Gene encoding the C- and N-terminal regions of SecE were constructed and placed under the control of the tac promoter on plasmids. The C-terminal region of SecE (SecE-C) was sufficient for suppression of the secEcs phenotype, confirming the results of Schatz et al. (Schatz, P. J., Bieker, K. L., Ottemann, K. M., Silhavy, T. J., and Beckwith, J. (1991) EMBO J. 10, 1749-1757). SecE-C allowed the overproduction of SecY, and its overproduction was achieved when the tac-secY gene, on a plasmid, was induced, indicating that the C-terminal region is the site of interaction of SecE with SecY and that the interaction makes the two Sec proteins stable. SecE-C was purified and used with SecY for the reconstitution of protein translocation activity. SecE-C was active in the functional reconstitution. The SecE-C/SecY-dependent protein translocation absolutely required SecA and ATP as the native translocation reaction did. Quantitative analysis revealed that SecE-C was 50% as active as intact SecE. The N-terminal region of SecE (SecE-N) also suppressed in vivo the defect caused by the secEcs mutation. SecE-N was, however, inactive in the overproduction of SecY. A possible oligomeric structure of SecE is discussed.

Extensive genetic studies have revealed that several genes are involved in protein translocation across the cytoplasmic membrane of Escherichia coli. They are the secA, secB, secD, secE, secF, and secY genes (1-5). Of these, the secD, secE, secF, and secY genes encode integral membrane proteins of the cytoplasmic membrane (3, 4, 6). All Sec proteins encoded by these genes have been overproduced with the aid of gene manipulation techniques (7, 8) and purified (9, 10). Recently, the reconstitution of proteoliposomes active in protein translocation from the purified Sec proteins was achieved, which made it possible to directly demonstrate the involvement of the SecE and SecY proteins in the translocation reaction (9-12). No biochemical evidence as to the functions of SecD and SecF in protein translocation has been obtained, however.

The interaction between SecE and SecY in the cytoplasmic membrane has been demonstrated. Three genetic strategies have been used to demonstrate the SecE/SecY interaction:

1. Suppressors-directed inactivation, Sec titration, and the use of synthetic lethality. The following physiological and biochemical evidence also supports the interaction between the two Sec proteins. 1) Overproduction of SecY is possible only when SecE is overproduced (7); 2) SecE and SecY can be copurified under certain conditions, and the complex can be reconstituted into proteoliposomes to exhibit translocation activity (12); and (3) SecE can be coimmunoprecipitated with SecY an anti-SecY antibody (12). It is highly likely, therefore, that SecE and SecY interact with each other to form a complex that constitutes the essential part of the protein translocation machinery.

SecE is a rather small protein with a molecular mass of 13.6 kDa (4) and has three membrane-spanning regions (4). Schatz et al. (14) recently demonstrated that a truncated SecE possessing only the third membrane-spanning region is sufficient for cell growth and in vivo protein translocation. The molecular mass of this region is only 6.1 kDa. In the present work, we demonstrated that the C-terminal fragment of SecE, which was almost the same as that studied by Schatz et al., was active in vivo in the overproduction of SecY. Conversely, the overproduction of this SecE fragment was supported by the high expression of the secY gene, suggesting that the C-terminal region of SecE is the site of interaction with SecY. Furthermore, we demonstrated that the purified C-terminal fragment was active in the reconstitution of protein translocation activity, i.e., proteoliposomes reconstituted from SecE, the C-terminal fragment of SecE, and phospholipids were translocationally active in the presence of SecA and ATP. Evidence suggesting the oligomeric nature of SecE is also presented.

**Experimental Procedures**

**Bacterial Strains**—E. coli MC4100 (15), PR520 (MC4100 secEcsE501) (16), and SM7000 (ompT secEcsE501 argE::TnlO) were used. The last strain was derived from W3110 M25 (17).

