Evidence That SecB Enhances the Activity of SecA†

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ABSTRACT: In *Escherichia coli*, SecA is a critical component of the protein transport machinery which powers the translocation process by hydrolyzing ATP and recognizing signal peptides which are the earmark of secretory proteins. In contrast, SecB is utilized by only a subset of preproteins to prevent their premature folding and chaperone them to membrane-bound SecA. Using purified components and synthetic signal peptides, we have studied the interaction of SecB with SecA and with SecA−signal peptide complexes in vitro. Using a chemical cross-linking approach, we find that the formation of SecA−SecB complexes is accompanied by a decrease in the level of cross-linking of SecA dimers, suggesting that SecB induces a conformational change in SecA. Furthermore, functional signal peptides, but not dysfunctional ones, promote the formation of SecA−SecB complexes. SecB is also shown to directly enhance the ATPase activity of SecA in a concentration-dependent and saturable manner. To determine the biological consequence of this finding, the influence of SecB on the signal peptide-stimulated SecA/lipid ATPase was studied using synthetic peptides of varying hydrophobicity. Interestingly, the presence of SecB can sufficiently boost the response of signal peptides with moderate hydrophobicity such that it is comparable to the activity generated by a more hydrophobic peptide in the absence of SecB. The results suggest that SecB directly enhances the activity of SecA and provide a biochemical basis for the enhanced transport efficiency of preproteins in the presence of SecB in vivo.

Studies on secretion in *Escherichia coli* have revealed the existence of several proteins which constitute the transport machinery and are required for efficient protein transport. Among these, SecA, the translocation ATPase, is of particular interest because it powers the translocation process by hydrolyzing ATP and recognizes signal peptides which are the earmark of secretory proteins (*1*−*3*). Furthermore, precursor proteins associated with the Ffh−4.5S RNA complex (the *E. coli* signal recognition particle) or the chaperone SecB are thought to be transferred to SecA at the translocon, suggesting that SecA plays a pivotal role during both SRP-dependent and SecB-dependent protein transport (*3*, *4*).

In contrast to SecA which is critical for cell viability (*5*), inactivation of the *secB* gene is not lethal and transport defects are observed for only a subset of preproteins (for a review, see ref *6*). These include maltose-binding protein (MBP), OmpA, OmpF, and PhoE. On the other hand, transport of PhoA, ribose-binding protein (RBP), and β-lactamase is not disabled in the absence of SecB. Several studies have been conducted to identify the properties which confer SecB dependence on some proteins, yet no consensus sequence or consistent region within this group of proteins has been found to be responsible.

One model suggests that the rate of preprotein folding governs SecB utilization (*7*). It has been proposed that the formation of a complex between SecB and its substrate depends on a kinetic partitioning between the rate of folding of the substrate relative to its rate of association with SecB. Functional signal peptides can retard preprotein folding, thus enhancing SecB binding opportunities (*8*). Upon binding, SecB prevents the premature folding of preproteins and chaperones them to membrane-bound SecA in an export-competent state. The interaction of SecA and SecB is mediated primarily through the 22 C-terminal amino acids of SecA (*9*), though at least one other region of SecA may also be involved (*10*). Interestingly, the interaction of these two proteins occurs with a higher affinity when a signal peptide is also present, suggesting that signal peptide−SecA binding may promote transfer of the preprotein to SecA (*11*).

The results of several studies are consistent with the hypothesis that SecB utilization is related to the kinetics of preprotein folding, and these have been recently reviewed (*12*). Other observations are not readily accounted for by this model. For example, SecB exhibits extremely fast association kinetics with substrates, suggesting that SecB can even interact with a range of cytoplasmic proteins (*13*, *14*). Furthermore, when preMBP is mutated to fold slower, the mutant (MBPV8G/I9A) exhibits diminished SecB dependence even though it is predicted to be more accessible for SecB binding (*15*). Indeed, the mutants MBPΔ57−145 and MBP2261 fold slower and consequently do bind SecB better than their wild-type counterpart, yet they are less SecB-

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dependent (*16*). Therefore, SecB binding cannot necessarily be equated with SecB dependence. Signal peptide mutants of the SecB-dependent protein MBP (*17*), the SecB-independent protein PhoA (*18*), and the SecB-independent protein RBP (*19*) indicate that reducing the signal peptide efficiency results in an enhanced reliance on SecB, though these mutants would be expected to fold more rapidly, since they are without the benefit of a highly hydrophobic signal peptide. These results point to the possibility that SecB may play an additional role in facilitating preprotein transport.

We have previously identified a short sequence motif that includes basic residues and which confers SecB dependence on the SecB-independent protein, PhoA, in vivo (*20*). The impact of the basic motif on SecB dependence decreases as the distance between its location and the signal peptide increases, potentially reflecting the extent to which the motif interferes with productive interactions between the signal peptide and the transport machinery. Intriguingly, the PhoA mutants which are most sensitive to the loss of SecB are those most sensitive to the inhibition of SecA via azide treatment (*20*). Furthermore, signal peptide mutants of PhoA with reduced hydrophobicity have been shown to interact less efficiently with SecA in vitro (*3*, *21*) and exhibit an enhanced requirement for SecB in vivo (*18*). This strong parallelism suggests the possibility that SecB may promote interactions of the signal peptide and SecA which would otherwise be less than optimal.

