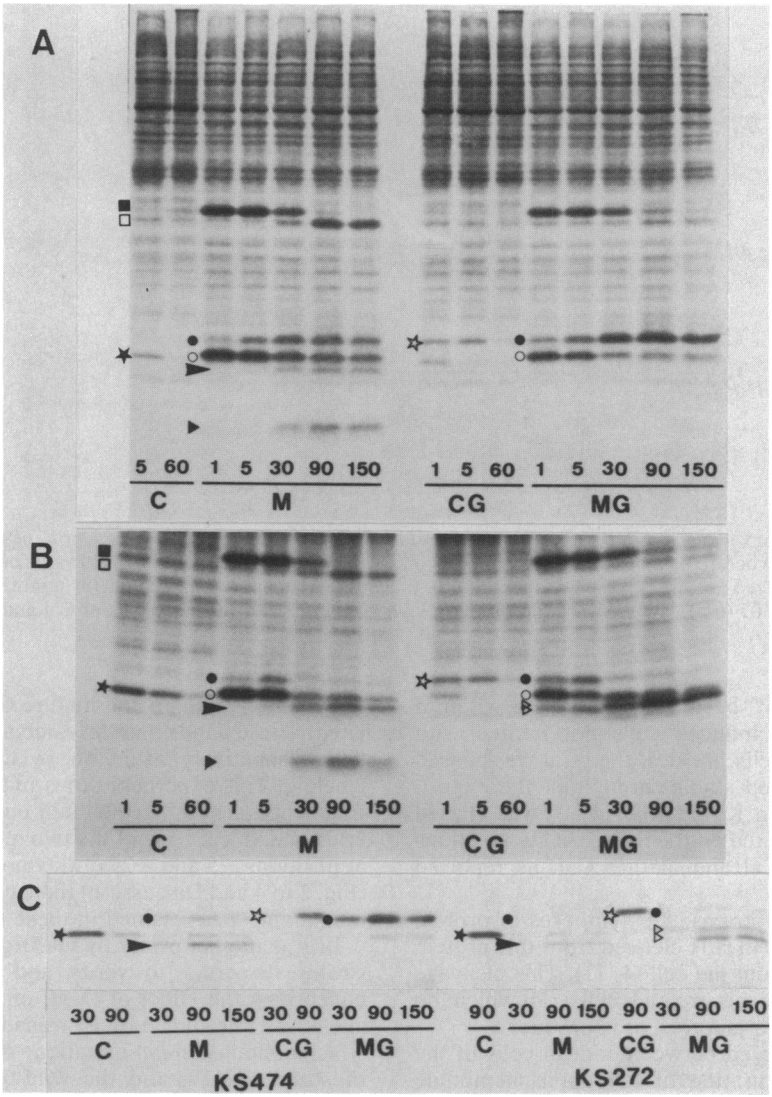

FIG. 1. Kinetics of Cal modification and maturation in a *degP41* strain. Cells were induced with mitomycin (M) or not induced (C) before being treated with globomycin (MG, CG) or left untreated. (A) After 30 min of induction, *degP41* strain KS474(pAT1) cells were pulsed for 10 s with [^{35}S]methionine. After various times of chase as indicated (in minutes), samples were taken and analyzed by urea-SDS-PAGE. The fluorogram is presented. (B) Same experiment as above but with KS272(pAT1) cells. Part of the fluorogram is presented. (C) After 20 min of induction, KS474(pAT1) and KS272(pAT1) cells were labeled with [2-^{3}H]glycerol. After various times of labeling, samples were taken and applied to a urea-SDS-PAG. Part of the fluorogram is presented. The two forms of Lpp are indicated as mature form (★) and modified precursor form (☆). The various forms of Cal are indicated as precursor (○), modified precursor (●), mature (▬►), signal sequence (►), and proteolytic fragment (⧐), and the two forms of AT1, are indicated as intact protein (■) and truncated protein (□).

