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Published in:
Journal of Bacteriology

Publication date:
1999

JOURNAL OF BACTERIOLOGY, Nov. 1999, p. 7021–7027 Vol. 181, No. 22
0021-9193/99/$04.00+0

Preprotein Translocation by a Hybrid Translocase Composed of *Escherichia coli* and *Bacillus subtilis* Subunits

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Received 6 July 1999/Accepted 9 September 1999

Bacterial protein translocation is mediated by translocase, a multisubunit membrane protein complex that consists of a peripheral ATPase SecA and a preprotein-conducting channel with SecY, SecE, and SecG as subunits. Like *Escherichia coli* SecG, the *Bacillus subtilis* homologue, YvaL, dramatically stimulated the ATP-dependent translocation of precursor PhoB (prePhoB) by the *B. subtilis* SecA-SecYE complex. To systematically determine the functional exchangeability of translocase subunits, all of the relevant combinations of the *E. coli* and *B. subtilis secY*, *secE*, and *secG* genes were expressed in *E. coli*. Hybrid SecYEG complexes were overexpressed at high levels. Since SecY could not be overproduced without SecE, these data indicate a stable interaction between the heterologous SecY and SecE subunits. *E. coli* SecA, but not *B. subtilis* SecA, supported efficient ATP-dependent translocation of the *E. coli* precursor OmpA (proOmpA) into inner membrane vesicles containing the hybrid SecYEG complexes, if *E. coli* SecY and either *E. coli* SecE or *E. coli* SecG were present. Translocation of *B. subtilis* prePhoB, on the other hand, showed a strict dependence on the translocase subunit composition and occurred efficiently only with the homologous translocase. In contrast to *E. coli* SecA, *B. subtilis* SecA binds the SecYEG complexes only with low affinity. These results suggest that each translocase subunit contributes in an exclusive manner to the specificity and functionality of the complex.

In the gram-negative bacterium *Escherichia coli*, secretory proteins are transported from the cytosol to the periplasm by the translocase (13, 16, 53). The translocase consists of the membrane-peripheral ATPase SecA, which is bound with high affinity to a heterotrimeric integral membrane protein complex composed of the SecY, SecE, and SecG subunits. The SecYEG complex is thought to function as a preprotein-conducting channel in the inner membrane (36). In *E. coli*, some preproteins associate first with the export-dedicated chaperone SecB, which stabilizes the preprotein in the cytosol and targets it to the membrane-bound SecA (18). SecA drives the stepwise translocation of the preprotein across the membrane by nucleotide-modulated cycles of SecA membrane insertion and deinsertion (17, 44, 49). SecA, SecY, and SecE are the essential components of the translocase and are needed for the viability of *E. coli*. SecG is dispensable in vivo but stimulates translocation in vitro (39, 40). SecG can be isolated as part of a stable complex together with SecY and SecE from the *E. coli* inner membrane (7, 8, 21). In addition, translocation involves the products of the *secD* and *secF* genes, both of which code for integral membrane proteins with large periplasmic domains (20). SecD and SecF are also not essential for the viability of *E. coli*, but they can associate with the SecYEG complex and functionally substitute for the SecG protein (14).

The translocase complex of gram-positive bacteria like *Bacillus subtilis* is homologous to the system found in *E. coli*. In recent years, the components of the *B. subtilis* translocase, i.e., SecA (*divA*) (41, 42), SecY (45), SecE (25), and SecG (52), have been identified genetically. The *B. subtilis* SecD and SecF subunits (6) form a single polypeptide in the membrane. SecB appears to be absent in gram-positive bacteria.

Except for the results of some in vivo studies using conditional lethal *sec* mutants, few data on the functional interaction between translocase subunits in a heterologous complex are available. SecA of the gram-positive bacterium *Staphylococcus carnosus* complements the temperature-sensitive *B. subtilis divA* (*secA*) mutant, but it cannot functionally replace *E. coli* SecA (28). *E. coli* SecA fails to complement the *B. subtilis divA* mutant (46). Under conditions of low expression, *B. subtilis* SecA can complement SecA mutants in *E. coli* K-12 strains (29) but not in an *E. coli* B strain (34). This finding shows that the complementation by *B. subtilis* SecA can be very critical. Chimeras of the *E. coli* and *B. subtilis* SecA proteins have been reported, and one of these is able to effectively complement the *E. coli secA* mutants. This chimera consists of the first 242 amino acids of *B. subtilis* SecA, including the ATP-binding site and the carboxy-terminal part of *E. coli* SecA (34). *B. subtilis* SecY is unable to restore the growth defect of *E. coli secY24* at the nonpermissive temperature, but it does support translocation of the precursor OmpA (proOmpA) (38). Likewise, *B. subtilis* SecE was shown to complement a cold-sensitive *E. coli secE* strain (25). The *B. subtilis* SecG and SecDF proteins are unable to complement the cold-sensitive growth phenotypes of the corresponding *E. coli* mutant proteins (6, 52). In consideration of these data, it appears that one or more Sec proteins function in a host-specific manner.