To examine this hypothesis, we have studied the interaction of SecB with SecA and SecA−signal peptide complexes in vitro using purified components and synthetic signal peptides to preclude any contribution from the mature region of the preprotein. We find that SecB promotes formation of a SecB−SecA−signal peptide ternary complex. Moreover, SecB directly enhances the ATPase activity of SecA. As a consequence, the presence of SecB can enhance the SecA/lipid ATPase activity produced by a signal peptide of intermediate hydrophobicity to productive levels which otherwise, in the absence of SecB, might be insufficient to support transport in vivo. The finding that SecB enhances SecA ATPase activity is consistent with previous observations that SecB increases the efficiency of preprotein export in vivo (*18*, *19*, *22*).

EXPERIMENTAL PROCEDURES

Materials. SecA was purified from strain BL21.14 pCS1 as described by Mitchell and Oliver (*23*). Strain TG1 (pREP4, pQE9*secB*) was used for the overproduction of $His_{(6)}$-SecB. Previous studies have found the behavior of $His_{(6)}$-SecB to be comparable to that of the untagged, wild-type SecB (*11*). SecB was purified using a Ni−NTA agarose column under nondenaturing conditions as described by the manufacturer (Qiagen, Valencia, CA) and subsequently dialyzed with buffer A [25 mM Tris-HCl (pH 7.5), 25 mM KCl, and 0.5 mM EDTA], supplemented with 5 mM β-mercaptoethanol and 0.5 mM PMSF.[1] For cross-linking reactions, SecA and SecB were dialyzed with buffer B [50 mM HEPES-KOH (pH 7.0), 30 mM KCl, 30 mM NH_4Cl, and 0.5 mM magnesium acetate]. β-Mercaptoethanol and PMSF were omitted when SecA and SecB were prepared for cross-linking reactions. Protein concentrations were determined by the Bradford assay at 595 nm (*24*). ATP, reactive blue 4 agarose, DOPC, and DOPG were purchased from Sigma (St. Louis, MO). Acetone-precipitated and ether-extracted *E. coli* phospholipids were from Avanti Polar Lipids, Inc. (Alabaster, AL). BS^3, DSS, EDAC, and sulfo-HSAB were from Pierce (Rockford, IL). SecA and SecB antisera were gifts from D. Oliver and C. Kumamoto, respectively. The ECL Western blotting kit and Superdex 200 resin were from Amersham Pharmacia Biotech (Piscataway, NJ). The synthetic signal peptides were prepared as reported previously (*2*, *21*). The sequences are as follows: WT (sequence of the wild-type alkaline phosphatase signal peptide), MKQSTIALALLPLLFTPVTKAC-NH_2; 3K4L, MKQKKLALAAAALALSSSASAC-NH_2; 1K2L, MKQQQ-AALAAAALAASSSASAC-NH_2.

SecA ATPase Assay. ATPase assays were carried out as described previously in buffer B, supplemented with 0.5 mg/mL BSA, 1 mM DTT, 4 mM ATP, and typically 40 μg/mL SecA in a total reaction volume of 50 μL (*1*, *2*, *21*). SecB in the concentration range of 0−425 μg/mL was added as appropriate. For reactions involving membrane-bound SecA, small unilamellar vesicles were formed by sonication in 1 mM DTT using a Branson sonifier and used at 320 μg/mL phospholipids. Flotation analysis confirmed that >95% of the SecA was stably associated with the liposomes. The reactions were initiated by addition of ATP, and the mixtures were incubated at 37 °C for 40 min. The amount of inorganic phosphate released was determined as reported previously (*1*, *21*, *25*). The peptide-stimulated SecA/lipid ATPase activity is determined as the difference in the rate of ATP hydrolysis in the presence and absence of peptide.

Cross-Linking Reactions with SecA and SecB. SecA (2 μg) and the appropriate amount of SecB (indicated in the figures) were mixed in buffer B on ice in a total reaction volume of 10 μL. After preincubation for 30 min on ice, cross-linking was initiated, unless otherwise mentioned, with 0.6 mM BS^3 for 90 min on ice and quenched by adding 1 μL of quenching buffer [1 M glycine and 100 mM $NaHCO_3$ (pH 8.5)] on ice, and the solution was mixed with 10 μL of gel loading solution [125 mM Tris-HCl (pH 6.8), 20% glycerol, 10% β-mercaptoethanol, 4% SDS, and 0.02% bromophenol blue] and analyzed by 6% SDS−PAGE (*26*) and silver staining or Western blotting.

