Vol. 217, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 5, 1995 Pages 217-223

PREFERENTIAL INTERACTION OF SEC-G WITH SEC-E STABILIZES AN UNSTABLE SEC-E DERIVATIVE IN THE *ESCHERICHIA COLI* CYTOPLASMIC MEMBRANE

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Received October 27, 1995

SUMMARY: SecY, SecE and SecG form a membrane part of the protein translocation machinery. A SecG-overproducing plasmid was constructed by placing the *secG* gene under the control of the *tac* promoter. From the extent of SecG overproduction, the number of SecG molecules in one normal cell was estimated to be about 1,000, which is similar to those of SecY and SecE. Overproduction of SecG stabilized the overproduction of SecE-C, an unstable truncated derivative of SecE, as effectively as SecY does. SecG overproduction also stabilized the overproduction of SecY. However, the SecG-dependent stabilization of SecY was less potent than the SecE-dependent stabilization. These results indicate that SecG preferentially interacts with SecE, which associates with SecY, the SecG-SecE-SecY complex thus being formed in the cytoplasmic membrane.

Protein translocation across the *Escherichia coli* cytoplasmic membrane involves seven Sec factors (1-3). A central part of the translocation machinery is composed of SecA, a peripheral membrane ATPase, and three integral membrane factors, SecY, SecE and SecG (4-9). In addition, SecB functions as a molecular chaperone for a subset of precursor proteins (10), and SecD and SecF function at a late stage of the translocation (11-13).

SecY, SecE and SecG form a complex in the membrane (6, 14-16), to which SecA delivers a precursor in an ATP-dependent manner (17). The subunit stoichiometry of the complex remains to be clarified, although the complex has been suggested to contain one molecule each of SecY and SecE (18). The mode of

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0006-291X/95 $12.00

subunit interaction in the complex also remains unknown. However, various indirect data indicating subunit interaction have been reported (14, 19-23). The most striking observation is the stabilization of overproduction of SecY on simultaneous overproduction of SecE (19, 23) or SecF (22). It has been shown that SecY is a substrate for proteases such as FtsH (24), and is immediately digested unless it associates with SecE or SecF. As a result, the stoichiometry between SecY and SecE remains constant. The numbers of SecY and SecE molecules were found to be similar in one normal cell (25, 26). In contrast, neither the number of molecules of SecG in one normal cell nor a Sec factor interacting with SecG in the complex has been determined.

MATERIALS AND METHODS

Plasmids. pTG1, that carries the *tac-secG* gene, was constructed as follows. pGE1 (3) carrying *secG* was cleaved with *Kpn*I, followed by partial digestion with *Alu*I. A 430 bp *Alu*I-*Kpn*I fragment that contains codons 5-110 of SecG was isolated, mixed with synthetic oligonucleotides (5'-GATCCTAGGAGGTTTAAATTT*ATG*TATGAAG-3' and 3'-GATCCTCCAAATTTAAATACATACTTC-5'; the initiation codon is italicized), that contain the ideal SD sequence followed by codons 1-4 of SecG, and then cloned into the *Bam*HI-*Kpn*I site of pUSI2 (27), a high copy vector carrying the *tac* promoter -*lac* operator and *lacI*. pTG1, thus constructed, carries *secG* under the control of the *tac* promoter . To construct pE6 that carries *tac-secE-C* (*secEΔ8-75*), a 280 bp *Mlu*I-*Kpn*I fragment of pE2 (21) containing codons 76-127 of SecE was treated with Klenow enzyme, mixed with synthetic oligonucleotides (5'-GATCCTAGGAGGTTTAAATTT*ATG* AGTGCGAATACCGAAG-3' and 3'-GATCCTCCAAATTTAAATACTCACGCTTATGGCTT C-3'; the initiation codon is italicized), that contain the ideal SD sequence followed by codons 1-7 of SecE, and then cloned into the *Bam*HI-*Kpn*I site of pUSI2. This SecE-C was shorter than SecEΔ7-66 constructed previously (21) but suppressed the *secE* null mutation (28) (data not shown). To construct pTEG61 that carries *tac-secE-C-tac-secG*, an 800 bp *Eco*RI-*Bgl*II fragment of pTG1 was cloned into the *Sma*I-*Bgl*II site of pE6, that lies just downstream of the *Kpn*I site. The *Eco*RI digestion was followed by treatment with Klenow enzyme. pMAN809 and pMAN510 carry *tac-secE* and *tac-secY*, respectively (19). The latter also carries a runaway replicon (19). pMAN480 (22) is a control vector for pMAN510. These plasmids were used to transform *E. coli* W3110 M25 (*ompT*) (29).