The complementation experiments described herein mainly score for restoration of growth and do not address the catalytic activities of the heterologous complexes formed. To study the host specificities of translocase subunits in a more systematic manner, we have expressed all relevant combinations of the three major integral membrane subunits, SecY, SecE, and SecG, of the *E. coli* and *B. subtilis* translocases in *E. coli*. Membranes harboring these hybrid translocase complexes were analyzed for their preprotein translocation activities in

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TABLE 1. Bacterial strains and plasmids used

Strain or plasmid	Relevant properties[a]	Reference
Strains		
E. coli DH5α	*recA Δlac*	22
E. coli SF100	*recA Δlac ΔompT*	3
B. subtilis DB104	*NprE18 aprEΔ3*	54
Plasmids		
pET324	Amp^r expression vector	47
pET340	*Ec-secY Ec-secE Ec-secG*	47
pET811	*Ec-secY Bs-secE Ec-secG*	This work
pET812	*Bs-secY Bs-secE Ec-secG*	This work
pET813	*Bs-secY Ec-secE Ec-secG*	This work
pET819	*Bs-secY Bs-secE*	This work
pET821	*Ec-secY Bs-secE Bs-secG*	This work
pET822	*Bs-secY Bs-secE Bs-secG*	This work
pET823	*Bs-secY Ec-secE Bs-secG*	This work
pET825	*Ec-secY Ec-secE Bs-secG*	This work
pET865	*Bs-secY*	This work

[a] *Ec*, *E. coli*, *Bs*, *B. subtilis*.

the presence of *E. coli* or *B. subtilis* SecA. The results suggest that each subunit of the translocase is directly involved in defining the host specificity of the complex.

MATERIALS AND METHODS

Materials. *E. coli* SecA (10, 12), *B. subtilis* SecA (48), proOmpA (11), and His-precursor PhoB (prePhoB) (51) were purified as described previously. *E. coli* SecA, *B. subtilis* SecA, proOmpA, and His-prePhoB were labeled with carrier-free ^{125}I (Amersham), Little Chalfont, Buckinghamshire, United Kingdom) with Iodo-Beads (Pierce Rockford) (51).

Strains and construction of plasmids. Strains and plasmids used are shown in Table 1. Overproduction of the SecY, SecE, and SecG proteins was performed with *E. coli* SF100 as described previously (47). The synthetic operon containing *B. subtilis* and *E. coli* SecY, SecE, and SecG under the control of an IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible *trc* promoter was constructed basically as described by van der Does et al. (47). Individual genes were amplified from the chromosomes of *E. coli* DH5α or *B. subtilis* DB104 (54) by PCR with primers containing the appropriate restriction sites and ribosome-binding sites. Nucleotide sequences of the cloned genes were checked on a Vistra DNA sequencer 725 (Amersham) with an automated sequencing kit from Amersham.

Isolation of IMVs. Cells overexpressing the various combinations of SecY, SecE, and SecG were harvested by centrifugation, washed, and resuspended in 50 mM Tris-HCl, pH 8.0. The cell suspension was passed three times through a French press at 16,000 lb/in^2 to obtain inside-out inner membrane vesicles (IMVs), and the cell debris was removed by low-spin centrifugation (10,000 × *g* for 5 min). Membranes were collected by high-spin centrifugation (200,000 × *g*, 1 h) and resuspended in buffer containing 1 mM dithiothreitol (DTT), and subsequently the inner membranes were separated from the outer membranes by sucrose density gradient centrifugation (47). IMVs were frozen in liquid N_2 and stored at −80°C. Protein content was determined by the *DC* protein assay (Bio-Rad).

For translocation and SecA-binding reactions, IMVs were treated with a polyclonal antibody (PAb) raised against *E. coli* SecA. One hundred microliters of IMVs (10 mg/ml) was incubated and mixed continuously for 1 h with 20 μl of PAb (47) and subsequently spun through a sucrose cushion (25% sucrose, 50 mM Tris [pH 8.0], 1 mM DTT) (44).

In vitro translocation assay. In vitro translocation of ^{125}I-proOmpA or ^{125}I-prePhoB into *E. coli* IMVs was assayed by the accessibility of the precursors to added proteinase K (12, 47). Reactions were performed in 50 μl of a solution containing 50 mM HEPES–KOH (pH 7.6), 30 mM KCl, 0.5 mg of bovine serum albumin per ml, 10 mM DTT, 2 mM Mg–acetate, 2 mM ATP, 10 mM phosphocreatine–phosphate, 50 μg of creatine kinase per ml, IMVs (10 μg of membrane protein), and, where indicated in the figures, purified *E. coli* or *B. subtilis* SecA (2 μg, unless indicated otherwise). Translocation was initiated by the addition of 1 μl of ^{125}I-labeled proOmpA or ^{125}I-labeled prePhoB (in 6 M urea–50 mM Tris, pH 7.2), which corresponds to about 0.2 μg of protein. After 30 min of incubation at 37°C, the mixture was chilled on ice and treated with proteinase K (1 mg/ml) for 15 min. Samples were precipitated with 7.5% trichloroacetic acid, acetone washed, and resuspended in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) sample buffer and separated on SDS–10% (prePhoB) or –12% (proOmpA) polyacrylamide gel (31), followed by autoradiography by exposure to Kodak Biomax MR film.