1 min after IPTG addition. Cells were labeled with [^{35}S]methionine (1,000 Ci/mmol) at 30 μCi/ml (1 μCi = 37 kBq) for 10 s and chased with cold methionine (500 μg/ml). Labeling with [2-^{3}H]glycerol was carried out at 125 μCi/ml (1 Ci/mmol). [^{35}S]methionine and [2-^{3}H]glycerol were purchased from Amersham Corp. In routine experiments, after various times of labeling, 10-μl samples of the cultures were mixed with 10 μl of sample buffer, heated, and applied to the gel. Sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGs) and urea-SDS-PAGs were electrophoresed and treated as described previously (5).

RESULTS

Stability of the lipid-modified precursor of Cal in a *degP* mutant. Cal synthesis by strains KS474 and KS272 carrying plasmid AT1 was compared by using a pulse-chase of 10 s with [^{35}S]methionine followed by electrophoresis and fluorography (Fig. 1). In mitomycin-induced cells of both strains, the precursor form of Cal (pCal) was one of the most strongly labeled proteins after the first minute of chase, along with the truncated colicin A of 20 kilodaltons (kDa), protein AT1, also encoded by the plasmid. Within 5 min, pCal had begun to be chased into a form of higher mass (pCal^{m}), which is lipid modified (3, 4). In the *degP* mutant KS474, the level of pCal^{m} increased with further chase, while in KS272, this form itself had been completely chased within 30 min. However, in both strains, mature Cal and the signal peptide began to accumulate after 30 min of chase.

In induced cells treated with globomycin, no Cal maturation takes place, as described previously (3), since the SPase

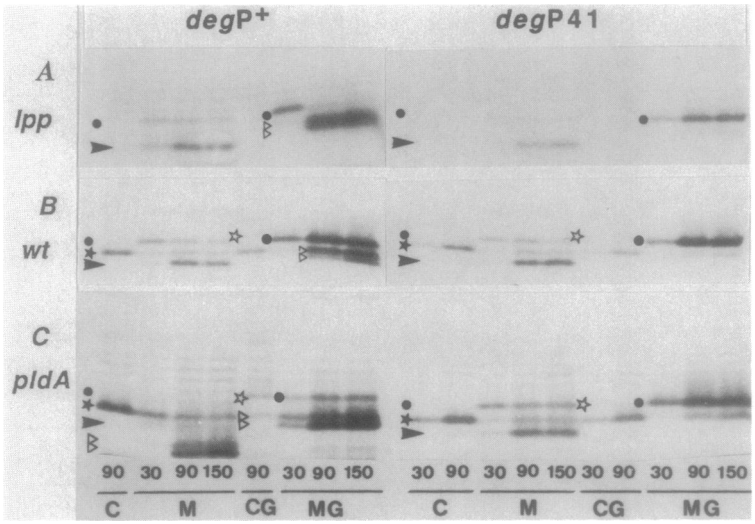

FIG. 2. Stability of pCal^m in various *degP41* strains carrying pAT1. Cells containing either a *degP*$^+$ or *degP41* allele were not induced (C) or induced with mitomycin (M) before being treated with globomycin (CG, MG) or left untreated. After 20 min of induction, cells were labeled with [2-^{3}H]glycerol and analyzed on urea-SDS-PAGs at various times after labeling as indicated (in minutes). The relevant part of the fluorogram is shown. (A) JE5505 (*lpp*). (B) W3110 (wild type). (C) CBM (*pldA*). The various forms of Cal and Lpp are indicated as in Fig. 1.

II is blocked. Again, pCal^m began to accumulate within 5 min of chase, just as in induced cells not treated with globomycin. In the *degP* cells, most of the pCal was chased into pCal^m, which remained stable throughout the experiment. In contrast, in strain KS272, the pCal^m was chased into two forms which migrated on the SDS-PAG to positions very close to those of pCal and mature Cal, as reported previously (3, 4).

