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Export of the Outer Membrane Lipoprotein Is Defective in *secD*, *secE*, and *secF* Mutants of *Escherichia coli*

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The export of major outer membrane lipoprotein has been found to be affected in *secD*, *secE*, and *secF* mutants of *Escherichia coli*, which are defective in protein export in general. After a shift to the nonpermissive temperature, the kinetics of accumulation of prolipoprotein and pre-OmpA protein was indistinguishable from that of pre-OmpA protein accumulation in the *secD* and *secF* mutants but different in the *secE* mutant. The prolipoprotein accumulated in the *secD*, *secE*, and *secF* mutants at the nonpermissive temperature was not modified with glyceride. We conclude from these results and those of previous studies that the export of lipoprotein requires all common *sec* gene products except the SecB protein, i.e., the SecA, SecD, SecE, SecF, and SecY proteins.

Current understanding of the essential features of protein secretion in bacteria has been obtained from biochemical and genetic studies with the gram-negative bacterium *Escherichia coli*. Many proteins synthesized in *E. coli* are exported from the cytoplasm to the periplasm and the outer membrane. These proteins are initially synthesized as precursor proteins which contain signal peptides (20 to 30 amino acids) in the form of N-terminal extensions. Efficient export of these precursor proteins has been shown to require proteins which can be divided into three groups according to their respective functions. The first group includes the chaperones, such as the SecB protein (21), the GroEL protein (22, 23), and the DnaK protein (25), which facilitate the targeting of the preproteins to the export machinery and maintain the precursor proteins in a translocation-competent conformation (6, 21, 36). The second group consists of the products of five *sec* genes—SecA (24), SecD (9, 10), SecE (32), SecF (10), and SecY (18, 20)—which constitute the protein export machinery (for reviews, see references 1 and 30). The exact functions of most of these gene products are not known. The third group of gene products required for protein export corresponds to the signal peptidases, which cleave the signal peptide during or after translocation of the precursor proteins. In bacteria, there are at least two signal peptidases, signal peptidases I (38, 39) and II (8, 34). They are required for the release of exported proteins from the cytoplasmic membrane of *E. coli* (7, 17).

Lipoproteins are distinct from nonlipoproteins in that they are processed by lipoprotein-specific signal peptidase (signal peptidase II) following lipid modification of the precursor proteins (15). The major outer membrane lipoprotein (Braun's lipoprotein) (4) is synthesized as a precursor protein (prolipoprotein) and processed by signal peptidase II after modification with glycerol and fatty acids (15, 35). Since the translocation is an early event in protein export, it is reasonable to assume that lipoproteins and nonlipoproteins may share a common pathway of protein translocation across the cytoplasmic membrane. We have previously demonstrated that the export of lipoprotein requires functional SecA and SecY proteins (14) but not SecB protein (14, 37). We have studied the export of lipoprotein in an *E. coli* mutant [*secD1*(Cs)] containing a cold-sensitive allele in the *secD* locus and have shown that prolipoprotein accumulates at the nonpermissive temperature (37). Recently, this *secD1*(Cs) allele has been shown to be defective in both *secD* and *secF* (10). It has been suggested that SecD and SecF proteins form a complex in the inner membrane which functions at a late step in protein export (10). The *secE* (*prlG*) gene encodes an integral inner membrane protein (32) which appears to interact with the SecY (PrlA) protein to form the translocator in the cytoplasmic membrane of *E. coli* (2, 3, 5).

In this paper, we have studied and compared the export of lipoprotein with that of a nonlipoprotein in individual *E. coli* mutants containing a new cold-sensitive allele of *secD*, a cold-sensitive allele of *secE*, and a cold-sensitive allele of *secF*. Our results indicate that the export of lipoprotein in *E. coli* requires functional SecD, SecE, and SecF proteins at steps prior to the modification and processing of prolipoprotein.