SecA binding. Binding of SecA to IMVs was assayed essentially as described previously (24). IMVs (10 μg of membrane protein) were suspended in 100 μl of translocation buffer (50 mM HEPES–KOH [pH 7.6], 30 mM KCl, 0.5 mg of bovine serum albumin per ml, 10 mM DTT, 2 mM Mg-acetate) and incubated for 15 min on ice with 1 nM ^{125}I-labeled *E. coli* or *B. subtilis* SecA and the concentration of nonlabeled SecA indicated in the figures. Samples were subsequently loaded on a sucrose cushion (0.25 mM sucrose in translocation buffer) and fractionated by centrifugation (10 min, 30 lb/in^2 in a Beckman Airfuge, 4°C). The amounts of ^{125}I-labeled SecA in the supernatant and the pellet were quantitated with a gamma counter.

Miscellaneous methods. To measure precursor-stimulated SecA ATPase activity, IMVs were treated with 4 M urea as described before (19, 24). IMV bearing overexpressed SecY, SecE, and SecG proteins were analyzed by SDS–15% PAGE (31), stained with Coomassie brilliant blue stain, or blotted on polyvinylide difluoride membranes (Millipore) with a semidry blotter (Bio-Rad). Immunodetection was carried out with PAb raised against SecY, SecE, or SecG of *E. coli* (47) or SecE (a PAb raised against a synthetic peptide of *B. subtilis* SecE [KDVGKEMKKV] by Research Genetics) or SecG of *B. subtilis* (51, 52). The PAb raised against *B. subtilis* SecY was a generous gift of R. Freudl (Forschungs Institute, Jülich, Germany).

RESULTS

***B. subtilis* SecG stimulates in vitro prePhoB translocation by SecYE.** Recently, we have reported the in vivo identification of the product of the *yvaL* gene as the *B. subtilis* homologue of SecG (52). For *E. coli*, SecG has been found to dramatically stimulate the in vitro SecYE-mediated translocation of various precursor proteins (7, 8, 21). To further substantiate that the *B. subtilis* YvaL protein is a SecG homologue, its ability to stimulate preprotein translocation into IMVs bearing the *B. subtilis* SecYE complexes was determined. To circumvent the instability of SecY in *B. subtilis* due to proteolytic degradation (51), the *B. subtilis* SecY and SecE proteins were overproduced in *E. coli* SF100, the host, and coexpressed with (pET822) or without (pET819) YvaL. SDS-PAGE and Coomassie brilliant blue staining of the IMVs isolated from SF100 cells transformed with pET819 showed high-level expression of the *B. subtilis* SecY and SecE proteins (Fig. 1A, lane 9) compared to the levels of expression in IMVs of the host strain transformed with the empty vector (pET324) (lane 1). Identical results were obtained with IMVs derived from cells harboring

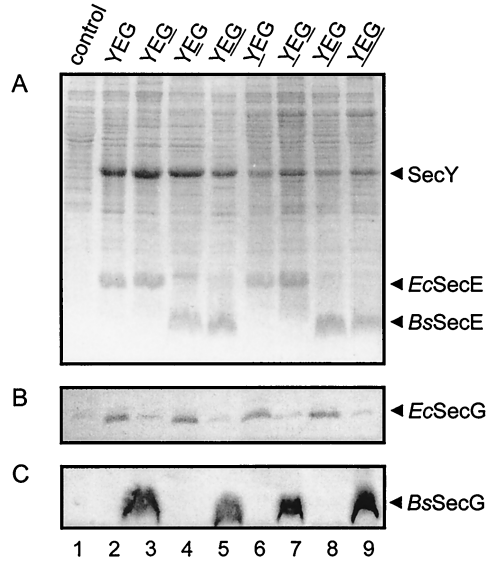

FIG. 1. Overproduction of *E. coli* and *B. subtilis* SecY, SecE, and SecG proteins in *E. coli* SF100 cells. (A) Coomassie brilliant blue-stained SDS–15% polyacrylamide gel of membranes derived from SF100 cells bearing a control plasmid (wild type) and the hybrid SecYEG complex; the loci of *B. subtilis* Sec proteins are underlined. (B and C) Immunoblots of *E. coli* SF100 membranes developed with PAbs raised against synthetic polypeptides corresponding to SecG of *E. coli* (B) and *B. subtilis* (C).