Pulse−Chase Analysis. Expression of mutant alkaline phosphatases under the control of the *lac* promoter was carried out as described previously (*18*). The sequences of the signal peptide region of these PhoA mutants are as follows: 3L, MKQSTLAAAAALALATPVTKA; 4L, MKQ-STLALAAAALALATPVTKA; 5L, MKQSTLALALALA-LATPVTKA; WT, MKQSTIALALLPLLFTPVTKA. Overnight cultures of $SecB^+$ (MC4100) and $SecB^-$ (CK1953) strains were grown in M63 medium supplemented with thiamine hydrochloride (2 μg/mL) and glycerol (0.4%), and containing 50 μg/mL chloramphenicol and then subcultured at a dilution of 1:50 in the same medium. Cells were then grown at 37 °C to logarithmic phase and harvested at an

[1] Abbreviations: DOPC, dioleoylphosphatidylcholine (C18:1); DOPG, dioleoylphosphatidylglycerol (C18:1); BSA, bovine serum albumin; BS^3, bis(sulfosuccinimidyl)suberate; DSS, disuccinimidyl suberate; DTT, dithiothreitol; EDAC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; IPTG, isopropyl β-D-thiogalactopyranoside; MOPS, 4-morpholinepropanesulfonic acid; NHS, *N*-hydroxysuccinimide; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethanesulfonyl fluoride; sulfo-HSAB, *N*-hydroxysulfosuccinimidyl 4-azidobenzoate.

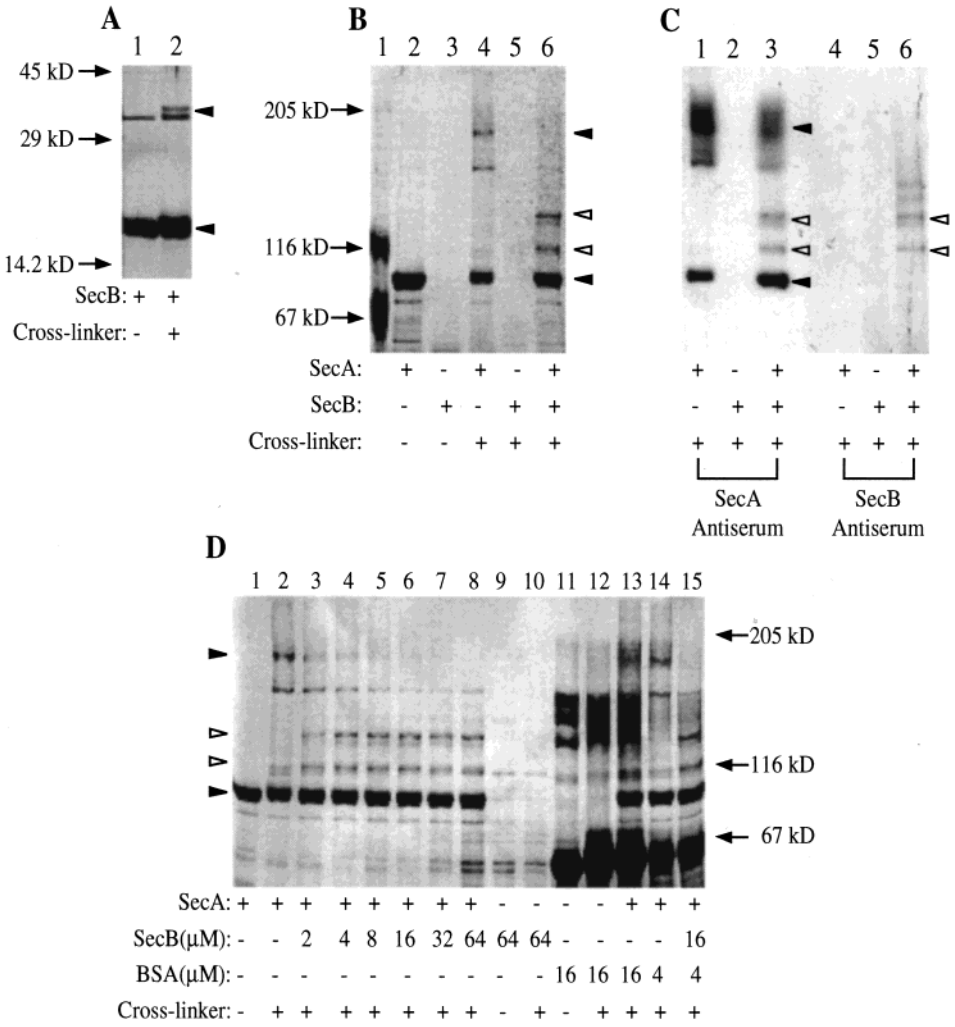


FIGURE 1: Cross-linking of SecA and SecB. The cross-linking reaction was performed with 0.6 mM BS^3 in buffer B [50 mM HEPES-KOH (pH 7.0), 30 mM KCl, 30 mM NH_4Cl, and 0.5 mM magnesium acetate], containing SecA and/or SecB in a total reaction volume of 10 μL. All other conditions were as described in Experimental Procedures. Samples were electrophoresed in 15% (A) or 6% (B−D) SDS−PAGE and silver-stained (A, B, and D) or blotted to Hybond C membranes (C). The positions of the monomeric (16.6 kDa) and dimeric (33 kDa) forms of SecB (A) or monomeric (100 kDa) and dimeric (200 kDa) forms of SecA (A−D) are denoted with black arrowheads. The SecA−SecB cross-linked species are denoted with white arrowheads. (A) SecB (2.7 μg, 16 μM) was used. A small amount of SecB resistant to reducing agents yields the dimeric form observed in lane 1. (B) SecA (2 μg, 2 μM) and SecB (2.7 μg, 16 μM) were added where indicated. (C) Western blot of cross-linked samples using SecA antiserum or SecB antiserum. (D) SecA (2 μg, 2 μM) and various amounts of SecB (0−10.7 μg or 0−64 μM) were used. Where indicated, 4 or 16 μM BSA was added.