**Materials**—Restriction endonucleases, T4 DNA ligase, and SP6 RNA polymerase were obtained from Takara Shuzo Co., Ltd. ATP and creatine kinase were from Boehringer Mannheim. IPTG was from Nacalai Tesque. Octyl glucoside was purchased from Dojindo Laboratories. Proteine K was from Merek, and Tras"S-label (a mixture of 70% 35S)methionine and 20% 35S)cysteine, 100 Ci/mmL) was obtained from ICN. Anti-SecY and anti-SecE antisera were raised against synthetic peptides corresponding to the Met-Arg region of SecY (18) and the Lys-Lys region of SecE (4), respectively, as described (7, 19).

**Plasmids**—pUS12 is a control vector carrying the lacI gene and the tac promoter (20). pMAN809 carries the lacI and tac-secE genes (7). pMAN510 carries the tac-secY gene and a runaway replicon (7).
pMAN803 is a pBR322-based plasmid containing a 0.79-kilobase pair XhoI-KpnI fragment including the secE gene (7). POAD26 encodes the gene for prnOmpA D26, which is under the control of the SP6 promoter (9).

Construction of Plasmids pE1 and pE2—For the construction of pE1, pMAN809 was digested with BamHI and KpnI. The small 540-base pair BamHI-KpnI fragment carrying the secE gene was further digested with AluI into four fragments. The AluI fragments were then ligated with the large BamHI-KpnI fragment of pMAN809. Of the plasmids thus constructed, the one that lacked the 180-base pair AluI fragment encoding residues 7-66 of SecE was selected. This plasmid, pE1, expresses the tac-secE-N gene encoding a SecE with an amino-terminal deletion (SecE-C).

For the construction of pE2, a stop codon (TGA) was introduced at codon 74 of the secE gene in pMAN809 by site-directed mutagenesis. A synthetic oligonucleotide (5'-dTTTGGCCTTGACGCGTACCGA-3') and the oligonucleotide-directed in vitro mutagenesis system of Amersham Corp. were used. Therefore, plasmid pE2 thus constructed carries the tac-secE-E gene encoding a SecE with a carboxy-terminal deletion (SecE-N). The chemical structures of SecE-C and SecE-N are shown in Fig. 1, together with that of intact SecE and its possible orientation in the membrane.

Bacterial Cultivation—All bacterial cells were grown in L broth medium. When the harbored plasmid, pMAN510, the medium was supplemented with kanamycin (30 μg/ml). When they were harbored by other plasmids, ampicillin (50 μg/ml) was added.

SDS-PAGE and Immunoblot Analysis of SecE, SecE Derivatives, and SecY—A gel composed of 12.5% acrylamide, 0.2% N,N'-methylenebisacrylamide and 0.5% SDS in 100 mM sodium phosphate (pH 7.2) was used according to Hussain et al. (21). All samples were applied to the gel without boiling. Immunoblot analysis was performed as follows. After electrophoresis, the gel was soaked in 100 mM sodium phosphate (pH 7.2), 20% (v/v) methanol for 10 min. Proteins were then transferred from the gel to a nitrocellulose membrane filter with the aid of a semi-dry electrophoretic transfer cell (Bio-Rad) in accordance with the manufacturer’s instructions. The blot was blocked with 10% horse serum in TBS buffer (50 mM Tris-HCl (pH 7.5) and 150 mM NaCl) for 15 min at room temperature and then reacted for 30 min with an antisera solution. TBS buffer was used for the dilution of antisera. After washing the membrane filter several times with TBS buffer supplemented with 0.05% Tween 20, anti-rabbit IgG antiserum conjugated with alkaline phosphatase was added, and then a 30-min incubation was performed. After washing several times, SecE or SecY on the blot was visualized using a Piloblot system (Promega Biotec).

Preparation of Cytoplasmic Membrane Vesicles and Phospholipids—Everted cytoplasmic membrane vesicles were prepared as described (22), as were SecE-containing phospholipids (19).

Purification of SecA, SecE, and SecY—SecA and SecE were purified from SecA- and SecE-overproducing cells, respectively, as described (9, 23). SecY was purified from SecE-SecY-overproducing cells as described (10).