MATERIALS AND METHODS

Materials. [^{35}S]methionine (1,000 to 1,200 Ci/mmol) was purchased from ICN Biomedicals, Inc., Irvine, Calif. [9,10-^{3}H]palmitic acid (60 Ci/mmol) and [2-^{3}H]glycerol (11.5 Ci/mmol) were from DuPont-NEN Research Products, Boston, Mass. Other chemicals used were obtained from commercial sources.

Bacterial strains and medium. Bacterial strains used in this study included CG-2 (MC1000 *phoR*) (9), KJ173 [MC1000 *phoR secD29*(Cs) *zaj*::Tn*10*] (10), KJ188 [MC1000 *phoR secE501*(Cs) *zijRK498*::Tn*5*) (27), and KJ184 [MC1000 *phoR secF62*(Cs) *zaj*::Tn*10*] (10), which were derived from MC1000 F$^-$ *araD139* Δ(*ara-leu*)*7679 galE galK* Δ(*lac*)*X74 rpsL thi* (9). These strains were generous gifts from Jon Beckwith (Boston, Mass.). Luria-Bertani medium was used for subculture, and M9 minimal medium supplemented with 0.4% glucose and thiamine (100 μg/ml) was employed for labeling experiments.

Labeling experiments. Pulse-labeling and pulse-chase experiments were used to study the kinetics of the maturation of lipoprotein and OmpA protein. Cultures were grown in

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M9 minimal medium at the permissive temperature (37°C) with continuous agitation in a rotary shaker. At the mid-logarithmic phase of growth (A_{600} = 0.4 to 0.5), the cultures were shifted to the nonpermissive temperature and incubation was continued for 1 to 3 h. In some experiments, the cultures were returned to the permissive temperature and incubation was continued for an additional 1 h. For pulse labeling, 1-ml portions of culture were withdrawn at various intervals and labeled with 40 μCi of [^{35}S]methionine for 1 min. For pulse-chase labeling, 1-ml portions of culture were pulse-labeled for 15 s and chased for 15, 30, and 45 s after the addition of methionine to a final concentration of 0.4%. In both labeling protocols, the incorporation of [^{35}S]methionine into cellular proteins was terminated by the addition of 100% (wt/vol) trichloroacetic acid to a final concentration of 10%. The trichloroacetic acid precipitates were washed with acetone and solubilized with 1% sodium dodecyl sulfate (SDS) in 10 mM sodium phosphate buffer (pH 7.0) at 100°C, and the solubilized samples were immunoprecipitated with antisera against purified lipoprotein or OmpA protein as described previously (16).

To detect the lipoprotein covalently bound to peptidoglycan, steady-state labeling was employed. Five-milliliter cultures were shifted to the nonpermissive temperature for 2 h and then labeled with 200 μCi (250 μg) of [^{35}S]methionine for 2 h. The cells were harvested by centrifugation at 10,000 × *g* for 10 min at 4°C, washed with 10 mM phosphate-buffered saline (pH 7.0), and disrupted with a sonicator (60 W, three times for 1 min each). The crude sonicate was centrifuged at 5,000 × *g* for 10 min at 4°C, and the supernatant fraction was further centrifuged at 200,000 × *g* for 1 h at 4°C. The pellet was resuspended in 500 μl of 1% SDS in 10 mM sodium phosphate buffer (pH 7.0), incubated in a boiling water bath for 20 min, and then centrifuged at 200,000 × *g* for 1 h at room temperature. The supernatant solution was subjected to immunoprecipitation with antiserum against purified lipoprotein (16). The pellet was resuspended in 1 ml of 4% SDS in 5 mM sodium phosphate buffer (pH 7.0), incubated in a boiling water bath for 20 min, and centrifuged at 200,000 × *g* for 1 h at room temperature. The pellet was washed twice with distilled water, resuspended in 200 μl of 10 mM sodium phosphate buffer (pH 7.0), and digested with lysozyme (200 μg/ml) for 20 to 24 h. The lysozyme-digested murein-lipoprotein complex containing the bound-form lipoprotein and the immunoprecipitated sample from the supernatant (the free-form lipoprotein) were analyzed by polyacrylamide gel electrophoresis (PAGE).