optical density at 600 nm of 0.8. Cells were washed twice with MOPS containing 40 mM KH_2PO_4 and resuspended in the same medium supplemented with amino acids (20 mg/mL, excluding methionine) and 0.4 mM IPTG. Cells were incubated at 37 °C for 15 min to induce the expression of alkaline phosphatase. $SecB^+$ (MC4100) and $SecB^-$ (CK1953) strains were pulse labeled with [^{35}S]methionine for 15 s in the presence of 0.4 mM IPTG and chased with excess unlabeled methionine as indicated. Samples were immunoprecipitated (*18*), separated by 7.5% SDS−PAGE, and analyzed for the extent of precursor processing with a phosphorimager (Bio-Rad).

RESULTS

To probe the interaction of SecB and SecA, we sought a system in which stable complexes could be conveniently studied. We were able to trap complexes by cross-linking in aqueous solution and make relative comparisons of the conditions which promoted or disrupted the SecA−SecB complex interaction. We used the water-soluble cross-linker, BS^3, at relatively low concentration (0.6 mM) and temperature (0 °C) to minimize nonspecific reactions. Using 15% SDS−PAGE to resolve the low-molecular mass range, the cross-linked products observed with SecB alone in the reaction correspond to its monomeric (16.6 kDa) and dimeric forms (33.2 kDa) (Figure 1A). SDS stable, tetrameric SecB complexes are probably not observed because the overall cross-linking efficiency is low and these would require more extensive linkage.

The comparable analysis with SecA alone also shows its monomeric (100 kDa) and dimeric species (200 kDa) on a 6% SDS−PAGE gel. When SecA and SecB are incubated together, two new bands are observed which migrate with apparent molecular masses that are greater than that of SecA alone (Figure 1B). Western blotting of a duplicate gel using antisera against either SecA or SecB indicates that these bands consist of SecA−SecB complexes (Figure 1C). The appearance of multiple bands could be accounted for by different ratios of bound SecB to SecA, or more likely, these

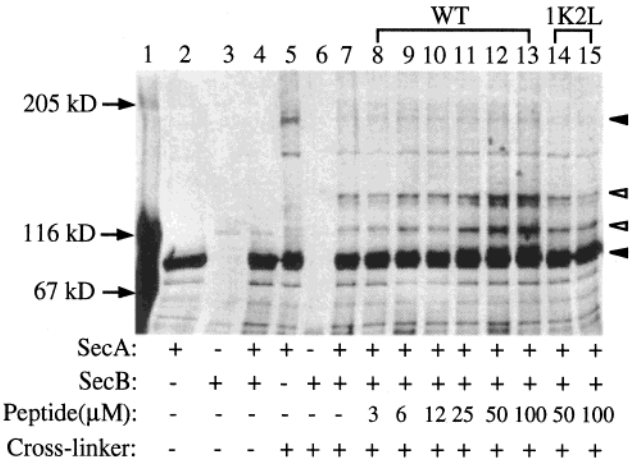
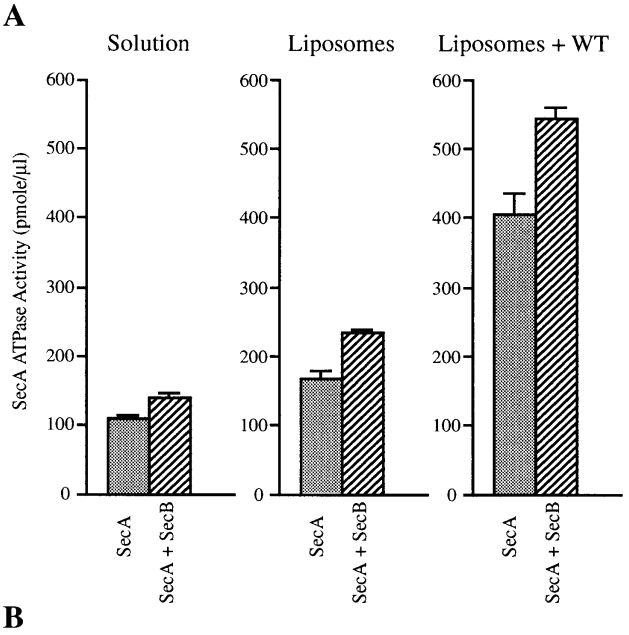