Purification of SecE-C—SM7000 cells harboring both pE1 and pMAN510 were induced with 1.5 mM IPTG at early log phase. After induction for 2 h at 37 °C, the cells were collected. Cytoplasmic membrane vesicles were prepared from these cells as described (22). The membrane vesicles were then solubilized with 2.5% octyl glucoside containing 10 mM potassium phosphate (pH 6.0) and 10 mM MgCl₂ on ice for 10 min at a concentration of 1 mg of protein/ml. The sample was then centrifuged at 140,000 × g at 4 °C for 30 min. Under these conditions, 94% of SecE-C was solubilized, whereas the extent of solubilization of the total protein was only 38%. The solubilized membrane vesicles were then applied to a 10-50% sucrose density gradient of 2 ml each, containing SecE-C (fractions 38-41 in Fig. 7) were collected; and then the buffer in the preparation was replaced with 20 mM Tris acetate (pH 9.0), 2.5% octyl glucoside, 10% (w/v) glycerol on a fast desalting column (1 × 10 cm; Pharmacia). The SecE-C preparation was applied to a 0.5-20% column of Mono P (Pharmacia) anion exchange, that had been equilibrated with 20 mM Tris acetate (pH 9.0), 2.5% octyl glucoside, 10% (w/v) glycerol. The column was developed at a flow rate of 0.5 ml/min with a linear gradient of 0-1 M NaCl in the same buffer. SecE-C appeared in the flow-through fraction. This fraction was concentrated to 300 μg/ml by membrane filtration and then applied to a Superose 12 HR size-exclusion column (1 × 30 cm) that had been equilibrated with 50 mM potassium phosphate (pH 6.95), 150 mM NaCl, 2.5% octyl glycoside, 10% (w/v) glycerol. The column was developed with the same buffer at a flow rate of 0.5 ml/min. SecE-C was determined by SDS-PAGE, and fractions containing it were collected and then concentrated to 100 μg/ml. These procedures are summarized in Table 1.

In Vitro Transcription and Translation—prnOmpA D26, a truncated promoter, was synthesized in vitro in the presence of Tran35S-label (0.46 mCi/ml) as described previously (24). 35S-Labeled pOMPA D26 was partially purified as reported (25).

Reconstitution of Proteoliposomes and Assaying of Protein Translocation Activity—The reconstitution of proteoliposomes active in protein translocation from purified SecY and SecE or its derivative was performed as described (10). Protein translocation activity was measured in the presence of SecA and ATP using 35S-labeled pOmpA D26 as a substrate. The translocated protein was detected as a protease K-resistant band on an SDS-polyacrylamide gel by fluorography (24). The efficiency of translocation was calculated by densitometric scanning of fluorograms with a Shimadzu Chromatoscan CS-830.

RESULTS

Truncated SecE Derivatives Lacking Either N- or C-terminal Region Suppress secEcs Mutation—We constructed plasmids carrying genes encoding the truncated SecE derivatives SecE-C and SecE-N (Fig. 1). For the construction of secE-N, a stop codon (UGA) was introduced at codon 74 of the secE gene. This insertion caused not only the introduction of a stop codon, but also frameshifting in the following region. The expression of intact SecE from this gene is therefore highly unlikely.

E. coli PR520 cells possessing a mutant secE gene (secE-E501) were transformed with these plasmids. The transformants were inoculated onto solid L broth medium in the absence or presence of IPTG, an inducer of the tac promoter. The plates were incubated at 30 °C for 2 days (Fig. 2). Although parent MC4100 cells harboring pUS12, a control vector, grew well at 20 °C, growth of PR520 cells harboring pUS12 was hardly detected. This cold-sensitive phenotype was suppressed by pMAN803, which carries the wild-type secE gene. pBR322, the control vector for pMAN803, did not suppress the cold-sensitive phenotype. In the absence of IPTG, pE1, which encodes SecE-C, partially suppressed the cold-sensitive phenotype, whereas pE2, which encodes SecE-N, did not. In the presence of IPTG, both pE1 and pE2 suppressed the cold-sensitive phenotype strongly. Since the secE-E501 mutation

![Fig. 1. Chemical structures of SecE and its derivatives and putative orientation model of SecE in cytoplasmic membrane.](image-url)
Fig. 2. Suppression of secEcsE501 mutation by SecE derivatives. MC4100 (secE*) or PR520 (secEcsE501) cells were transformed with the indicated plasmids. The transformants were streaked onto L broth/ampicillin plates supplemented with (right) or without (left) 1.5 mM IPTG and then incubated for 2 days at 20 °C.