In induced cells of both strains, the syntheses of protein AT1 were similar. About 2 kDa is cleaved from this protein at the time of its release from the cell (4, 11). This cleavage does not occur in globomycin-treated cells, in which no release takes place since no mature Cal is produced.

No difference was observed between control cells of the two strains in which maturation of the outer membrane lipoprotein, Lpp, had occurred within the first minute of chase. It migrated on the gel at the same position as pCal. After globomycin treatment of control cells, the modified precursor form of the lipoprotein, pLpp^m, accumulated in large amounts within the first minute of chase and was slowly degraded with time.

Cal synthesis in these strains was also studied by [2-^{3}H]glycerol labeling (Fig. 1C), which allows the detection of the two lipid-modified forms of Cal, pCal^m and mature Cal, and of Lpp, pLpp^m and mature Lpp. The synthesis of Lpp was strongly inhibited during mitomycin induction, as reported before (3). The appearance of the acylated forms of Cal, however, varied according to the strain labeled. In the KS474 strain, pCal^m and Cal were both observed after 30 min of labeling. Their levels varied little with time, and they were both present after 150 min of labeling. In contrast, in KS272, only mature Cal could be clearly observed after 90 and 150 min of labeling.

The difference in the metabolism of Cal between the two strains was more apparent following globomycin treatment of the induced cells. In KS474, pCal^m was present in large amounts after 30 min of labeling, while it could hardly be detected in the parental strain KS272. With time, the pCal^m level increased in KS474 cells, whereas in KS272 cells pCal^m was not observed and instead two bands which migrated at the positions of Lpp and mature Cal appeared and accumulated. These bands had the same apparent masses (6 and 4.5 kDa, respectively) as the ones visualized by [^{35}S]methionine labeling. This experiment thus indicated that they represent fragments of pCal^m which still contain the attached diglyceride and at least one of the two methionine residues located at positions −5 and +25 of the molecule. Later experiments (Fig. 2 to 4 and Discussion) indicated that in fact only the −5 methionine was retained in these fragments.

Degradation of pCal^m by the DegP protease in *lpp* and *pldA* strains. In order to verify and extend the observations concerning the effect of DegP on Cal stability, the *degP41* allele was introduced by P1 transduction into strain JE5505, which contains an *lpp* mutation; strain CBM, a *pldA* mutant of strain W3110; and the wild-type strain W3110. These *degP41* strains were then transformed with pAT1 to study Cal synthesis by labeling with [2-^{3}H]glycerol (Fig. 2).

In the *lpp degP41* and *lpp degP*$^+$(pAT1) strains, no band corresponding to Lpp or pLpp^m was observed in either control or induced cells, as expected. In induced cells, the two acylated Cal forms were present in similar amounts in the two strains; pCal^m could be observed after 30 min of labeling, and mature Cal was observed only after 90 min of labeling, when it became the major band observed. In induced cells treated with globomycin, the behavior of the labeled pCal^m was different for the two strains. In *lpp degP*$^+$ cells, it was observed after labeling for 30 min, whereas at later times two fragments of 6 and 4.5 kDa accumulated. In *lpp degP41* cells, the amount of labeled pCal^m increased continuously, and no smaller fragments were observed.

In control cells of the *degP41* and *degP*$^+$ derivatives of W3110(pAT1) and CBM(pAT1) cells, labeling of Lpp or pLpp^m was observed according to the presence or absence of globomycin, although in many cases the inhibition of Lpp processing was partial and pLpp^m could only be observed on overexposures of the fluorograms. A large decrease in Lpp synthesis was observed after mitomycin induction (Fig. 1). However, the forms of Cal observed in induced cells again differed according to the strain studied. In *degP*$^+$ and *degP41* W3110 strains, pCal^m was observed after 30 min of