FIGURE 2: Signal peptide dependence of SecA−SecB cross-linking. The cross-linking reaction conditions are as described in the legend of Figure 1 except that 16 μM SecB and varying amounts of signal peptide were used. DSS was used as a cross-linker. The positions of the monomeric and dimeric forms of SecA are denoted with black arrowheads. The SecA−SecB cross-linked species are denoted with white arrowheads. The sequences of the WT and 1K2L peptides are given in Experimental Procedures. When SecA is a component of the reaction mixture, it is always present at 2 μM. In those instances where there is extensive SecA−signal peptide complex formation (e.g., lanes 11−13), these can produce SecA−signal peptide cross-linked products which appear as a smear on the gel.

represent different conformations of the same complex induced by different cross-linking patterns as seen in previous comparable studies (*3*, *27*). Interestingly, the formation of the cross-linked SecA−SecB species is concomitant with a loss of cross-linked SecA dimer. These effects are striking when the cross-linked products are analyzed as a function of increasing SecB concentration, keeping the concentration of SecA constant. As shown in Figure 1D, over the concentration range that is employed, there is a gradual loss of the SDS stable, cross-linked form of the SecA dimer with a corresponding increase in the level of the slower migrating SecA−SecB species which plateaus at about 16 μM SecB. Changes in the faster migrating SecA−SecB species are less evident since both SecA and SecB alone have cross-linked products which comigrate in the same region. To rule out the possibility that the changes in cross-linked species in the presence of SecA and SecB are due to nonspecific interactions, BSA was used because it has a molecular mass similar to that of the SecB tetramer and a similar pI (4.0 and 4.8 for SecB and BSA, respectively) (*28*, *29*). When BSA is present instead of SecB, no disappearance of the cross-linked SecA dimer species is observed (Figure 1D, lanes 13 and 14). This indicates that BSA does not substitute for SecB and that the change in the level of cross-linked SecA dimer seen in the presence of SecB is not due to interference by additional protein. Since the BSA preparation has some high-molecular mass impurities, we were not able to distinguish these from possible SecA−BSA cross-linked species (expected molecular mass of 167 kDa) at a high BSA concentration (16 μM). However, at a lower BSA concentration (4 μM, Figure 1D, lane 14), no SecA−BSA complex is detectable, while the same or a lower concentration of SecB clearly shows SecA−SecB complex formation (Figure 1D, lane 4). Furthermore, when SecB is added to SecA in addition to BSA, the pattern of cross-linked species is similar to that of the comparable



B

	Phosphate released (pmole/μl)			% difference	
	-	+BSA	+SecB	by BSA	by SecB
SecA	111	116	140	5	26± 6
SecA + liposomes	167	175	235	5	41± 3
SecA + WT + liposomes	405	446	544	10	34 ± 4

FIGURE 3: Effect of SecB on the ATPase levels of SecA. (A) The reactions were performed in buffer A [50 mM Tris-HCl (pH 7.0), 30 mM KCl, 30 mM NH_4Cl, and 0.5 mM magnesium acetate], supplemented with 1 mM DTT and 4 mM ATP in a 50 μL reaction volume. Liposomes (small unilamellar vesicles; 320 μg/mL) from *E. coli* phospholipids were used where indicated. SecA (2 μg, 0.4 μM), SecB (2.7 μg, 3.2 μM monomer), or BSA (2.6 μg) was used. The final concentration of the wild-type signal peptide (denoted as WT) was 20 μM. All other conditions were as described in Experimental Procedures. (B) The results of panel A are summarized, and the effect of BSA is also shown for comparison. Each data point represents the average of three experiments ± the standard error.

reaction mixture without BSA. Similar results were obtained with other cross-linkers such as EDAC and DSS and at different temperatures (25 and 37 °C; data not shown).

Moreover, a sulfo-HSAB cross-linker which is bound to SecA before the addition of SecB produced comparable results, suggesting that the changes we observe are not due to the inaccessibility of the cross-linker (data not shown). It should also be noted that we have no indication that the actual levels of the SecA dimeric species are changing, only that the levels of the dimer that can be cross-linked in the presence of SecB are substantially reduced. However, this suggests the possibility that the conformation of SecA changes due to SecB.

It has been suggested that the presence of preprotein increases the affinity of SecB for inner membrane vesicles (*4*, *9*, *11*), presumably through an interaction with SecA. This implies that SecB may stabilize SecA in a conformation that is conducive to signal peptide interaction (and vice versa). In vitro cross-linking of SecA and SecB in the presence of a synthetic signal peptide, corresponding to the wild-type alkaline phosphatase signal sequence, was not enhanced