Fig. 3. Expression of SecE derivatives. SM7000 (ompT secEcsE501) cells were transformed with pUSI2 (lanes 1 and 2), pE1 (lanes 3 and 4), or pE2 (lanes 5 and 6). Each transformant was grown in liquid L broth/ampicillin medium in the absence (lanes 1, 3, and 5) or presence (lanes 2, 4, and 6) of 1.5 mM IPTG at 37 °C. At the late log phase, the cells were collected and treated with 5% trichloroacetic acid. Cellular proteins (20 µg) were then dissolved in 2% SDS and subjected to SDS-PAGE, followed by immunoblot analysis with anti-SecE antiserum. Purified SecE (100 ng) (lane 7) and purified lipoprotein (280 ng) (lane 8) were treated similarly. The migration positions of molecular mass markers are indicated.

Fig. 4. Cellular localization of SecE derivatives. SM7000 cells harboring either pE1 (left) or pE2 (right) were grown for 2 h at 37 °C in the presence of 1.5 mM IPTG. Cell fractionation was carried out as described previously (22). Individual fractions equivalent to 2 × 10^7 cells were subjected to SDS-PAGE, followed by immunoblot analysis with anti-SecE antiserum. The positions of SecE-C, SecE-N, and lipoprotein (LP) are indicated. The positions of molecular mass markers are also indicated. WC, whole cells; CP, cytosol plus periplasm; M, membrane fraction; OM, outer membrane fraction; CM, cytoplasmic membrane fraction.

lower level of SecE in the secEcsE501 background (lanes 1–6) (14).

E. coli cells expressing SecE-C and SecE-N were fractionated, and individual fractions were analyzed by SDS-PAGE. Protein profiles visualized by Coomassie Brilliant Blue staining verified proper fractionation (data not shown). The localization of SecE derivatives was then examined (Fig. 4). Both derivatives of SecE were localized in the cytoplasmic membrane, like intact SecE.

Effect of High Expression of secY Gene on Synthesis of SecE Derivatives—Intact SecE was overproduced when the tac-secE gene was induced with IPTG. The production of SecE-C in the presence of IPTG was, however, not as extensive as that of intact SecE, in spite of the fact that the secE-C gene was under the control of the tac promoter (Fig. 5, compare lanes 5 and 3). The overproduction of SecE-C was achieved with high expression of the secY gene as follows. pMAN510 carrying the tac-secY gene was introduced into E. coli cells together with pE1 carrying the tac-secE-C gene. The cells were then grown in the presence of IPTG and subjected to immunoblot analysis with anti-SecE antiserum. When the tac-secY gene was coinduced with the tac-secE-C gene, >10-fold overproduction of SecE-C, compared with the tac-secE-C gene alone, was achieved (Fig. 5). This strongly suggests that SecE-C interacts with SecY and is consequently stabilized. The amount of SecE-N, on the other hand, did not increase in the presence of the tac-secY gene.

Cells analyzed in Fig. 5 (lanes 2, 4, 6 and 8) harbored pMAN510, which carries secY and a runaway replicon. The amount of lipoprotein in these cells was lower than that in the pMAN510-free cells analyzed (lanes 1, 3, 5, and 7). Run-
positions of molecular mass markers, SecE, SecE-C, SecE-N, and to immunoblot analysis with anti-SecE antiserum. The migration were then treated as described in the legend to Fig. 3 and subjected to immunoblot analysis with anti-SecE antiserum. The migration positions of molecular mass markers, SecE, SecE-C, SecE-N, and lipoprotein (LP) are indicated.