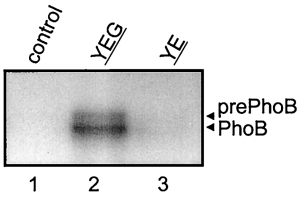

FIG. 2. In vitro translocation of ^{125}I-prePhoB into SecA-depleted IMVs of *E. coli* SF100 cells transformed with the control vector, pET324 (lane 1); with pET822, which allows overproduction of *B. subtilis* SecYEG (lane 2); or with pET819, which directs overproduction of *B. subtilis* SecYE (lane 3). Translocation activity was measured in the presence of ATP and *B. subtilis* SecA as described in Materials and Methods.

pET822 that expressed only SecY and SecE (data not shown). Immunoblotting was employed to demonstrate the expression of the *B. subtilis* YvaL protein (Fig. 1C, where YvaL is indicated as SecG). Next, the IMVs were analyzed for the translocation of the urea-denatured ^{125}I-labeled precursor of the *B. subtilis* alkaline phosphatase (prePhoB) (51). IMVs were treated with a PAb against SecA to inactivate endogenous membrane-bound *E. coli* SecA. Even when supplemented with *B. subtilis* SecA and ATP, IMVs derived from the host strain *E. coli* SF100 transformed with the empty vector were inactive for ^{125}I-prePhoB translocation (Fig. 2, lane 1). The presence of YvaL (lane 2) dramatically enhanced the ^{125}I-prePhoB translocation activity of *B. subtilis* SecYE (lane 3). No translocation was observed in the absence of *B. subtilis* SecA (see Fig. 4A, lane 9). These results further demonstrate that *B. subtilis* YvaL is functionally homologous to *E. coli* SecG.

Overproduction of hybrid *E. coli* and *B. subtilis* SecYEG complexes. Plasmid pET340 harbors a synthetic operon of the *E. coli secY*, *secE*, and *secG* genes under the control of an IPTG-inducible *trc* promoter and allows high-level functional overproduction of the SecYEG complex in *E. coli* (47). To analyze the functional interchangeability of the *E. coli* and *B. subtilis* translocase subunits, pET340 was used to construct hybrid SecYEG complexes. The *E. coli secY*, *secE*, and *secG* genes were systematically replaced by their *B. subtilis* counterparts (Table 1), which yielded a total of eight different SecYEG complexes, including the two homologous systems. SDS-PAGE and immunoblotting of IMVs were used to analyze the IPTG-induced overproduction of the subunits. Coomassie brilliant blue staining of the SDS-polyacrylamide gel revealed that, irrespective of the construct used, a high level of overproduction of the *E. coli* and *B. subtilis* SecY and SecE proteins could be achieved (Fig. 1). These protein bands were not visible in the control transformed with the expression vector only (Fig. 1, lane 1). The identities of the proteins were further verified by means of immunoblotting with peptide-specific antibodies (data not shown), permitting also the detection of overexpressed *E. coli* SecG (Fig. 1B) (40) and *B. subtilis* SecG (Fig. 1C). Based on the Coomassie brilliant blue staining, the expression level of the *B. subtilis* SecY protein appeared to be about 25% of that of the *E. coli* SecY protein. Consistent with its smaller molecular mass, i.e., 6.9 versus 13.6 kDa, *B. subtilis* SecE was found to migrate much faster by SDS-PAGE than *E. coli* SecE (Fig. 1A).

E. coli SecY can be stably produced only when it is coover-expressed with SecE (33). FtsH, a membrane-bound protease (1), degrades the uncomplexed form of SecY. By analogy, *B. subtilis* SecY could be overproduced in *E. coli* only when it was coexpressed with SecE (i.e., with pET819) and not in its absence (i.e., with pET865) (data not shown). Since all constructs showed a high level of overproduction of hybrid SecYEG complexes, we conclude that *E. coli* and *B. subtilis* SecY and SecE proteins form stable complexes.

Translocation activity of hybrid translocase. To establish whether the hybrid SecYEG complexes were functional, the SecA- and ATP-dependent translocation of ^{125}I-labeled *E. coli* proOmpA and *B. subtilis* prePhoB was analyzed. Since the IMVs bearing overexpressed SecYEG contained substantial amounts of tightly bound endogenous *E. coli* SecA, membranes were first incubated with PAbs raised against *E. coli* SecA to reduce the background translocation activity in the absence of added SecA (44). The efficiency of this treatment varied with the hybrid SecYEG complex, but in all cases the endogenous translocation activity could be reduced to a low level (Fig. 3A). When IMVs were supplemented with a saturating concentration of *E. coli* SecA, a substantial amount of ^{125}I-proOmpA (20 to 25% of total input) was translocated by the IMVs bearing overexpressed *E. coli* SecYEG (Fig. 3B, lane 2) and by the hybrid complexes that contained *E. coli* SecY with either the SecG (lane 3) or SecE (lane 4) subunit substituted for its *B. subtilis* counterpart. With all of the other hybrid SecYEG complexes, only a low translocation activity of proOmpA was observed (lanes 5 to 9). When instead of *E. coli* SecA, *B. subtilis* SecA was used, proOmpA translocation was inefficient with each of the hybrids (Fig. 3C). The small amount of translocated proOmpA in the presence of *B. subtilis* SecA was not processed by signal peptidase.