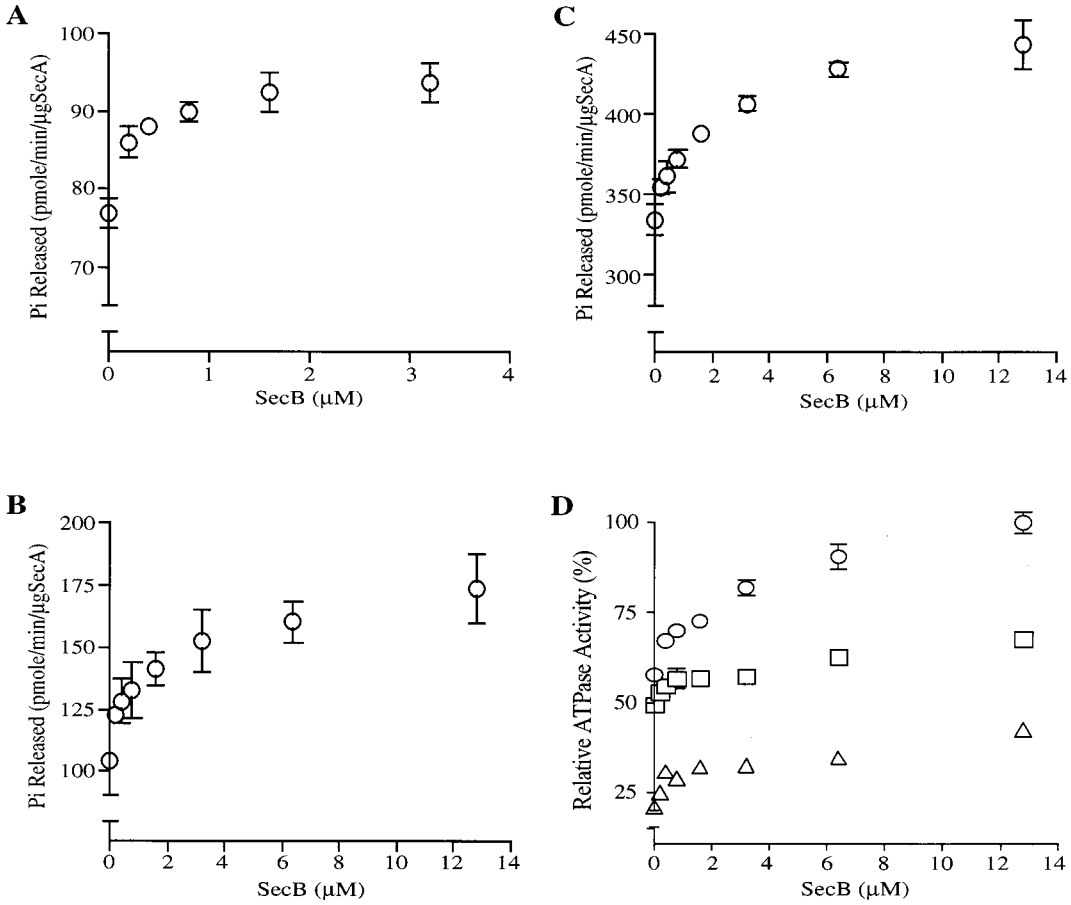


FIGURE 4: SecB concentration dependence of SecA ATPase levels. (A) SecA ATPase activities in solution. (B) SecA ATPase activities in liposomes. (C) WT signal peptide-stimulated SecA ATPase activities in liposomes. (D) Signal peptide-stimulated SecA ATPase activity in liposomes using the WT (○), 3K4L (□), and 1K2L (△) peptides was plotted relative to the maximal level of activity obtained with the WT peptide. Note, in most cases, the error was so small the error bar is within the symbol. See the legend of Figure 3 for reaction conditions. Each data point represents the average of three experiments ± the standard error.

using either of the water-soluble reagents, BS^3 or EDAC. However, when DSS, a hydrophobic analogue of BS^3, was used, we observed an enhancement in the level of the SecA−SecB cross-linked species as the concentration of the wild-type signal peptide was increased (Figure 2). This suggests the possibility that in the presence of the signal peptide, the hydrophobic contact surface area between SecA and SecB is increased. No such change is observed when the 1K2L signal peptide, which corresponds to a nonfunctional signal sequence, is used (Figure 2). Interestingly, the presence of either signal peptide does not further change the level of the cross-linked SecA dimer, suggesting that this is an effect due to SecB alone (Figure 2).

We observe enhancement in the levels of SecA−SecB cross-linking in the presence of increasing concentrations of the functional signal peptide, suggesting that the signal peptide promotes formation of a signal peptide−SecA−SecB ternary complex. In vivo, such complex formation could also theoretically be driven from the other direction, with increases in the local concentration of SecB promoting SecA−signal peptide complex formation. A parallel situation was shown for BiP associated with ER protein translocation (*30*). In the presence of Sec63p, BiP binds polypeptide substrates more readily. Furthermore, this is concomitant with an enhancement in BiP ATPase activity. To examine this possibility in our system, we studied the influence of SecB on the SecA ATPase activity in aqueous solution and in liposomes. As shown in Figure 3A, in the absence of SecB, the SecA ATPase activity is enhanced by incorporating SecA in liposomes (*1*), and further increased by the addition of functional signal peptides (*2*, *21*). Surprisingly, when SecB is also present at a SecA dimer to SecB tetramer molar ratio of ∼1:4, a further enhancement in the SecA ATPase activity is observed in both solution and liposomes. For SecA in solution, the addition of SecB resulted in a 26% increase in ATPase activity, while for liposome-bound SecA, there was a 41% increase in activity in the presence of SecB over the reaction under the same conditions without SecB (Figure 3B). This latter increase is even greater (more than 50%) at higher SecB concentrations (Figure 4). This represents an enhancement in ATPase levels that is comparable to the effect of lipid on SecA alone. It had previously been reported that SecB stimulates the translocation ATPase activity of SecA using *E. coli* inner membrane vesicles (*9*). That result was attributed to a targeting function of SecB; that is, by enhancement of the delivery of the preprotein to the translocon and maintenance of it in an unfolded export competent state, translocation activity was also enhanced. Here, using purified SecA and SecB, and even in the absence of preprotein, we show that SecB also directly enhances the catalytic activity of SecA.