Fractionation with alkali. For fractionation with alkali (28), 1-ml portions of culture pulse-labeled with 40 μCi of [^{35}S]methionine for 1 min were added to 5 ml of cold 0.1 N NaOH which had been freshly diluted from a 10 N NaOH stock solution. Samples were centrifuged at 10,000 × *g* for 15 min at 4°C, and the supernatant solutions were removed. The pellets were washed with 1 ml of 5% (wt/vol) trichloroacetic acid, and the proteins in the alkali-soluble supernatant fractions were precipitated by the addition of 100% (wt/vol) trichloroacetic acid to a final concentration of 10%. The trichloroacetic acid precipitates were washed with acetone, dried with heat, and then solubilized with 1% SDS in 10 mM sodium phosphate buffer (pH 7.0) at 100°C. The solubilized samples were immunoprecipitated with antiserum against purified lipoprotein as described previously (16).

PAGE. Lipoprotein and OmpA protein were analyzed by two different PAGE systems, Ito's gel system (19) and the Tricine-SDS-PAGE system developed by Schägger and Jagow (29). After electrophoresis, the gel was dried and an autoradiogram was made with Kodak X-Omat film, using an intensifying screen at −80°C. As standards, mature lipoprotein, modified prolipoprotein, and unmodified prolipoprotein were prepared according to procedures described previously (16). Briefly, the mature lipoprotein was prepared from wild-type (CG-2) cells, and the modified prolipoprotein was prepared from globomycin-treated wild-type (CG-2) cells. The unmodified prolipoprotein was prepared from MM18 cells which was induced with maltose for the synthesis of the MalE-LacZ hybrid protein (35).

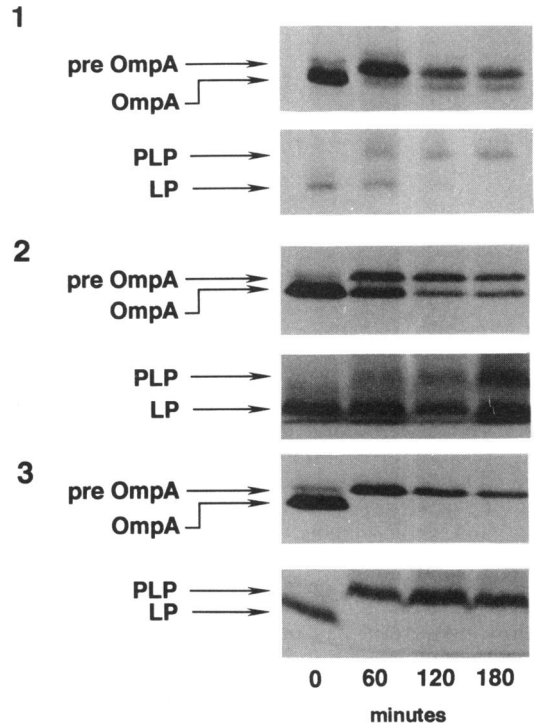

FIG. 1. Accumulation of prolipoprotein and pre-OmpA protein in *secD*, *secE*, and *secF* mutant cells after shift to the nonpermissive temperature (25°C). The *secD* (KJ173), *secE* (KJ188), and *secF* (KJ184) mutant cells grown at 37°C were shifted to 25°C, and incubation was continued. One-milliliter portions of the culture were withdrawn every 60 min and pulse-labeled with [^{35}S]methionine for 1 min. After precipitation with trichloroacetic acid, lipoprotein and OmpA protein were isolated by immunoprecipitation and analyzed by SDS-PAGE (Ito's gel system [19]). Panel 1, KJ173 *secD*; panel 2, KJ188 *secE*; panel 3, KJ184 *secF*. PLP, prolipoprotein; LP, mature lipoprotein.