In contrast to that of proOmpA, translocation of *B. subtilis* prePhoB depends much more critically on the origin of the translocase components (Fig. 4). *E. coli* SecA supported efficient translocation of prePhoB (15 to 20% of total input) when it was combined with the homologous SecYEG complex (Fig. 4B, lane 2), while it supported a low level of translocation (about 5%) when it was combined with the *B. subtilis* SecYEG complex (lane 9). On the other hand, *B. subtilis* SecA promoted translocation of prePhoB (20 to 25%) only when it was assayed together with the *B. subtilis* SecYEG complex (Fig. 4C, lane 9). Replacement of only one of the integral subunits of the translocase for its heterologous counterpart resulted in a nearly complete loss of prePhoB translocation activity. These data demonstrate that prePhoB translocation exhibits a very narrow requirement for the translocase subunits.

SecA translocation ATPase activity of hybrid translocase. The ATPase activity of SecYEG-bound SecA stimulated by a translocation-competent preprotein is termed "translocation ATPase" (32) because with the wild-type translocase it correlates with translocation activity. Previous studies have demon-

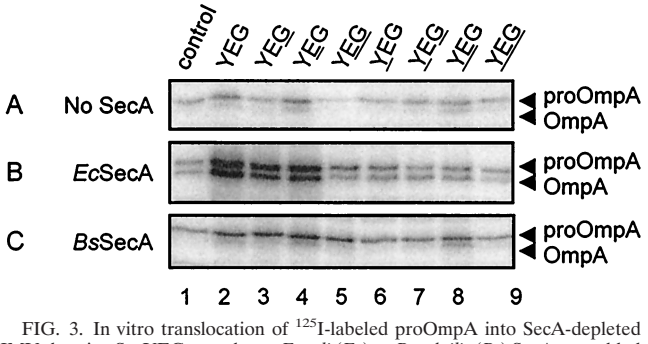

FIG. 3. In vitro translocation of ^{125}I-labeled proOmpA into SecA-depleted IMVs bearing SecYEG complexes. *E. coli* (*Ec*) or *B. subtilis* (*Bs*) SecA was added as indicated. The loci of *B. subtilis* Sec proteins are underlined. Translocation in the absence of added SecA reflects the activity of the remaining endogenous SecA. No translocation activity was observed in the absence of ATP. Experimental conditions were as described in Materials and Methods.

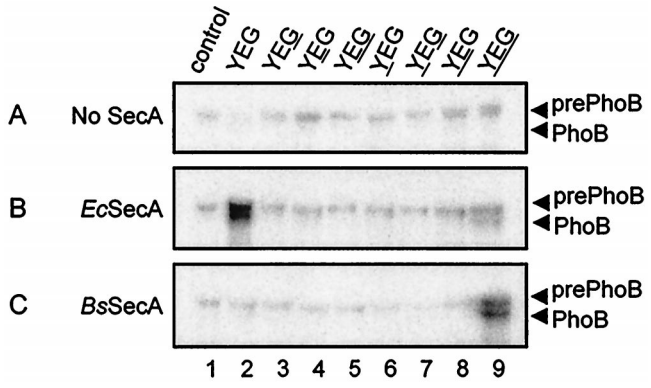

FIG. 4. In vitro translocation of ^{125}I-labeled prePhoB into SecA-depleted IMVs bearing SecYEG complexes. Experimental conditions were as described in the legend of Fig. 2 and Materials and Methods. The loci of *B. subtilis* SecA proteins are underlined. *Ec*, *E. coli*; *Bs*, *B. subtilis*.

strated that proOmpA is an exceptionally good substrate for SecA ATPase activity in comparison to many other *E. coli* preproteins (4). IMVs were treated with urea to reduce background ATPase activity, and the proOmpA-stimulated SecA ATPase was measured with wild-type *E. coli* IMVs or IMVs bearing the overexpressed *E. coli* or *B. subtilis* SecYEG complex. *E. coli* SecA supported a substantial translocation ATPase both with overexpressed *E. coli* SecYEG and with overexpressed *B. subtilis* SecYEG (Fig. 5). On the other hand, with *B. subtilis* SecA, only a low level of proOmpA-stimulated ATPase activity was observed (Fig. 5), consistent with its poor ability to translocate proOmpA. The prePhoB-stimulated ATPase activity of the *E. coli* or *B. subtilis* SecA was very low, irrespective the nature of the SecYEG complex (data not shown), even though prePhoB was translocated efficiently by the IMVs containing the homologous systems (Fig. 4).