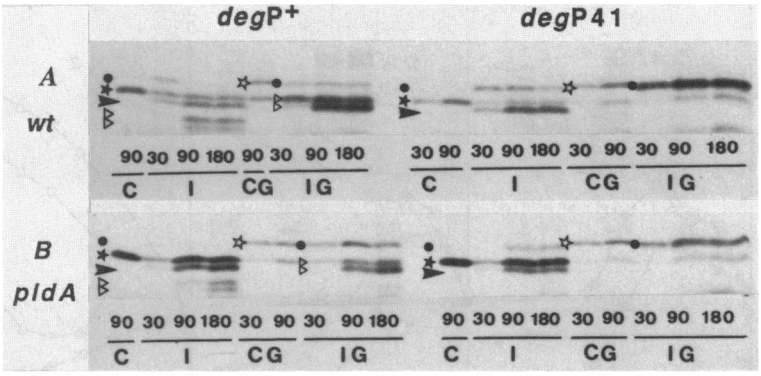

FIG. 3. Stability of acylated Cal forms in *degP41* strains carrying pCK4. Cells containing either a *degP*$^+$ or a *degP41* allele were not induced (C) or induced with IPTG (I) for 1 min before being treated with globomycin (CG, IG) or left untreated. After 10 min of induction, cells were labeled with [2-^{3}H]glycerol and analyzed by urea-SDS-PAGE at the times after labeling indicated (in minutes). W3110 (wild-type). (B) CBM (*pldA*). The various forms of Cal and Lpp are indicated as in Fig. 1.

labeling, and in addition mature Cal accumulated with time. In *degP*$^+$ CBM cells, pCal^m and mature Cal were barely detectable, and two acylated fragments of lower molecular mass than Cal (about 3 and 2.5 kDa) accumulated instead. These fragments were not detected by [^{35}S]methionine labeling (data not shown). In contrast, in *degP41* CBM cells, pCal^m was observed after 30 min of labeling, while mature Cal was observed after 90 min and then accumulated. Thus, the *degP41* mutation appeared to restore the appearance of mature Cal, which was otherwise further hydrolyzed in the *pldA* strain. Such fragments were not obtained in CBM(pColA9) cells, which, unlike cells containing pAT1, did not overproduce Cal (data not shown).

In induced *pldA* cells treated with globomycin, pCal^m labeling and behavior differed according to the presence or absence of the *degP41* allele. In *degP*$^+$ cells, pCal^m was labeled within 30 min, and by 90 min the two degradation fragments of 6 and 4.5 kDa were visible, as observed in the KS272 and JE5505 strains. In *degP41* cells, pCal^m was labeled with the same timing, and its level increased with time, as observed for the KS474 and JE5505 *degP41* strains. It should be noted that [2-^{3}H]glycerol incorporation was higher in *pldA* cells than in *pldA*$^+$ cells.

DegP proteolysis of Cal modified precursor is not related to mitomycin induction. In the experiments described above, proteolysis of pCal^m in *degP*$^+$ cells was observed after mitomycin addition. This drug induces a cascade of various repair phenomena in the cell known as the SOS response (14). In plasmid CK4, the *cal* gene is under the control of the *tac* promoter and is expressed after IPTG addition (4). The effect of the DegP protease on CBM and W3110 cells carrying this plasmid was examined.

In IPTG-induced *degP*$^+$ cells of either strain labeled with [2-^{3}H]glycerol, pCal^m was observed after 30 min but did not accumulate with time (Fig. 3). Mature Cal was also detected in 30 min, but its level increased with time concurrently with the formation of two acylated fragments of 3 and 2.5 kDa. These two fragments were not observed with [^{35}S]methionine labeling (not shown). They were not present in induced *degP41* cells, in which only pCal^m and Cal were present, the latter accumulating with time. Mature Lpp was strongly labeled in these *degP*$^+$ and *degP41* induced cells, since no shut-off of its synthesis occurred after IPTG induction.