RESULTS

Prolipoprotein accumulation in *secD*, *secE*, and *secF* mutants. To study the effect of *secD*, *secE*, or *secF* mutation on the export of lipoprotein and OmpA protein in *E. coli*, we pulse-labeled each of these mutants with [^{35}S]methionine for 1 min after shifting the culture to the nonpermissive temperature for various periods of time. Accumulation of prolipoprotein and pre-OmpA protein was observed for each of these mutants after the temperature shift to 25°C (Fig. 1), whereas no accumulation of the precursor of either protein was observed at 37°C (data not shown). In the *secE* mutant, the accumulation of prolipoprotein was greater with increasing time at 25°C; however, the extent of prolipoprotein accumulation was less than 43% even at 180 min after the

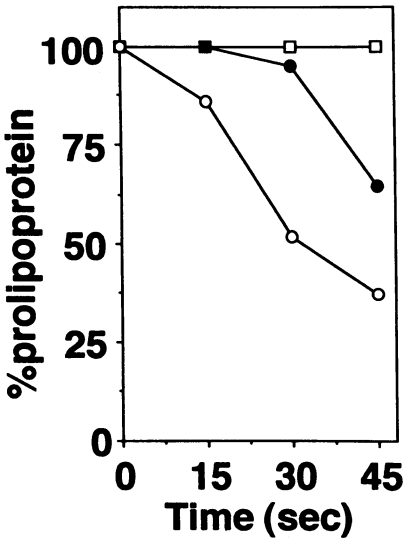

FIG. 2. Kinetics of lipoprotein maturation in *secD*, *secE*, and *secF* mutants at the nonpermissive temperature. The *secD* (KJ173), *secE* (KJ188), and *secF* (KJ184) mutant cells grown at 37°C were shifted to 25°C, and incubation was continued for 3 h. One-milliliter portions of the culture were pulse-labeled with [^{35}S]methionine for 15 s and chased for 15, 30, and 45 s after the addition of excess unlabeled methionine. After precipitation with trichloroacetic acid, lipoprotein was isolated by immunoprecipitation and analyzed by SDS-PAGE (Ito's gel system [19]). The bands corresponding to prolipoprotein and mature lipoprotein in the autoradiogram were quantitated with an image scanner connected to a computer. The percentage of prolipoprotein in the total (lipoprotein plus prolipoprotein) was calculated and adjusted by a factor of 0.67, inasmuch as prolipoprotein contains three residues of methionine compared with two methionine residues in the mature lipoprotein. Symbols: ●, percentage of prolipoprotein in KJ173 *secD*; ○, percentage of prolipoprotein in KJ188 *secE*; □, percentage of prolipoprotein in KJ184 *secF*.