Materials. SecG was purified from cells harboring pTG1 as described (6). Restriction endonucleases and DNA modifying enzymes were obtained from Takara Shuzo Co., Ltd. IPTG was from Nacalai Tesque. A Plotoblot system for visualization of immunoblots was purchased from Promega Biotec. Oligonucleotides were synthesized using a Beckman Oligo 1000 DNA synthesizer.

Other methods. SDS-PAGE and quantitative immunoblotting were performed as described (21). The bands on the immunoblots were quantitated by densitometric scanning with a Shimadzu Chromatoscanner CS-930. Anti-SecE (13), anti-SecG (6), and anti-SecY (30) antisera were used to detect the respective proteins.

RESULTS

The number of SecG molecules in one normal cell. A SecG-overproducing plasmid, pTG1, was constructed by placing the *secG* gene under the control of the *tac* promoter -*lac* operator. *E. coli* W3110 M25 cells harboring pTG1 overproduced SecG in an IPTG-dependent manner (Fig. 1). The level of SecG in the absence of IPTG was similar to that in the cells harboring a control vector, pUSI2. The extent of overproduction was estimated to be 200- to 400-fold by means of densitometric scanning of immunoblots obtained with appropriately diluted samples. A total membrane fraction was prepared from the SecG-overproducing cells and analysed by SDS-PAGE, followed by Coomassie Brilliant Blue-staining. The SecG content of the membrane fraction was found to be 15.7%, indicating that the SecG content of the membranes of normal cells should be 200- to 400-fold lower than this value. Using these values for the equation reported by Matsuyama et al. (25), the number of SecG molecules in one normal cell was estimated to be 650 to 1300. The number of SecG molecules was also determined by means of quantitative immunoblotting using purified SecG as a standard. From four independent determinations, the number of SecG molecules was estimated to be 1200 to 1400. Taken together, we concluded that about 1000 molecules of SecG are present in one normal *E. coli* cell.

SecG-overproduction supports the overproduction of SecE-C. SecE-C, a truncated derivative of SecE, lacks the N-terminal portion (8-75) of SecE, which comprises 127 amino acid residues, but is still functional and able to complement the *secE* null mutant (28). On the other hand, unlike the intact SecE, stable overproduction of SecE-C was dependent on the simultaneous overproduction of

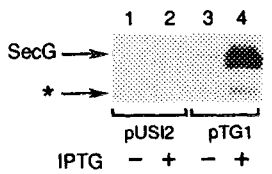

Fig. 1. Overproduction of SecG. *E. coli* W3110 M25 cells harboring pUSI2 (control vector, lanes 1 and 2) or pTG1 (*tac-secG*, lanes 3 and 4) were cultivated in LB/ampicillin at 37 °C. At the mid-log phase (4 x 10^8 cells/ml), IPTG (final 1.5 mM) was added (lanes 2 and 4) or not added (lanes 1 and 3). After 2-h cultivation at 37 °C, the cells were treated with TCA (final 5 %), and the precipitate containing 5 μg protein was analysed by SDS-PAGE and immunoblotting using the anti-SecG antibody. The asterisk indicates a 9-kDa proteolytic product of SecG.

SecY (21), suggesting that overproduced SecE-C is unstable unless it interacts with SecY. Since our previous observation suggested the interaction between SecE and SecG (6, 16), we examined whether or not SecG-overproduction supports that of SecE-C. When SecE-C was expressed together with SecG, the amount of SecE-C was more than 10-fold higher compared to that expressed in the absence of SecG expression (Fig. 2A). Furthermore, irrespective of whether SecY (Fig. 2B) or SecG is co-overproduced, a similar amount of SecE-C was overproduced, indicating that SecG stabilizes SecE-C as effectively as SecY does.

