Functional Signal Peptides Bind a Soluble N-terminal Fragment of SecA and Inhibit Its ATPase Activity*

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The selective recognition of pre-secretory proteins by SecA is essential to the process of protein export from Escherichia coli, yet very little is known about the requirements for recognition and the mode of binding of precursors to SecA. The major reason for this is the lack of a soluble system suitable for biophysical study of the SecA-precursor complex. Complicating the development of such a system is the likelihood that SecA interacts with the precursor in a high affinity, productive manner only when it is activated by binding to membrane and SecYEG. A critical aspect of the precursor/SecA interaction is that it is regulated by various SecA ligands (nucleotide, lipid, SecYEG) to facilitate the release of the precursor, most likely in a stepwise fashion, for translocation. Several recent reports show that functions of SecA can be studied using separated domains. Using this approach, we have isolated a proteolytically generated N-terminal fragment of SecA, which is stably folded, has high ATPase activity, and represents an activated version of SecA. We report here that this fragment, termed SecA64, binds signal peptides with significantly higher affinity than does SecA. Moreover, the ATPase activity of SecA64 is inhibited by signal peptides to an extent that correlates with the ability of these signal peptides to inhibit either SecA translocation ATPase or in vitro protein translocation, arguing that the interaction with SecA64 is functionally significant. Thus, SecA64 offers a soluble, well defined system to study the mode of recognition of signal peptides by SecA and the regulation of signal peptide release.

SecA plays a central role in bacterial protein export: recognition of the protein precursor and facilitation of its translocation across the membrane (1–4). Critical to this role is the ability of SecA to bind specifically to precursors with functional signal sequences. The structural origin of this binding is a major question, since many different sequences can act as signal sequences, but specific patterns of residues are required and define the targeting function (5). To date, no systems have been described that allow direct characterization of SecA signal peptide interactions without including other species that participate in the translocation reaction (membranes, SecY). Some success in dissecting the requirements for signal sequence recognition by SecA has been achieved by the use of idealized signal sequences in in vitro and in vivo protein translocation assays (6–8) and including, in some cases, cross-linking experiments as a measure of interaction with SecA (9, 10). For example, a recent study by Miller et al. (11) demonstrates interaction of synthetic signal peptides with SecA in a vesicle system. Still, the complexity of these systems has made it difficult to explore the intriguing structural question of how a variety of diverse signal sequences can be specifically bound by one protein. We have been attempting to simplify the interacting species, SecA and the precursor protein, to develop a system for detailed biophysical studies.

SecA is a large (901 amino acids), homodimeric, multifunctional protein with highly interdependent ligand binding activities (1–4). For example, pre-protein binding is stimulated by binding to phospholipids (12). Moreover, binding to pre-protein and lipid stimulates the ATPase activity of SecA, yet higher activity is seen (so-called “translocation ATPase”) in the presence of SecYEG, the putative membrane translocation pore (13). Several years ago, Wickner and co-workers (13, 14) reported that signal peptides inhibit the translocation ATPase of SecA in the presence of membranes and SecY. In contrast, the recent study by Miller et al. (11) finds that signal peptides stimulated SecA ATPase activity in the presence of lipids. In earlier work, we observed that signal peptides inhibit in vitro protein translocation (15), although the site of inhibition in the in vitro system has not been definitively established. In these latter effects, mutant signal peptides that have low in vivo activity (monitored by the proportion of the precursor protein that attains an extracytoplasmic location and is thus cleaved by signal peptidase) are less effective in modulating ATPase or translocation activities.

Clearly, it would be of great advantage to dissect the functions of SecA in order to carry out detailed studies of its interactions with ligands. Recent work from several laboratories suggests that the study of separated domains of SecA may provide an opportunity to analyze its functions in greater detail (16–19). Our particular interest is to elucidate the means by which SecA recognizes many different secreted proteins and to
clarify how precursor binding may be regulated. In the present study, we report the isolation and characterization of SecA64, a stable proteolytic fragment of SecA lacking about one-third of the sequence from the C terminus and 10 residues from the N terminus. Our studies with SecA64 suggest that it mimics the state of SecA that recognizes and binds precursors and is then active in translocation, as described above. This fragment corresponds closely with proteolytic fragments described previously (18, 19); comparable fragments have recently been expressed and characterized for some biochemical functions (16, 17). Like the N-terminal fragments described by these researchers, SecA64 has high ATPase activity, presumably because it lacks the proposed intramolecular regulatory region (16).

Since SecA64 has properties suggesting that it resembles the activated state of SecA critical for pre-protein delivery to the membrane, we were particularly interested in determining how signal sequence binding modulates its functions. We have used peptides corresponding to wild-type and mutant signal sequences from the Escherichia coli outer membrane proteins, LamB and OmpA, to explore signal sequence binding to SecA64. Additionally, we have compared the effects of the same signal peptides on intact SecA membrane and translocation ATPase activities as well as on in vitro protein translocation in a well defined assay system. We find that the signal peptides bind to SecA64 and inhibit its ATPase activity to an extent that parallels their ability to facilitate export in vivo.