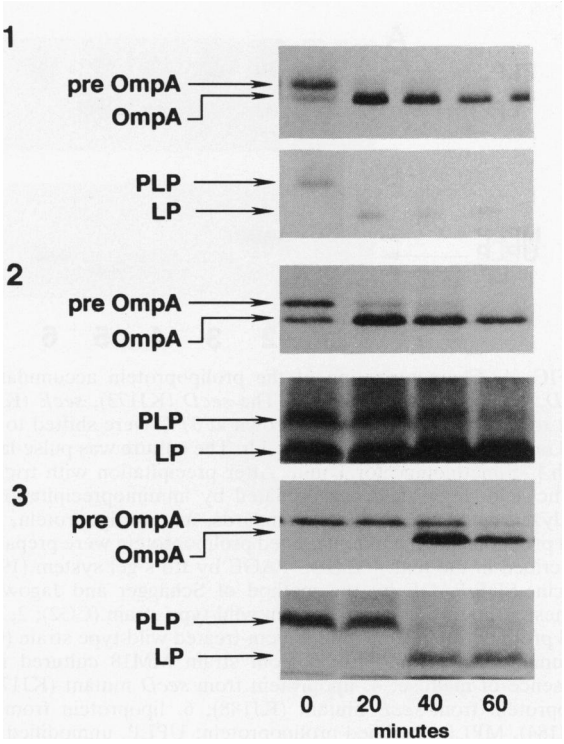

FIG. 3. Kinetics of the recovery of protein export in the *secD*, *secE*, and *secF* mutants after shift from the nonpermissive temperature to the permissive temperature. The *secD* (KJ173), *secE* (KJ188), and *secF* (KJ184) mutant cells grown at 37°C were shifted to 25°C, and incubation was continued for 3 h. The cultures were shifted back to 37°C, and one-ml portions were withdrawn every 20 min and pulse-labeled with [^{35}S]methionine for 1 min. After a precipitation with trichloroacetic acid, lipoprotein and OmpA protein were isolated by immunoprecipitation and analyzed by SDS-PAGE (Ito's gel system [19]). Panel 1, KJ173 *secD*; panel 2, KJ188 *secE*; panel 3, KJ184 *secF*. PLP, prolipoprotein; LP, mature lipoprotein.

temperature shift, while the accumulation of pre-OmpA protein was significantly greater at 60 min after the temperature shift. In contrast to the data obtained with the *secE* mutant, significant accumulation of prolipoprotein and pre-OmpA protein was observed for *secD* and *secF* mutants 60 min after the temperature shift to 25°C. With the *secF* mutant, very little mature lipoprotein was detected with 1-min pulse-labeling, suggesting that the maturation of lipoprotein was completely inhibited in the *secF* mutant at the nonpermissive temperature. To determine the extent of these defects, we carried out pulse-chase experiments at both the permissive and the nonpermissive temperatures. Figure 2 shows that after a 15-s pulse of the *secD*, *secE*, or *secF* mutant at 25°C, all the newly synthesized lipoprotein was prolipoprotein; this result was not observed with the wild-type cells or with mutants grown at the permissive temperature (data not shown). In the *secD* and *secE* mutants, the prolipoprotein was slowly and partially processed during a subsequent chase of up to 45 s at the nonpermissive temperature. In the *secF* mutant, this newly synthesized prolipoprotein remained totally unprocessed after a 45-s chase. This lack of processing of prolipoprotein in the *secF* mutant was confirmed in a pulse-chase experiment using a chase of up to 30 min (data not shown).

We studied how soon after a shift back to the permissive temperature the secretion defect in the *secD*, *secE*, or *secF* mutant was restored. Each of these mutants was preincubated at the nonpermissive temperature. At 20-min intervals after the culture was shifted to the permissive temperature, 1-ml portions of the culture were pulse-labeled with [^{35}S]methionine for 1 min. The recovery of the secretion was observed with all *sec* mutants (Fig. 3). The secretion defects in the *secD* and *secF* mutants were fully restored within 20 and 40 min, respectively, after the culture was shifted to the permissive temperature, and the kinetics of recovery was identical for lipoprotein and OmpA protein. In the *secE* mutant, the kinetics of restoration of the secretion of lipoprotein and OmpA protein appeared to be different. The percentage of prolipoprotein was found to be 59.8, 59.3, 58.4, and 51.6% of the total lipoprotein synthesized during the 1-min pulse at 0, 20, 40, and 60 min after the temperature shift, respectively. The corresponding values for the pre-OmpA protein were 57.9, 35.0, 30.0, and 0%. The secretion of lipoprotein was restored only partially, and more slowly than the secretion of the pre-OmpA protein, after the culture was shifted to the permissive temperature.

We examined whether the restoration of the secretion defect requires de novo protein synthesis. The *secF* mutant was preincubated at the nonpermissive temperature and shifted to the permissive temperature with or without chloramphenicol (30 μg/ml). At 20-min intervals after the culture