Effect of SecG-overproduction on the overproduction of SecY. Overproduction of SecY is stabilized when SecE (19, 23) or SecF (22) is simultaneously overproduced. To determine whether or not SecG overproduction is able to stabilize the SecY-overproduction, the expression of SecY was induced with or without the co-expression of SecG (Fig. 3). Induction of SecY alone caused an about 4-fold increase in the amount of SecY compared to the wild type. When SecG was co-expressed, the amount of SecY was about 20-fold higher than that in the case of the wild type cells. Since the overproduction of SecG affected the level of neither SecE nor SecF (data not shown), the increase in the amount of SecY upon SecG-expression is most likely caused by SecG-SecY interaction. However, SecG-

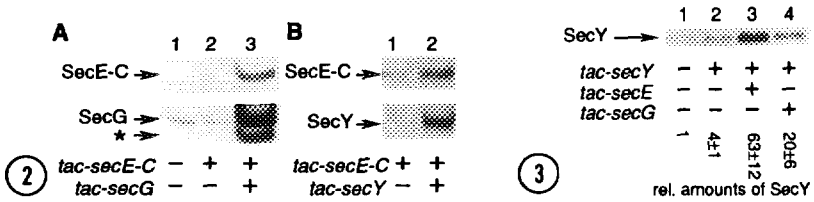

Fig. 2. SecG stabilizes SecE-C. W3110 M25 cells were transformed with pUSI2 (A, lane 1), pE6 (A, lane 2), pTEG61 (A, lane 3), pMAN480 and pE6 (B, lane 1) or pMAN510 and pE6 (B, lane 2). These cells were grown on LB/ampicillin (A) or LB/ampicillin plus kanamycin (B) to the mid-log phase (4 x 10^8 cells/ml), and then induced with 1.5 mM IPTG for 2 h at 37 °C. The cells were then precipitated with TCA and analysed by SDS-PAGE, followed by immunoblotting with anti-SecE (A and B), anti-SecG (A) or anti-SecY (B) antiserum. The genes controlled by the *tac* promoter are indicated at the bottom. The bands corresponding to SecY, SecE-C, SecG and the 9 kDa proteolytic product of SecG (asterisk) are also indicated.

Fig. 3. Effect of overproduction of SecG on SecY-overproduction. W3110 M25 cells harboring pMAN480 and pUSI2 (lane 1), pMAN510 (*tac-secY*) and pUSI2 (lane 2), pMAN510 and pMAN809 (*tac-secE*) (lane 3), or pMAN510 and pTG1 (*tac-secG*) (lane 4) were cultivated and induced as in Fig. 2. The cells were then processed as Fig. 1, and then SecY was visualized by immunoblotting. The relative amounts of SecY were determined in four independent experiments and are indicated at the bottom.

dependent overproduction of SecY was lower than the SecE-dependent overproduction, suggesting that the SecG-SecY interaction is less potent than the SecE-SecY interaction.

DISCUSSION

It has been established that the protein translocation machinery of *E. coli* comprises SecA and the SecY-SecE-SecG complex. To clarify the molecular mechanism underlying the translocation, it is essential to understand the mode of interaction among the four Sec factors and the subunit stoichiometry.

In this study, we established an SecG-overproducing system and found that the number of SecG molecules in one normal cell is similar to those of SecY and SecE (25, 26). The complex has been suggested to contain one molecule each of SecY and SecE (18). Taking these results together, we assume that the complex comprises one molecule each of the three subunits. SecA has been shown to function as a dimer (31, 32).

SecY and SecE have been shown to interact with each other in the complex (14, 18-21, 23). We examined the overproduction of an unstable SecE derivative and found that SecG stabilizes the derivative, most likely by interacting with it. SecG was also found to stabilize the overproduction of SecY. However, the SecY stabilization by SecG was less significant than that by SecE. Taken together, these results indicate that SecG preferentially interacts with SecE in the complex. SecE therefore should be placed at the center of the complex, and is thereby capable of interacting with both SecY and SecG. This is consistent with our previous observation that reduction of the SecE level causes a decrease in the level of not only SecY but also SecG (6). On the other hand, the function of SecE in protein translocation remains unknown. Only a portion of the second cytoplasmic region comprising less than 20 amino acid residues has been found to be essential for the SecE function as long as this region retains the proper topological arrangement on the membrane (33). SecE presumably plays a limited, but essential, role in protein translocation. Remarkable stimulation of protein translocation by SecG may be achieved through interaction of SecG with the second cytoplasmic region of SecE. The importance of the transmembrane region, which immediately follows the essential cytoplasmic region of SecE, for the SecE-SecG interaction has not been completely excluded, however.

ACKNOWLEDGMENTS

This work was supported by grants to H. T. from the Ministry of Education, Science and Culture of Japan. K. N. is a recipient of a JSPS Postdoctoral Fellowship.