If this finding is to have relevance for protein translocation, the presence of SecB should also enhance the level of signal peptide-induced SecA/lipid ATPase activity. Using the

A

	Phosphate released (pmole/μgSecA/min)	
	- SecB	+SecB
1K2L	2 ± 1	81 ± 1
3K4L	74 ± 14	165 ± 4
WT	265 ± 8	355 ± 6

B

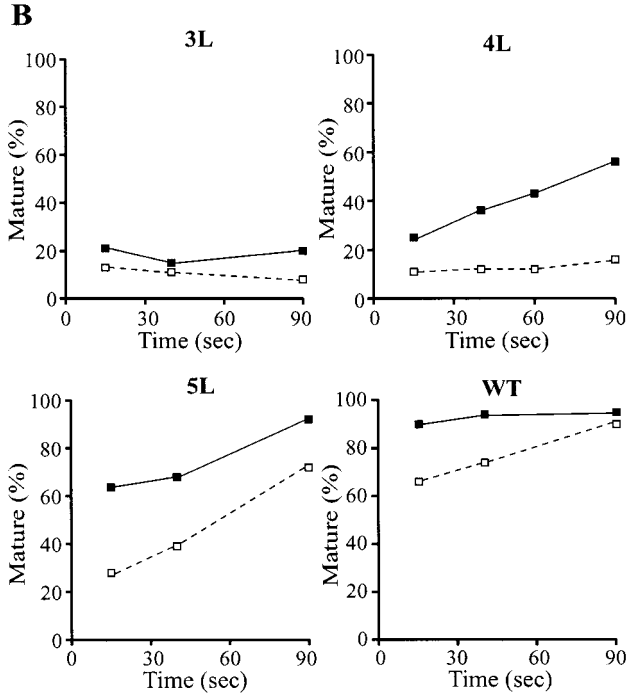


FIGURE 5: Effect of SecB on the SecA/lipid ATPase activity induced by signal peptides of varying hydrophobicity. (A) The reaction conditions were as described in the legend of Figure 3. The SecA ATPase activity in the presence of liposomes and in the absence of signal peptides is taken to be zero. The concentrations of SecA and SecB are 0.4 and 3.2 μM, respectively. The sequences of the WT, 3K4L, and 1K2L peptides used at 10 μM are given in Experimental Procedures. Each data point represents the average of three experiments ± the standard error. (B) Pulse−chase analysis of alkaline phosphatases with signal peptides of varying hydrophobicity in SecB$^+$ and SecB$^-$ strains. Cells of MC4100 (SecB$^+$) and CK1953 (MC4100 SecB$^-$) harboring plasmids encoding the indicated proteins were labeled with [^{35}S]methionine for 15 s and chased with excess cold methionine for 15, 40, and 90 s. The alkaline phosphatases were immunoprecipitated and analyzed as described in Experimental Procedures. White squares represent protein expression in the SecB$^-$ strain and black ones that in the SecB$^+$ strain. The hydrophobicity of the signal sequences was varied using various combinations of leucine and alanine residues comparable to those used in the synthetic peptides in panel A.

synthetic peptide corresponding to the wild-type alkaline phosphatase signal sequence to stimulate the SecA/lipid ATPase activity (*2*), we observed a 34% increase in the presence of SecB compared to that in its absence (Figure 3B). No such increase in activity was observed when BSA was added instead of SecB at a comparable concentration.

In Figure 4, the dependence of SecA ATPase activity on SecB concentration is shown. The effect on ATPase activity is dose-dependent and saturable under the three sets of conditions that were employed: SecA in solution, SecA in liposomes, and signal peptide-stimulated SecA in liposomes. Maximal levels of activity are reached at molar ratios of 1:2 in solution and 1:8 in liposomes (SecA dimer:SecB tetramer). This suggests that under the conditions employed here, SecB interacts more efficiently with SecA in solution than with liposomes.