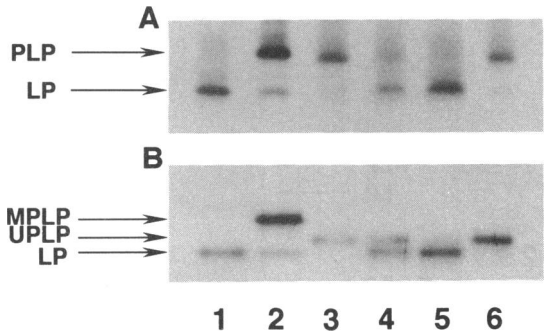

FIG. 4. Characterization of the prolipoprotein accumulated in *secD*, *secE*, and *secF* mutants. The *secD* (KJ173), *secE* (KJ188), and *secF* (KJ184) mutant cells grown at 37°C were shifted to 25°C, and incubation was continued for 3 h. The culture was pulse-labeled with [^{35}S]methionine for 1 min. After precipitation with trichloroacetic acid, lipoprotein was isolated by immunoprecipitation and analyzed by SDS-PAGE. As standards, mature lipoprotein, modified prolipoprotein, and unmodified prolipoprotein were prepared as described in the text. (A) SDS-PAGE by Ito's gel system (19); (B) Tricine-SDS-PAGE by the method of Schägger and Jagow (29). Lanes: 1, mature lipoprotein from wild-type strain (CG2); 2, modified prolipoprotein from globomycin-treated wild-type strain (CG2); 3, unmodified prolipoprotein from strain MM18 cultured in the presence of maltose; 4, lipoprotein from *secD* mutant (KJ173); 5, lipoprotein from *secE* mutant (KJ188); 6, lipoprotein from *secF* (KJ184). MPLP, modified prolipoprotein; UPLP, unmodified prolipoprotein; LP, mature lipoprotein.

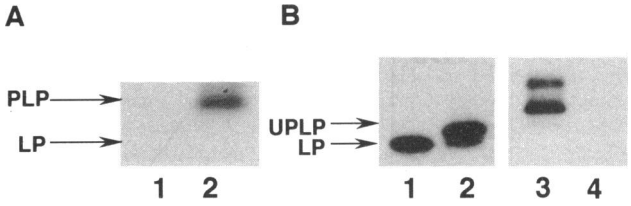

FIG. 5. Characterization of prolipoprotein accumulated in the *secF* mutant. (A) Alkaline fractionation of prolipoprotein accumulated in the *secF* mutant. The *secF* (KJ184) mutant cells grown at 37°C were shifted to 25°C, and incubation was continued for 1 h. The culture was pulse-labeled with [^{35}S]methionine for 5 min and then added to cold 0.1 N NaOH. After centrifugation, lipoprotein in the pellet and the supernatant was immunoprecipitated and analyzed by SDS-PAGE (Ito's gel system [19]). Lane 1, NaOH supernatant; lane 2, NaOH pellet. PLP, prolipoprotein; LP, mature lipoprotein. (B) Characterization of the bound-form lipoprotein in *secF* mutant cells. The *secF* (KJ184) mutant cells grown at 37°C were shifted to 25°C, and incubation was continued for 2 h. The cells were then labeled with 200 μCi of [^{35}S]methionine in the presence of unlabeled methionine (5 μg/ml) for an additional 2 h. Free-form and bound-form lipoproteins were prepared as described in the text. Lane 1, free-form lipoprotein of the wild type; lane 2, free-form lipoprotein of the *secF* mutant; lane 3, bound-form lipoprotein of the wild type; lane 4, bound-form lipoprotein of the *secF* mutant. UPLP, unmodified prolipoprotein.

was shifted to the permissive temperature, 1-ml portions of the culture were withdrawn, washed, and pulse-labeled with [^{35}S]methionine for 1 min. The recovery of the secretion of lipoprotein in the *secF* mutant after a shift back to the permissive temperature was not affected by chloramphenicol, suggesting that de novo protein synthesis is not required for the recovery of the lipoprotein secretion in the *secF* mutant.

Prolipoprotein accumulated in the *secD*, *secE*, and *secF* mutants is unmodified. Prolipoprotein that accumulated in the *secD*, *secE*, and *secF* mutants at the nonpermissive temperature was analyzed with two different PAGE systems in order to ascertain whether or not the prolipoprotein was modified with glyceride (Fig. 4). By Ito's gel system, both the modified and the unmodified prolipoprotein migrated more slowly than the mature lipoprotein (Fig. 4A), with the modified prolipoprotein migrating slightly more slowly than the unmodified prolipoprotein. In this gel system, prolipoprotein that accumulated in each of these *sec* mutants appeared to migrate to the same position as that of unmodified prolipoprotein. This was verified by using the Tricine-SDS-PAGE system, which gave a better separation of the unmodified and the modified prolipoproteins (Fig. 4B). The prolipoprotein that accumulated in the *secD* and *secF* mutants migrated to the same position as that of the unmodified prolipoprotein; with the *secE* mutant, however, the band was faint because of a lesser accumulation of the prolipoprotein.