Fig. 5. SecY-dependent overproduction of SecE derivatives. SM7000 cells transformed with the indicated plasmids were grown for 2 h at 37 °C in the presence of 1.5 mM IPTG. The cells were then treated as described in the legend to Fig. 3 and subjected to immunoblot analysis with anti-SecE antiserum. The migration positions of molecular mass markers, SecE, SecE-C, SecE-N, and lipoprotein (LP) are indicated.

away replication of the plasmid seems to cause the decrease in the amount of lipoprotein.

SecE-C Allows Overproduction of SecY—The overproduction of SecY is possible only when SecE is co-overproduced (Fig. 6, lanes 1–3) (7). The tac-secE-C gene also supported the tac-secE-dependent overproduction of SecY (lane 4), whereas the tac-secE-N gene was not effective in SecE overproduction (lane 5). Thus, it is highly likely that SecE-C and SecY each mutually support the overproduction of the other. This mutual dependence strongly suggests the presence of a firm interaction between SecY and SecE and that the C-terminal portion of SecE is responsible for the interaction.

SecE-C Can Replace Intact SecE for Translocation in Reconstituted Proteoliposomes—The cytoplasmic membrane fraction was prepared from SecE-C/SecY-overproducing cells, and purification of SecE-C was carried out. When the membrane fraction was solubilized as described under “Experimental Procedures,” SecE-C was solubilized almost completely (Table I; see also Fig. 8A), whereas SecY was not (data not shown). The resultant solubilized fraction was then subjected to Mono S column chromatography. The elution profile is shown in Fig. 7A. Individual fractions were analyzed for activity of SecE-C by SDS-PAGE, followed by Coomassie Brilliant Blue staining (Fig. 7B) or immunoblotting (Fig. 7C). The SecE-C-containing fractions were then reconstituted into proteoliposomes with or without purified SecY for examination of translocation activity. Proteoliposomes exhibited translocation activity only when they were reconstituted in the presence of SecY, and a parallelism was observed between the amount of SecE-C in these fractions and the translocation activity reconstituted (Fig. 7D). No intact SecE was detected in these fractions. We suggest, therefore, that SecE-C can replace intact SecE in the protein translocation. The SecE-C-dependent translocation activity was SecA-dependent (Fig. 7D).

Reconstitution Experiments with Purified SecE-C—The purification of SecE-C was continued. Mono S fractions 38–41 (Figs. 7A and 8A, lane 3) were collected and applied to a Mono P column. The flow-through fraction from the Mono P column (lane 4) was then subjected to gel filtration (lane 5). The purity of SecE-C in this final preparation was 81.7% (Fig. 8A and summarized in Table I). We then examined by immunoblot analysis whether or not this final preparation was contaminated by intact SecE. The amount (1.9 μg) of this preparation did not give any detectable band of SecE (Fig. 8B, lane 1). Since the minimum amount of SecE detectable under these conditions is ~4 ng (lanes 2–6), the maximum amount of intact SecE that could have contaminated the final SecE-C preparation should be <0.2%.

SecY was supplemented with different amounts of either purified SecE-C or SecE and then subjected to reconstitution into proteoliposomes, which were then assayed for translocation activity. Translocation activity was reconstituted in a manner dependent on the concentration of SecE-C or SecE added (Fig. 9, A and B). No translocation activity was reconstituted with either SecY alone or a SecE derivative alone. The proteoliposomes thus reconstituted were analyzed for their protein composition by SDS-PAGE (Fig. 9C). The recovery of SecE-C in proteoliposomes was considerably lower than that of intact SecE. Densitometric tracing of the gel (Fig. 9C) revealed that the recoveries of SecE-C and SecE in proteoliposomes were 10 and 30%, respectively. Based on the results presented in Fig. 9 (A–C), the initial rates of translocation of individual reconstituted proteoliposomes were plotted against the amounts of SecE-C and SecE (Fig. 9D). SecE-C was ~50% as active as intact SecE per molecule with regard to protein translocation activity, suggesting that the C-terminal region of SecE is functionally active in protein translocation.
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FIG. 7. Chromatography of SecE-C on Mono S column and reconstitution of protein translocation activity from purified SecY and Mono S fractions. A, membrane vesicles prepared from SecE-C/SecY-overproducing cells were solubilized and then the solubilized sample (20 ml, 7.6 mg of protein) was fractionated (2 ml each) on a Mono S column with the indicated gradient of NaCl (---) as described under "Experimental Procedures." B and C, 50 and 20 µl, respectively, of Mono S fractions 36-44 were subjected to SDS-PAGE and then analyzed by Coomassie Brilliant Blue staining (B) and immunoblotting with anti-SecE antiserum (C). The positions of SecE-C and molecular mass markers are indicated. D, aliquots (60 µl) of these fractions were reconstituted into proteoliposomes in the presence (C, Δ) or absence (□) of SecY (5.8 µg). The proteoliposomes were then subjected to the translocation assay in the presence (C, □)