***Bacillus subtilis* SecA binds SecYEG with low affinity.** In *E. coli*, SecYEG functions as a high-affinity membrane-binding site for SecA (24). The ability of the *E. coli* and *B. subtilis*

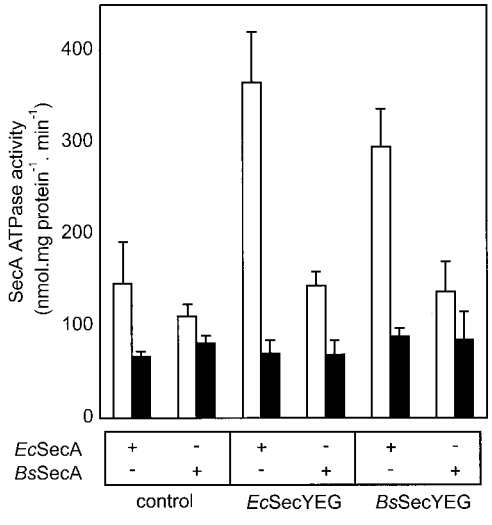

FIG. 5. SecA ATPase activity of IMVs derived from control cells and cells that overexpress the *E. coli* and *B. subtilis* SecYEG complexes. The ATPase activities of *E. coli* (*Ec*) and *B. subtilis* (*Bs*) SecA proteins were measured in the presence (open bars) and absence (filled bars) of proOmpA. IMVs were treated with 4 M urea to inactivate endogenous SecA and other ATPases.

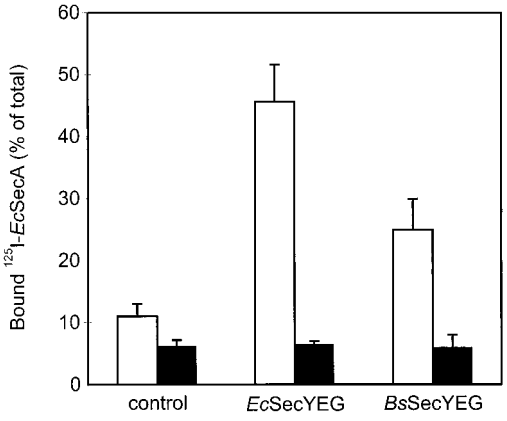

FIG. 6. Binding of *E. coli* ^{125}I-SecA to IMVs derived from control cells and cells that overproduce the *E. coli* (*Ec*) and *B. subtilis* (*Bs*) SecYEG complexes in the absence (open bars) or presence (filled bars) of a 500-fold excess of nonlabeled SecA. ^{125}I-SecA was used at a final concentration of 1 nM. Endogenous levels of SecA were removed by treatment of the IMVs with a PAb directed against SecA as described in Materials and Methods.

SecYEG complexes to bind ^{125}I-labeled SecA (at a 1 nM concentration) was determined. IMVs bearing overexpressed *E. coli* or *B. subtilis* SecYEG supported high levels of binding of *E. coli* ^{125}I-SecA compared to that of the wild-type control (Fig. 6). ^{125}I-SecA binding was effectively reduced to the background level by the addition of a 500-fold excess of nonlabeled SecA. In contrast, it was not possible to discern specific binding of ^{125}I-labeled *B. subtilis* SecA to any of the SecYEG complexes (data not shown). This result suggests a much lower affinity for the SecYEG complex than that of *E. coli* SecA, even though the translocation assays demonstrated that the *B. subtilis* SecA is active (Fig. 4). To investigate this phenomenon further, we determined the ability of *B. subtilis* SecA to compete with *E. coli* ^{125}I-SecA for binding to the *E. coli* and *B. subtilis* SecYEG complexes. Increasing amounts of nonlabeled *E. coli* SecA efficiently chased ^{125}I-SecA bound to *E. coli* SecYEG (Fig. 7). However, *B. subtilis* SecA appeared far less efficient in this chase. The IMVs retained, even at a 250-fold

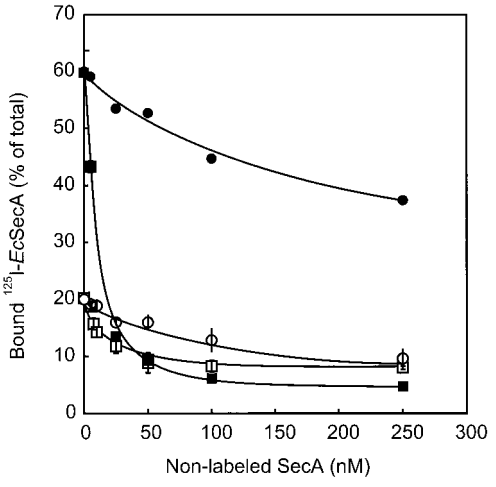

FIG. 7. Binding of *E. coli* ^{125}I-SecA to IMVs bearing the overexpressed *E. coli* (filled symbols) and *B. subtilis* SecYEG (open symbols) complexes in the presence of various concentrations of non-labeled *E. coli* (■ and □) or *B. subtilis* (● and ○) SecA. ^{125}I-SecA was used at a final concentration of 1 nM. Concentrations of SecA indicated are for the monomer. *Ec*, *E. coli*.