After globomycin treatment of induced cells, the results of [2-^{3}H]glycerol labeling were essentially the same in W3110 as in CBM cells. In *degP*$^+$ cells, pCal^m was rapidly synthesized, and with time two fragments of 6 and 4.5 kDa appeared that could also be detected by [^{35}S]methionine labeling (not shown). In *degP41* cells, pCal^m was labeled within 30 min, and its level increased with time.

DegP proteolysis of mutant forms of pCal^m. Extensive site-directed mutagenesis of mature Cal has been performed in a study of the effect of point, deletion, and frameshift mutations on its activity (8). The frameshift mutant FS2 contains the first 16 amino acids of mature Cal and 11 unrelated amino acids which replace the carboxyl-terminal 17 residues of the wild-type protein. The point mutation A2 yields Cal with a substitution of glutamic acid for arginine at position 7 of the mature protein. Both of these mutant Cal proteins were shown to be acylated.

The action of the DegP protease on the modified precursor forms of these Cal mutants was examined. After globomycin treatment of induced cells and labeling with [2-^{3}H]glycerol, the pCal^m form of the FS2 and A2 mutants accumulated as expected (Fig. 4). However, that of the mutant FS2 was stable in both *degP*$^+$ and *degP41* W3110 strains, while in the A2 mutant degradation products appeared in W3110 *degP*$^+$ but not in W3110 *degP41* cells. Similar results were observed with [^{35}S]methionine labeling (not shown).

Cal function in *degP* cells. The Cal protein fulfills at least four functions in the cells. It promotes the release of colicin A as well as various other cellular proteins, quasi-lysis of the culture, death of the cells, and activation of the outer membrane phospholipase A. The influence of DegP on these functions was compared in *degP*$^+$ and *degP41* strains.

The protein contents of the cells and culture supernatants of the various strains containing pColA9 were analyzed by

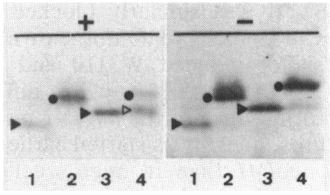

FIG. 4. Stability of acylated forms of mutant Cal proteins. Wild-type W3110 cells (+) and W3110 *degP41* cells (−) carrying either pFS2 (lanes 1 and 2) or pA2 (lanes 3 and 4) were induced with mitomycin and treated with globomycin (lanes 2 and 4), or left untreated (lanes 1 and 3) and labeled for 3 h with [2-^{3}H]glycerol before being analyzed by urea-SDS-PAGE. The various forms of Cal are indicated as in Fig. 1.