DISCUSSION

Schatz et al. (14) genetically demonstrated that the C-terminal segment of SecE (SecEΔ7-78), possessing only one of its three hydrophobic membrane-spanning domains, is sufficient for the suppression of cold-sensitive defects caused by the secEcsE501 mutation. In the present work, we constructed, on a DNA level, a similar SecE derivative (SecE-C) and confirmed their results. SecEΔ7-78 has a deletion of residues 7-78, whereas SecE-C has a deletion of residues 7-66, hence possessing an additional 12 residues at the N terminus. This additional region is hydrophilic and neutral and localized on the cytosolic surface of the cytoplasmic membrane (4). We assume that SecE-C and SecEΔ7-78 can be discussed on the same basis. In these SecE derivatives, the membrane-spanning hydrophobic stretch is preceded by positively charged residues. This may contribute to insertion of the hydrophobic stretch into the membrane in the correct orientation.

In addition to the suppression of the cold-sensitive phenotype caused by the secEcsE501 mutation, SecE-C was also functionally active as intact SecE in other respects: namely, high expression of the secE-C gene allowed the overproduction of SecY, which cannot be overproduced alone (7); and SecE-C is 50% as active as intact SecE in the reconstitution of translocation activity from purified Sec proteins, indicating that SecE-C is as active as intact SecE on almost an equimolar or absence (Δ) of SecA using proOmpA D26 as a substrate. The translocation reaction was carried out at 37 °C for 15 min. The amounts of SecE-C in the individual fractions were estimated from the results shown in C and are indicated by open bars.

FIG. 8. Purification of SecE-C. A, fractions obtained at individual purification steps were analyzed by SDS-PAGE with Coomassie Brilliant Blue staining. The positions of SecE-C and molecular mass markers are indicated. Lane 1, cytoplasmic membrane fraction derived from secEcsE501-overproducing cells (62 µg); lane 2, octyl glucoside supernatant of the cytoplasmic membrane fraction (25 µg); lane 3, Mono S fraction (3.9 µg); lane 4, Mono P flow-through fraction (2.5 µg); lane 5, Superose 12 HR fraction (1.7 µg). B, 1.9 µg of the Superose 12 HR fraction were subjected to immunoblot analysis with anti-SecE antiserum (lanel). To detect possible contamination by intact SecE, purified SecE at different concentrations was also subjected to immunoblot analysis with anti-SecE antiserum (lanes 2-6). Lanes 2-6 contained 100, 33, 11, 3.7, and 1.2 ng of SecE, respectively. The positions of SecE, SecE-C, and molecular mass markers are indicated.