We further characterized the prolipoprotein accumulated in the *secF* mutant by labeling with [2-^{3}H]glycerol or [9,10-^{3}H]palmitic acid. The mutant cells were shifted to the nonpermissive temperature for 1 h and then were incubated with or without globomycin (100 μg/ml) for 20 min. A small portion of each culture was pulse-labeled with [^{35}S]methionine for 1 min, and the remainder of the culture was further incubated with [2-^{3}H]glycerol (200 μCi) or [9,10-^{3}H]palmitic acid (100 μCi) for 2 h at the nonpermissive temperature. These labeled samples were treated with trichloroacetic acid, immunoprecipitated, and analyzed by PAGE. In the wild-type cells incubated with or without globomycin, we could see bands labeled with [2-^{3}H]glycerol or [^{3}H]palmitate corresponding to the [^{35}S]methionine-labeled mature lipoprotein or modified prolipoprotein. With or without globomycin, the prolipoprotein accumulated in the *secF* mutant was not modified with lipid. In the region of the gel corresponding to the [^{35}S]methionine-labeled unmodified prolipoprotein, no band was observed for samples labeled with [2-^{3}H]glycerol or [^{3}H]palmitate (data not shown), indicating that the prolipoprotein that accumulated in the *secF* mutant was not even modified with glycerol. This result suggests that at the nonpermissive temperature, the *secF* mutation prevented the unmodified prolipoprotein from gaining access to the modification enzymes, and no lipid-modified prolipoprotein was detected even in the presence of globomycin, which specifically inhibits signal peptidase II.

We studied the localization of the prolipoprotein that accumulated in the *secF* mutant at the nonpermissive temperature by using an alkali fractionation method (28). [^{35}S]methionine-labeled cells were treated with 0.1 M cold NaOH and centrifuged. Lipoprotein was isolated from both the NaOH pellet and the supernatant fraction and analyzed by PAGE. As shown in Fig. 5A, the prolipoprotein that accumulated in the *secF* mutant was recovered in the NaOH pellet. This result suggests that the unmodified prolipoprotein was associated with the membrane. The labeled unmodified prolipoprotein in the *secF* mutant remained totally unprocessed with a chase of up to 40 min at the permissive temperature (data not shown). Since the lipoprotein secretion defect was fully restored 40 min after the temperature shift to 37°C, this result indicates that the unmodified prolipoprotein accumulated in the *secF* mutant cannot regain access to the modification and processing enzyme even after the mutant cells have restored normal secretion functions.

We have also studied whether or not the prolipoprotein accumulated in the *secF* mutant is covalently linked to peptidoglycan. As shown in Fig. 5B, no bound-form prolipoprotein was detected for the *secF* mutant with steady-state labeling at the nonpermissive temperature.