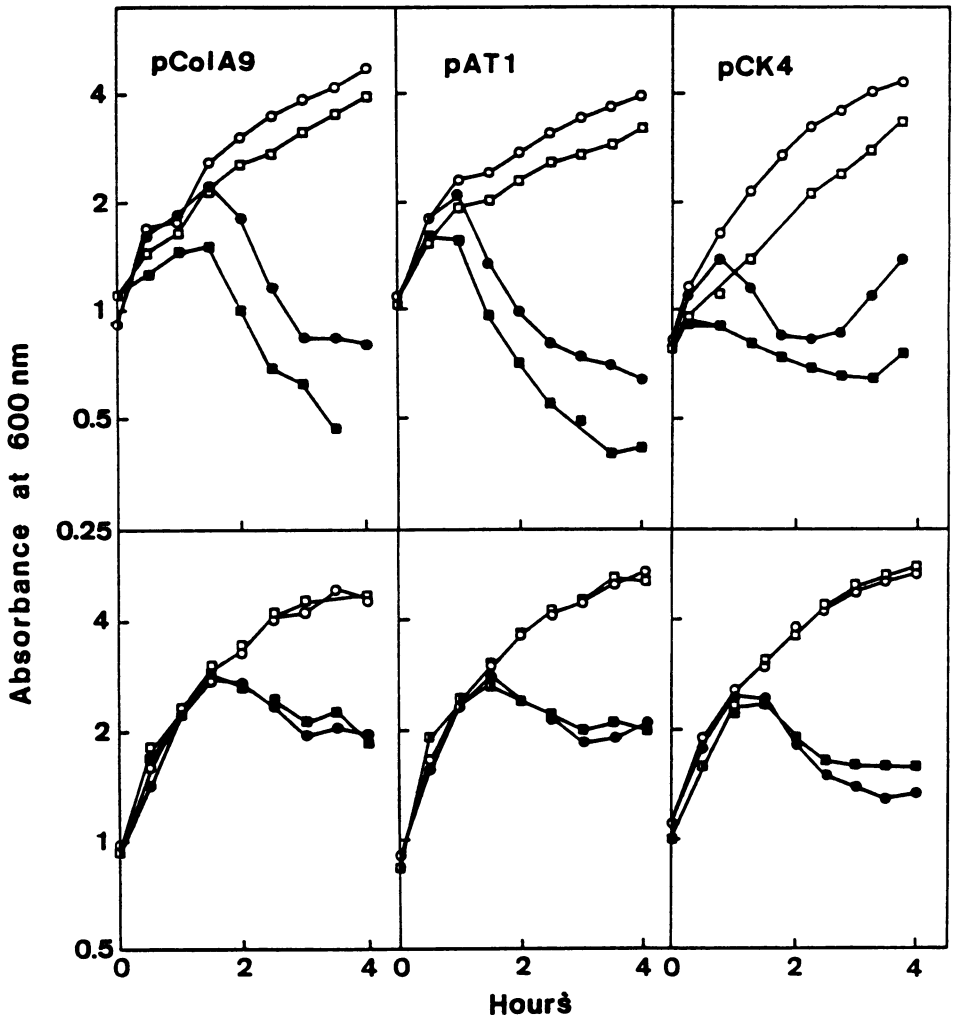

FIG. 5. Effect of the *degP41* mutation on quasi-lysis of cells containing various Cal-producing plasmids. W3110 (top) and CBM (bottom) cells with (■, □) or without (●, ○) the *degP41* mutation were grown in LB and induced (■, ●) or not induced (□, ○). The A_{600} of the cultures was measured at various times after induction.

SDS-PAG electrophoresis. Release of colicin A was similar in both strains, but surprisingly it appeared that W3110 *degP41* cells were leaky, releasing appreciable quantities of cellular protein even before induction. When the plasmidless strains were examined, these results were confirmed for W3110 *degP41* as well as the original *degP41* strain KS474 but not for the CBM *degP41* strain (not shown). Thus, the presence of a *pldA* mutation restored the wild-type phenotype. The release of colicin A has been shown to be inhibited in *pldA* cells (3). It was similarly blocked in *degP*$^+$ and *degP41* CBM(pColA9) cells (data not shown).

The quasi-lysis of induced W3110 and CBM cultures carrying either pColA9, pAT1, or pCK4 differed according to the presence or absence of the *degP41* mutation (Fig. 5). For W3110 cultures, quasi-lysis started earlier and was more pronounced in *degP41* than in *degP*$^+$ cells. In contrast, quasi-lysis was similar and much reduced (although not absent) in both *degP41* and *degP*$^+$ CBM cultures.

The lethality of Cal induction to cells was measured by loss of colony-forming ability (Table 1). For cells carrying pColA9 or pAT1, the presence of a *degP41* mutation decreased the survival of W3110 but not CBM cells. The same results were obtained for cells carrying pCK4. As noted previously (4), in all cases the presence of the *pldA* mutation (i.e., in CBM and CBM *degP* cells) lowered but did not eliminate the mortality of induced cells.