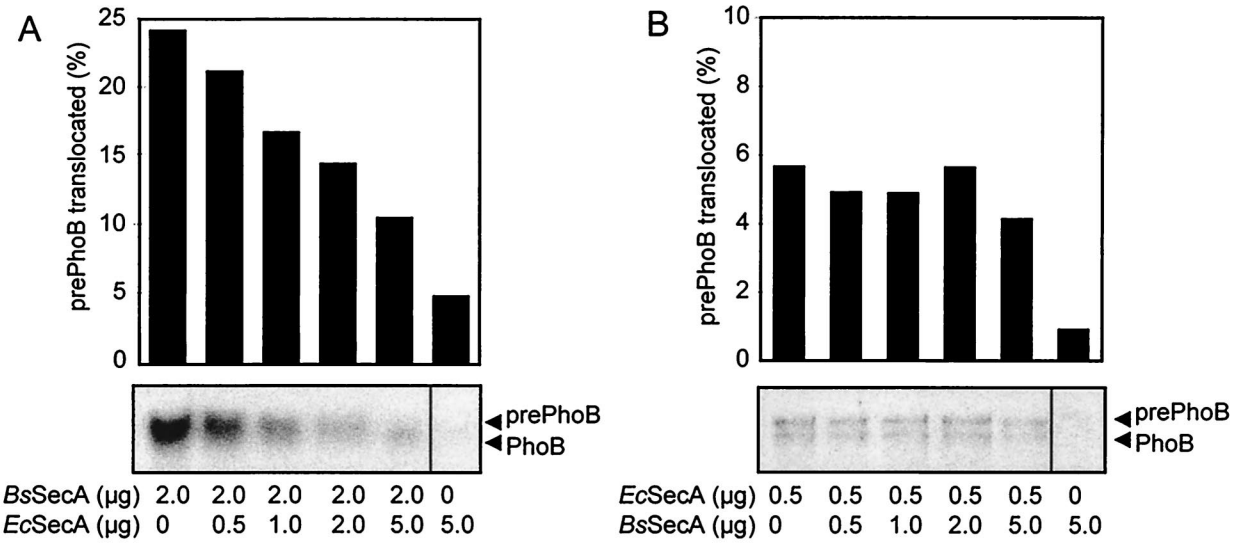

FIG. 8. Translocation of prePhoB into IMVs bearing the overexpressed *B. subtilis* (A) or *E. coli* (B) SecYEG complex in the presence of various amounts of *E. coli* (*Ec*) and *B. subtilis* (*Bs*) SecA. PrePhoB was used at 2 μg per translocation reaction mixture.

excess, up to 60% of specifically bound *E. coli* ^{125}I-SecA. Also the *E. coli* ^{125}I-SecA that bound to *B. subtilis* SecYEG was more efficiently chased by nonlabeled *E. coli* SecA than by *B. subtilis* SecA. The lower level of specific binding of *E. coli* ^{125}I-SecA observed with IMVs bearing *B. subtilis* SecYEG is consistent with the lower level of expression of *B. subtilis* SecY than that of *E. coli* SecY (Fig. 1). It is concluded that *E. coli* SecA binds the SecYEG complex with a much higher affinity than *B. subtilis* SecA.

The functional consequence of the low-affinity binding of *B. subtilis* SecA to the SecYEG complex was further assessed in a competition experiment between *E. coli* SecA and *B. subtilis* SecA for the translocation of prePhoB. Since efficient translocation of prePhoB is observed only with a homologous translocase (Fig. 4), addition of a heterologous SecA may prevent translocation by the formation of a nonfunctional complex. To avoid depletion of the preprotein substrate, a large amount of prePhoB was added to the translocation reaction (about 0.7 μM, which equals the highest concentration of the competing SecA dimer used). The *B. subtilis* SecA-dependent translocation of prePhoB into IMVs bearing *B. subtilis* SecYEG was progressively inhibited by increasing amounts of *E. coli* SecA (Fig. 8A). In the reverse experiment, *B. subtilis* SecA was hardly capable of inhibiting the *E. coli* SecA-dependent translocation of prePhoB in *E. coli* SecYEG IMVs (Fig. 8B), even though *E. coli* SecA was present at a subsaturating amount (0.5 μg) while *B. subtilis* SecA was added at a 10-fold excess. These results are consistent with the notion that *E. coli* SecA binds SecYEG with a higher affinity than does *B. subtilis* SecA.

DISCUSSION

Most of our knowledge on the catalysis of bacterial preprotein translocation is based on studies of *E. coli*. Here we report on the formation and activities of hybrid translocase complexes composed of subunits originating from *E. coli* and *B. subtilis*. Our data demonstrate that the translocase subunits of these bacteria cannot be unconditionally exchanged and provide evidence for host-specific functions. Each translocase subunit seems to contribute in an exclusive manner to the specificity and functionality of the complex.