DISCUSSION

The results obtained in the current and earlier studies clearly show that the export of Braun's lipoprotein requires all known *sec* genes except *secB*, i.e., *secA*, *secD*, *secE*, *secF*, and *secY*. The SecB-independent export of Braun's lipoprotein may be a special case for this particular lipoprotein, since the export of pullulanase has been shown to require intact *secB* gene as well (26). Among the *sec* genes identified and characterized, four (*secY*, *secE*, *secD*, *secF*) encode membrane proteins (10, 18, 32). It has been postulated that some of these membrane proteins, especially the SecY and SecE proteins, form a complex which functions as the translocator in the inner membrane. Whether these proteins form a pore in the membrane or simply function to facilitate protein export is not known. Earlier studies have shown that the export pathways for lipoproteins and nonlipoproteins are not compartmentalized (11–13), and our study supports the idea that the export of lipoprotein utilizes the same translocator used by nonlipoproteins. The SecD and SecF proteins share amino acid sequence homology (10). Two findings have suggested that these two proteins play a role at a late step in protein translocation. First, no allele of *secD* and *secF* genes has been found as a suppressor of signal sequence mutations of exported proteins (33), suggesting that these two gene products interact with the exported proteins at a step(s) after the recognition of the signal sequence by the export machinery has taken place. Secondly, on the basis of their deduced amino acid sequences, these two proteins appear to have large periplasmic domains, a feature not found in SecE and SecY proteins (10). Our finding that the prolipoprotein that accumulated in the *secD* and the *secF* mutants is not modified suggests that the modification of prolipoprotein takes place after it has encountered a productive interaction with the SecD and SecF proteins.

It is noteworthy that the effects of the *secE* mutation on the export of OmpA protein and lipoprotein were kinetically and quantitatively different, while the effects of the *secD* and *secF* mutations on the export of these two proteins were indistinguishable. Since the *secE501*(Cs) allele of the mutant we used in this study is not located in the coding region of the SecE protein but in the putative translation initiation site (31), it may affect the synthesis of the SecE protein but not its structure. The observed difference may reflect a different requirement of the amount of the SecE protein for an optimal export of OmpA protein and lipoprotein.

The prolipoprotein accumulated in the *secF* mutant at the nonpermissive temperature is not modified with lipid. Since the unmodified prolipoprotein was recovered from the NaOH pellet and was not covalently bound to the peptidoglycan, it is likely that the unmodified prolipoprotein remains associated with the inner membrane. Neither lipid modification nor processing of prolipoprotein is required for its covalent attachment to peptidoglycan (40); thus, the failure of the unmodified prolipoprotein present in the *secF* mutant to be covalently linked to the peptidoglycan is consistent with its being located in the inner membrane and not accessible to the enzyme involved in the formation of the murein-bound form of lipoprotein. Our results suggest that the SecD and SecF proteins function at a step before the prolipoprotein undergoes posttranslational modifications and processing. The reason why this unmodified prolipoprotein cannot be further modified even after the SecF protein function has been restored is unknown.

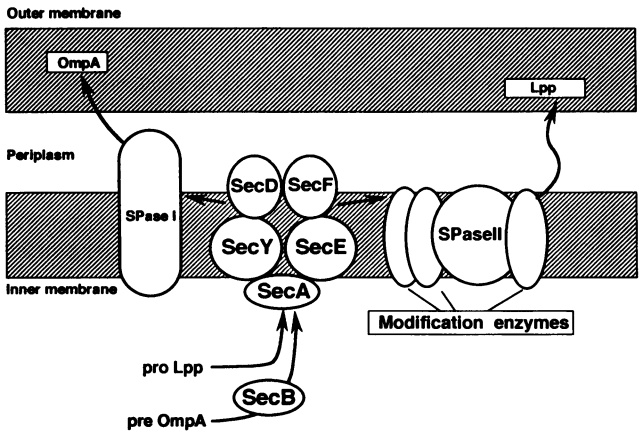

FIG. 6. A common export pathway for lipoprotein and nonlipoprotein precursors in *E. coli*. Export of prolipoprotein requires functional SecA, SecY, SecE, SecD, and SecF proteins but does not require a functional SecB protein. The divergence of the export pathway for lipoproteins and nonlipoproteins occurs after the preproteins have encountered functional SecD and SecF proteins. Lpp, lipoprotein; SPase I, signal peptidase I; SPase II, signal peptidase II.

Figure 6 shows our current understanding of the export of lipoproteins based on our results and those of previous studies. It is not clear whether all these membrane proteins (SecY, SecE, SecD, and SecF) form a single complex as a translocator or whether the SecD and SecF proteins function sequentially after the preprotein has been translocated by the SecY-SecE complex. The divergence of the export pathway for lipoproteins and nonlipoproteins occurs after the step defined by the SecD and SecF proteins.

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