REFERENCES

1. Bieker, K. L., Phillips, G. J., and Silhavy, T. J. (1990) J. Bioenerg. Biomembr. 22, 291-310.
2. Schatz, P. J., and Beckwith, J. (1990) Annu. Rev. Genet. 24, 215-248.
3. Nishiyama, K., Hanada, M., and Tokuda, H. (1994) EMBO J. 13, 3272-3277.
4. Wickner, W., Driessen, A. J. M., and Hartl, F.-U. (1991) Annu. Rev. Biochem. 60, 101-124.
5. Mizushima, S., Tokuda, H., and Matsuyama, S. (1992) In Membrane Biogenesis and Protein Targeting (W. Neupert and R. Lill, Eds.), pp. 21-32, Elsevier Science Publishers, Amsterdam, The Netherlands.
6. Nishiyama, K., Mizushima, S., and Tokuda, H. (1993) EMBO J. 12, 3409-3415.
7. Oliver, D. (1993) Mol. Microbiol. 7, 159-165.
8. Driessen, A. J. M. (1994) J. Membr. Biol. 142, 145-159.
9. Tokuda, H. (1994) FEBS Lett. 346, 65-68.
10. Kumamoto, C. (1991) Mol. Microbiol. 5, 19-22.
11. Gardel, C., Johnson, K., and Beckwith, J. (1990) EMBO J. 9, 3209-3216.
12. Bieker-Brady, K., and Silhavy, T. J. (1992) EMBO J. 11, 3165-3172.
13. Matsuyama, S., Fujita, Y., and Mizushima, S. (1993) EMBO J. 12, 265-270.
14. Brundage, L., Hendrick, J. P., Schiebel, E., Driessen, A. J. M., and Wickner, W. (1990) Cell 62, 649-657.
15. Akimaru, J., Matsuyama, S., Tokuda, H., and Mizushima, S. (1991) Proc. Natl. Acad. Sci. USA 88, 6545-6549.
16. Hanada, M., Nishiyama, K., Mizushima, S., and Tokuda, H. (1994) J. Biol. Chem. 269, 23625-23631.
17. Hartl, F.-U., Lecker, S., Schiebel, E., Hendrick, J. P., and Wickner, W. (1990) Cell 63, 269-279.
18. Joly, J. C., Leonard, M. R., and Wickner, W. (1994) Proc. Natl. Acad. Sci. USA 91, 4703-4707.
19. Matsuyama, S., Akimaru, J., and Mizushima, S. (1990) FEBS Lett. 269, 96-100.
20. Bieker, K. L., and Silhavy, T. J. (1990) Cell 61, 833-842.
21. Nishiyama, K., Mizushima, S., and Tokuda, H. (1992) J. Biol. Chem. 267, 7170-7176.
22. Sagara, K., Matsuyama, S., and Mizushima, S. (1994) J. Bacteriol. 176, 4111-4116.
23. Baba, T., Taura, T., Shimoike, T., Akiyama, Y., Yoshihisa, T., and Ito, K. (1994) Proc. Natl. Acad. Sci. USA 91, 4539-4543.
24. Kihara, A., Akiyama, Y., and Ito, K. (1995) Proc. Natl. Acad. Sci. USA 92, 4532-4536.
25. Matsuyama, S., Fujita, Y., Sagara, K., and Mizushima, S. (1992) Biochim. Biophys. Acta 1122, 77-84.
26. Bassilana, M., and Wickner, W. (1993) Biochemistry 32, 2626-2630.
27. Shibui, T., Uchida, M., and Teranishi, Y. (1988) Agric. Biol. Chem. 52, 983-988.

28. Schatz, P. J., Bieker, K. L., Ottemann, K. M., Silhavy, T. J., and Beckwith, J. (1991) EMBO J. 10, 1749-1757.
29. Sugimura, K. (1988) Biochem. Biophys. Res. Commun. 153, 753-759.
30. Nishiyama, K., Kabuyama, Y., Akimaru, J., Matsuyama, S., Tokuda, H., and Mizushima, S. (1991) Biochim. Biophys. Acta 1065, 89-97.
31. Akita, M., Shinkai, A., Matsuyama, S., and Mizushima, S. (1991) Biochem. Biophys. Res. Commun. 174, 211-216.
32. Cabelli, R. J., Dolan, K. M., Qian, L., and Oliver, D. B. (1991) J. Biol. Chem. 266, 24420-24427.
33. Murphy, C. K., and Beckwith, J. (1994) Proc. Natl. Acad. Sci. USA 91, 2557-2561.