A previous study of *E. coli* has shown that SecY can be stably overexpressed only together with SecE (33). The formation of a complex of SecE with SecY prevents the degradation of SecY by FtsH (27). FtsH is a membrane-integrated ATP-dependent metalloprotease that degrades incorrectly folded or assembled cytosolic and inner membrane proteins (1). Likewise, stable overproduction of *B. subtilis* SecY in *E. coli* was possible only when the protein was coexpressed with either *B. subtilis* or *E. coli* SecE. Therefore, the proper interaction between the SecE and SecY subunits is a prerequisite for the overproduction of hybrid SecYEG complexes that are composed of *B. subtilis* and *E. coli* subunits. Both the *E. coli* and *B. subtilis* SecE proteins stabilize SecY, irrespective of the origin of SecY. Even though a stable SecY-SecE interaction was apparent for each of the heterologous pairs, none of the hybrid complexes supported prePhoB translocation while the homologous translocase complexes were highly active. These data indicate that SecE not only functions in the stabilization of SecY but also fulfills a catalytic function. Efficient translocation of proOmpA strictly required *E. coli* SecA, but with the integral subunits a great flexibility was apparent. Apparently, precursor substrates differ in their levels of dependency on the translocase subunits, while each of the subunits may critically contribute to the specificity of the complex.

The subunit swapping experiments with the *E. coli* and *B. subtilis* translocases demonstrate that structural and functional aspects of the translocase subunit interactions can be separated. With SecY and SecE being essential subunits of the translocase, the SecY-SecE interaction has been studied in great detail. The SecE proteins of *E. coli* and *B. subtilis* are rather distinct. *E. coli* SecE is a 13.6-kDa integral membrane protein with three transmembrane segments (TMS) (43), whereas *B. subtilis* SecE is a small membrane protein of 6.9 kDa with only one TMS (25). *B. subtilis* SecE is homologous to the carboxy-terminal portion of *E. coli* SecE, which corresponds to the minimal functional size of this protein. The first two TMS of *E. coli* SecE can be deleted without loss of function (37, 43), whereas the third TMS can be replaced by a related sequence of the *B. subtilis* SecE (30) or by an unrelated TMS (37). Residues essential for the function of SecE are located in the highly conserved cytoplasmic region (30, 37). This domain is

thought to interact with the fourth cytosolic loop of SecY (2). The second periplasmic loop of SecE interacts with the first periplasmic loop of SecY (23), allowing the proximity of TMS 2 of SecY to TMS 3 of SecE (26). It remains to be determined which of these interacting sites is involved in the catalytic function of SecE. In this respect, it is of interest that the reduced specificities observed for *prlA* mutants of SecY (5) correlate with a loosened interaction between the SecY and SecE subunits (15) and a tighter binding of SecA (50).

The low translocation activity of *B. subtilis* SecA with proOmpA may relate to poor recognition of this preprotein substrate. *B. subtilis* SecA can interact with the signal sequence of proOmpA (9), but its ATPase activity is only poorly stimulated by complete proOmpA (this study). proOmpA may thus not be properly recognized by SecYEG-bound *B. subtilis* SecA, and this in turn may prevent SecA from functionally associating with SecYEG. In vivo experiments indicated that proOmpA can be translocated by *B. subtilis* (35), but the conditions used differed from those used in the in vitro experiments, as SecDF and the proton motive force may add to the efficiency of translocation. In the in vitro system, no processing of proOmpA was observed. One may speculate that at the initiation of translocation, *B. subtilis* SecA exposes the signal sequence cleavage site less efficiently to the *E. coli* signal peptidase than does *E. coli* SecA.

B. subtilis SecA binds the *E. coli* SecYEG only with very poor affinity. Although this was noticed before (48), we can now relate this poor binding affinity to the functionality of the complex in an in vitro translocation assay. The observation that *E. coli* SecA efficiently competes with *B. subtilis* SecA for binding to the *B. subtilis* SecYEG complex is remarkable. In the in vitro translocation reaction, this competition resulted in the formation of an inactive complex of *E. coli* SecA and *B. subtilis* SecYEG. Although inhibition may also relate to competition for the available preprotein, this possibility seems less likely, as in our experiments the precursor was added in excess relative to the level of SecA. The remarkable difference between the SecYEG binding affinities of *E. coli* and *B. subtilis* SecA also makes in vivo complementation studies of temperature-sensitive *E. coli* SecA mutants more difficult to interpret (28, 29, 34, 41, 46). The presence of residual *E. coli* SecA, either active or nonactive, may prevent heterologous SecA from interacting efficiently with SecYEG. Future studies should reveal whether the dramatic difference in binding affinities for SecYEG reflects a mechanistic difference in the way *E. coli* and *B. subtilis* SecA support translocation.

ACKNOWLEDGMENTS

We thank Chris van der Does, Erik Manting, Andreas Kaufmann, and Martin van der Laan for valuable suggestions.

These investigations were supported by CEC Biotech grants BIO2 CT 930254 and BIO4 CT 960097.

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