DISCUSSION

This study demonstrates that the DegP protease hydrolyzes the acylated precursor of Cal, pCal^m, an intermediate in the biosynthesis of the Cal lipoprotein. This degradation is clearly observed in globomycin-treated cells, in which pCal^m cannot be processed by SPase II to mature Cal and the signal peptide. Under these conditions, pCal^m was cleaved by the DegP protease into two fragments of 6 and 4.5 kDa, which retained the lipid-modified amino terminus of pCal^m. These two lipopeptides accumulated with time. This proteolytic breakdown occurred in each of the strains tested, wild-type, *pldA*, and *lpp*, and no matter which Cal-encoding plasmid was carried by the cells, pColA9 or the Cal-overproducing plasmids pAT1 and pCK4. It was observed after induction of Cal by either mitomycin or IPTG, indicating that it is not related to the SOS response. The hydrolysis of pCal^m by the DegP protease also seems to occur to some extent in cells which have not been treated with globomycin, since the level

TABLE 1. Cell survival after Cal induction[a]

Strain and plasmid	Time of induction (h)	No. of CFU			
		degP$^+$ host		*degP41* host	
		Control	Cal induced	Control	Cal induced
W3110					
pColA9	0	1.2×10^9		1×10^9	
	3	4×10^9	1×10^7	2.8×10^9	2×10^6
pAT1	0	1.2×10^9		1.1×10^9	
	3	4×10^9	1.6×10^7	2×10^9	3×10^6
pCK4	0	5.8×10^8		2.7×10^8	
	3	3.1×10^9	7×10^3	2.6×10^9	1×10^3
CBM					
pColA9	0	6.7×10^8		6×10^8	
	3	4×10^9	9×10^7	3.5×10^9	1.2×10^8
pAT1	0	6.7×10^8		7.7×10^8	
	3	4.4×10^9	1.4×10^8	3.7×10^9	1.4×10^8
pCK4	0	4.4×10^8		4×10^8	
	3	3×10^9	4×10^5	3.2×10^9	1×10^4

[a] Number of colony-forming bacteria after Cal induction in *degP*$^+$ and *degP41* isogenic strains. The indicated strains carrying the various plasmids were grown in LB medium and induced with mitomycin or IPTG as appropriate. The cells were diluted in LB and plated on LB agar plus ampicillin.

of pCal^m was always higher in *degP* than in wild-type cells. However, in this case the breakdown products were not evident, suggesting that they were further hydrolyzed.

As observed in this and earlier pulse-chase studies with [^{35}S]methionine, the processing of pCal^m by SPase II appears to be a rate-limiting step in the biosynthesis of mature Cal. This slow rate of maturation is presumably due either to the structure of Cal itself or to poor contact between Cal and SPase II owing to differences in their localization in the cell envelope. In any case, it is presumably the long life of this assembly intermediate of Cal in the envelope, especially in cells which have been treated with globomycin, which allows it to become a substrate for the DegP protease. SPase II has been shown to be located in the inner membrane (25), and DegP is apparently localized either in the periplasm or at the periplasmic side of the inner or outer membrane (24), whereas sucrose density gradient analysis of Cal-producing cells indicated that while mature Cal was present in both membranes, pCal^m was found exclusively in the inner membrane (unpublished observations).

In the induced *degP*$^+$ cells which overproduced Cal, two Cal fragments of about 3 and 2.5 kDa were observed. They contained the diglyceride part of pCal^m but neither the −5 nor the +25 methionine residue. They were not observed in *degP* cells or in globomycin-treated *degP*$^+$ cells. All of these data suggest that these fragments are produced from processing by SPase II of the pCal^m fragments produced by the DegP protease. The cleavage of pCal^m by the DegP protease would occur in the C-terminal part of the molecule, before the +25 methionine residue, leaving the signal peptide intact. Then, these truncated pCal^m forms would be matured by Spase II, giving the observed fragments, which would consist of truncated mature Cal molecules (Fig. 6). Accumulation of the Cal signal peptide observed by [^{35}S]methionine labeling (data not shown) was found to be similar in the *degP*$^+$ and the *degP* strains studied, favoring this interpretation. It would also be consistent with the resistance to DegP cleavage of the pCal^m of the mutant FS2, which contains a completely altered carboxy-terminal sequence. That the observed fragments arise from DegP cleavage followed by maturation and not the reverse is suggested by the stability of mature Cal once it has accumulated (Fig. 1 to 3). Thus, truncated pCal^m can be processed by SPase II, but Cal mutants which contain only the signal sequence and first 16 or 18 amino acids of the mature protein have been shown to be neither acylated nor processed (8). This implies that the assembly of these mutant Cal proteins is blocked either at or before the acylation of the precursor. It should be noted, however, that Luirink et al. found that truncated fragments of the cloacin DF13 lysis protein that contained as few as 4 amino acids of the mature protein were both acylated and processed (17).