The enhancement of ATPase activity due to the interaction of SecB with SecA may contribute to the SecB dependence of preproteins with more weakly hydrophobic signal peptides observed in vivo (*17*, *18*). Using conditions similar to those in which we established ternary complex formation involving SecB, SecA, and the signal peptide (Figure 2), we examined the effect of SecB on the SecA/lipid ATPase activity in the presence of synthetic peptides with varying hydrophobicities. These peptides include the wild-type alkaline phosphatase signal peptide and two model peptides which vary in the combination of leucine and alanine residues in the core region. The 3K4L peptide is moderately hydrophobic; a preprotein with a similar signal sequence functions, albeit with reduced efficiency, in vivo in the presence of SecB but accumulates as a precursor in the absence of SecB (*18*). The 1K2L peptide is of low hydrophobicity; a preprotein with a comparable signal sequence accumulates as a precursor in vivo both in the presence and in the absence of SecB (*18*). As shown in Figure 4D, the enhancement in signal peptide-stimulated SecA ATPase activity is dependent on SecB concentration for all three peptides that are employed, though the absolute magnitude of the effect differs. In the absence of SecB, the extent of signal peptide-induced SecA/lipid ATPase activity is dependent on the hydrophobicity of the signal peptide (*2*, *21*). In each case, the addition of SecB resulted in a significant enhancement of the ATPase levels generated in vitro (Figures 4D and 5A), and this is reflected in the in vivo transport activity of alkaline phosphatase with the corresponding signal peptides in SecB$^+$ and SecB null strains (Figure 5B). Interestingly, although the 1K2L peptide-induced activity is enhanced in the presence of SecB in vitro, the maximum level of SecA/lipid ATPase produced is similar to that generated by the 3K4L peptide in the absence of SecB (Figures 4D and 5A). As shown in Figure 5B, in the comparable cases in vivo, these are insufficient to generate transport activity on a meaningful time scale. In vivo, a preprotein carrying the 4L signal peptide is translocated in a strict SecB-dependent manner (Figure 5B). These results are consistent with the notion that even in the presence of SecB a threshold level of SecA activity must be generated to result in reasonable levels of transport (*18*, *31*). Furthermore, these results suggest that the ability of SecB to enhance SecA activity directly has an important biological consequence; namely, it rescues export of preproteins with signal peptides that are less than ideal.

DISCUSSION

It has been suggested that SecB plays at least two roles in SecB-dependent protein secretion. One is that SecB binds to preproteins through their mature portion, keeping the preproteins from premature folding (*7*). The other proposed role is that SecB targets preproteins to membrane translocation sites through binding to membrane-associated SecA (*4*). It has been reported that SecB−preprotein complexes have a higher affinity for inner membrane vesicles than does SecB alone (*4*, *9*). A model which could account for these

observations involves binding of SecB−preprotein complexes to SecA with the generation of a conformational change in SecA. This could, in turn, enhance binding of the signal peptide to SecA such that transfer of the preprotein readily occurs (*11*). The presence of SecB ensures that the SecA ATPase activity is maximally activated and that efficient translocation will proceed.

Significant changes in SecA ATPase levels in the presence of SecB were observed for SecA in liposomes as well as in aqueous solution. The results presented in Figure 3 indicate that the signal peptide−SecA−SecB ternary complex represents the most active form of SecA ATPase. This suggests that an additional function of SecB may be to enhance the catalytic activity of SecA and promote interactions of SecA with the signal peptide of the preprotein. This provides an explanation for the observation that preproteins which interact with the transport pathway with reduced efficiency require SecB (*17*, *18*). Most compelling is the finding that identical proteins which differ only in the hydrophobicity of their signal peptides exhibit a continuum with respect to SecB dependence in vivo. That is, preproteins with more hydrophobic signal peptides do not require SecB, while those with more weakly hydrophobic signal peptides do (*18*). Furthermore, the latter examples are more sensitive to impaired SecA function due to azide treatment than the former (*20*), pointing to the possibility that SecB can rescue inefficient signal peptide−SecA interactions. The data presented in Figure 5 demonstrate that the presence of SecB can elevate the SecA ATPase activity produced by a moderately hydrophobic signal peptide to levels consistent with what is required to achieve transport in vivo. This may explain the strict SecB dependence observed in vivo for a preprotein with this signal peptide (*18*, *22*).

We propose that in addition to its chaperone activity, SecB modulates the activity of SecA to enhance preprotein translocation efficiency. This hypothesis is consistent with the findings that deletion of SecB is not lethal, that only a subset of preproteins depends markedly on SecB for transport (*6*), and that even SecB-dependent proteins can be transported in its absence, but with significantly reduced efficiency (*32*). Perhaps all preproteins utilize SecA−SecB complexes; loss of SecB simply does not result in a detectable defect for SecB-"independent" proteins because their interaction with SecA is, even in its absence, sufficient. This does not mean that only those preproteins with inefficient signal peptides should require SecB. Several factors could lead to impaired interaction of preproteins with SecA. These include rapid preprotein folding rates, overexpression of exported proteins, interfering sequences or structures within the early mature region of the preprotein, and signal peptides of only moderate hydrophobicity. Previously, the presence of SecB was found to stimulate protein transport (*28*, *33*), and this result was attributed to the SecB-assisted delivery of the preprotein to SecA at membrane-bound translocation sites (*4*). Our results suggest the observed increase in the level of transport in the presence of SecB is not merely a matter of efficient targeting to the translocon but is also due to a direct effect of SecB on the ATPase activity of SecA.

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