or absence (Δ) of SecA using proOmpA D26 as a substrate. The translocation reaction was carried out at 37 °C for 15 min. The amounts of SecE-C in the individual fractions were estimated from the results shown in C and are indicated by open bars.
Fig. 9. Reconstitution of translocationally active proteoliposomes from SecE-C and SecY. A and B, SecE-C or intact SecE, respectively, was supplemented with (C, △, □, ●) or without (▲) 3.8 μg of SecY and then reconstituted into proteoliposomes. The amounts of SecE-C used for reconstitution were 6 (○, △), 4 (△), 2 (□), and 0 (●) μg. The amounts of SecE used for reconstitution were 3 (○), 2 (△), 1 (□), and 0 (●) μg. The reconstituted proteoliposomes were recovered by centrifugation and suspended in 110 μl of buffer containing 50 mM potassium phosphate (pH 7.5) and 150 mM NaCl. Aliquots (90 μl) of the proteoliposome suspension were mixed with 30 μl of the ATP-containing solution (9), and the mixture was incubated at 37 °C for 3 min. The translocation reaction was started by adding prewarmed proOmpA D26 (24 μl) at 37 °C. At the indicated times, 25 μl of the reaction mixture were withdrawn and analyzed for proOmpA D26, which was translocated. The amount of proOmpA D26 translocated into proteoliposomes was expressed as a percentage of the input proOmpA D26. C, an aliquot (20 μl) of the reconstituted proteoliposome suspension was precipitated with trichloroacetic acid (final concentration of 10%) and then analyzed by SDS-PAGE with Coomassie Brilliant Blue staining. The amounts of SecE-C and SecE recovered in the reconstituted proteoliposomes were determined by densitometric scanning of the individual bands and are indicated at the bottom of the gel. The positions of SecY, SecE, SecE-C, and molecular mass markers are also indicated. D, the initial rates of translocation obtained from the results shown in A and B are plotted against the amounts (estimated from the result shown in C) of SecE-C (○) or SecE (△) incorporated into 1 ml of proteoliposomes suspension. Molecular masses of 7.4 and 13.6 kDa for SecE-C and SecE, respectively, were used for estimation.

basis. It is certain, therefore, that the C-terminal region of SecE possessing the third membrane-spanning stretch plays a major role in protein translocation.

SecE-C was, however, different from intact SecE in its stability in cells. Although intact SecE is quite stable in cells, even when it is overproduced alone (7), the overproduction of SecE-C was possible only under high expression of the secY gene. It seems likely that the N-terminal portion is required for the stable assembly of SecE into the membrane. It is interesting in this respect that the efficiency of the incorporation of SecE-C into reconstituted proteoliposomes was quite low. Although SecEΔ7-78 complements the cold sensitivity caused by the secEcsE501 mutation, it cannot alone support cell growth in the cold (14). This may also be due to the unstable nature of truncated SecE.

SecE-N suppressed the cold-sensitive phenotype of the secEcsE501 mutation, whereas it did not support the overproduction of SecY. Its overproduction was not supported by SecY either. It is unclear at present whether or not SecE-N exhibits some activity in reconstituted proteoliposomes, either by itself or together with SecE or SecE-C. One possible explanation for the suppression of the cold-sensitive phenotype is that SecE may function as an oligomeric form. In the secEcsE501 mutant, the level of SecE is too low to grow normally at low temperature (14). If SecE and SecE-N can form an oligomer that is functionally active, SecE-N could suppress the cold-sensitive phenotype. Furthermore, since the activity of SecE-C was about half that of SecE, it is likely that the N-terminal portion of SecE plays a role in enhancing the activity exhibited by the C-terminal portion. The forma-
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...ation of an oligomer from a low level of SecE and an overproduced level of SecE-N, therefore, may result in an increase of the total activity of SecE in the cell and permit cell growth at low temperature even though SecE-N alone is entirely inactive. The oligomeric nature of SecE was suggested by its elution position on size-exclusion chromatography (9). A possible oligomeric structure was also previously discussed in connection with the paradoxical nature of SecEΔ7-78 (14).

The interaction between SecE and SecY has been suggested genetically, physiologically, and biochemically (7,12,13). The SecE-dependent overproduction of SecY is one of the direct and lines of evidence for this interaction (7). We observed in the present work that the overproduction of SecY was supported by high expression of the secE-C gene and vice versa, whereas the secE-N gene was entirely inactive in SecY overproduction. It is most likely, therefore, that the C-terminal region is the site of interaction of SecE with SecY.

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