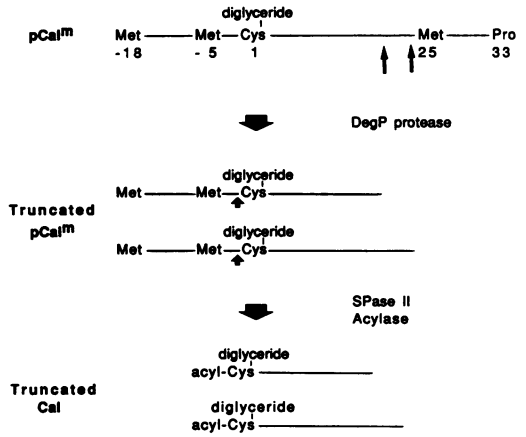

FIG. 6. Proposed pathway of degradation of lipid-modified precursor Cal by the DegP protease. Arrows indicate the enzyme cleavage sites. The acylation of the amino terminus has not been demonstrated but has been shown to occur for other lipoproteins (26).

The substrate specificity for cleavage by the DegP protease which is demonstrated by its failure to cleave the acylated FS2 precursor may be a determining factor in the stability of other colicin lysis proteins which are homologous but not identical to Cal (6). The degradation of pCal^m observed here, however, could explain difficulties encountered in the demonstration of assembly intermediates or even the mature form of other lysis proteins (1, 10, 18, 20, 21).

The effects of Cal on induced cells were more dramatic in *degP* cells than in the wild type (Fig. 5 and Table 1); the release of protein from the cells occurred earlier, and quasi-lysis and lethality were more pronounced. These increased effects are presumably due to the presence of a higher level of mature Cal in *degP* than in *degP*$^+$ cells. In this sense, the DegP protease may be considered a detoxifying agent for the cell, preventing the formation of a lethal product.

Although the original *degP* mutation was isolated as one which reduced the proteolysis of several fusion and deletion proteins (24), no other normal physiological substrates for this enzyme activity have as yet been identified. Recent studies have indicated that the *htrA* gene product is identical to DegP (12). The *htrA* locus was identified as a gene whose expression is required for the growth of *E. coli* at elevated temperature, and although it is not a member of the classical group of heat shock proteins, its expression is increased at high temperatures (13). It is thus tempting to speculate that this protein functions to deal with undesirable consequences of growth at elevated temperatures, as are other heat shock proteins (some of which are proteases), and that the damage to the envelope caused by Cal or simply the presence of Cal mimics these consequences and thus elicits the same response. Further studies are in progress to determine the

effect of mutations at other heat shock loci on both the stability and functions of Cal.

The studies reported here also suggest that the *degP* gene product and the outer membrane phospholipase A interact either directly or indirectly in a manner which controls the activity of this phospholipase. We found that the *degP* mutants were leaky and that this phenotype was suppressed by an additional mutation in the *pldA* gene. Phospholipase activity is normally tightly regulated in the cell, and two of the most-efficient means of activating this enzyme are heat shock (23) and colicin lysis proteins (3, 18, 22), which in fact require the enzyme for the efficient release of colicin. This suggests a possible relationship between the functions of Cal and the effects of heat shock on the cell.

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