EXPERIMENTAL PROCEDURES

Reagents—Unless specifically mentioned, standard laboratory reagents were purchased from Sigma, Fisher, or Aldrich. The source of the lipids used in the ATPase and circular dichroism (CD) experiments is Avanti Polar Lipids, Inc. Typically the lipid mixture used consisted of a 3:1 molar ratio of L-

\[ \text{-dioleoyl phosphatidylcholine} \] and \[ \text{L-}[\text{2-hydroxy-1,1-bis(hydroxymethyl)ethyl} \]glycine. LamB, \( \lambda \) phage receptor protein; OmpA, outer membrane protein A; PMSF, phenylmethylsulfonyl fluoride; WT, wild type; Tricine, N-[5-hydroxy-1-bis(hydroxymethyl)ethyl]glycine.

\[ \text{ATPase activity was estimated by either of two methods (24, 25), which were found to give consistent results. To test the effect of lipid-} \]

binding on activity, purified protein was incubated in the assay buffer at a concentration of 300 nM or by a second widely used protocol (22), with some minor modifications. A mixture of synthetic phospholipids \[ \text{L-}[\text{2-hydroxy-1,1-bis(hydroxymethyl)ethyl} \]glycine. ATPase activities as well as on in vitro protein translocation in a well defined assay system. We find that the signal peptides bind to SecA64 and inhibit its ATPase activity to an extent that parallels their ability to facilitate export in vivo.

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Signal Peptide Recognition by Translocation Active SecA

RESULTS

Limited proteolysis of SecA with α-chymotrypsin in the presence of ATP gives rise to a 64-kDa polypeptide that is remarkably stable (Fig. 1A). During the course of chymotryptic digestion, the ATPase activity of SecA and its proteolytic fragments increases severalfold, whereas lipid stimulation of the ATPase activity decreased during the same time period (Fig. 1B). We have isolated and purified the 64-kDa fragment, which we term SecA64, and found that the elevated ATPase activity co-purifies with SecA64. Decreases in the ATPase activity of the sample increases (Fig. 1B). The addition of ATP results in the stabilization of a 64-kDa band, which co-migrates with SecA in a gel of the digestion. Lane 1 contains molecular weight markers, lane 2 contains 1.8 µg of SecA incubated in buffer for 120 min in the absence of protease, lane 3 contains the same amount of protein incubated for 120 min in the presence of 0.14 mg/ml α-chymotrypsin. Lane 4 contains SecA treated under the same conditions but in the presence of 15 mM ATP. The addition of ATP results in stabilization of a 64-kDa band, which is indicated by the lower arrow. ATPase activity alone or in the presence of 150 µg/ml 1,2,3-trioleoyl phosphatidylcholine:1,2-dioleoyl phosphatidylglycerol small unilamellar vesicles. The assay of ATPase activity was carried out as described in the text. As digestion proceeds, the ATPase activity of the sample increases (shaded bars). In the presence of lipids, the ATPase activity is stimulated relative to the activity in the absence of lipids (early time points), but the difference decreases as SecA becomes depleted and SecA64 accumulates (black bars). C, Top, Tricine-SDS-polyacrylamide gel electrophoresis of fractions from Sephacryl S-200 column run in 300 mM guanidine (aminomethanamide) hydrochloride, buffer (10 mM Tris-HCl, pH 8.5, 30 mM KCl, 300 mM guanidine (aminomethanamide) hydrochloride, 1 mM PMSF, 1 mM DTT) at 4°C. Each fraction was precipitated in 15% trichloroacetic acid and resuspended in sample buffer. Lane 1, SecA; lane 2, α-chymotrypsin digested material; lanes 3–14, column fractions 1–12. Bands due to SecA and SecA64 are indicated by arrows. Bottom, ATPase activities of column fraction show that the highest activity co-purifies with SecA64.

FIG. 1. Limited α-chymotrypsin digestion of SecA in the presence of ATP results in stabilization of a 64-kDa band that has elevated ATPase activity. A, SDS-polyacrylamide gel electrophoresis gel of the digestion. Lane 1 shows molecular weight markers, lane 2 contains 1.8 µg of SecA incubated in buffer for 120 min in the absence of protease. Lane 3 contains the same amount of protein incubated for 120 min in the presence of 0.14 mg/ml α-chymotrypsin. Lane 4 contains SecA treated under the same conditions but in the presence of 15 mM ATP. The addition of ATP results in stabilization of a 64-kDa band, which is indicated by the lower arrow. ATPase activity alone or in the presence of 150 µg/ml 1,2,3-trioleoyl phosphatidylcholine:1,2-dioleoyl phosphatidylglycerol small unilamellar vesicles. The assay of ATPase activity was carried out as described in the text. As digestion proceeds, the ATPase activity of the sample increases (shaded bars). In the presence of lipids, the ATPase activity is stimulated relative to the activity in the absence of lipids (early time points), but the difference decreases as SecA becomes depleted and SecA64 accumulates (black bars). C, Top, Tricine-SDS-polyacrylamide gel electrophoresis of fractions from Sephacryl S-200 column run in 300 mM guanidine (aminomethanamide) hydrochloride, buffer (10 mM Tris-HCl, pH 8.5, 30 mM KCl, 300 mM guanidine (aminomethanamide) hydrochloride, 1 mM PMSF, 1 mM DTT) at 4°C. Each fraction was precipitated in 15% trichloroacetic acid and resuspended in sample buffer. Lane 1, SecA; lane 2, α-chymotrypsin digested material; lanes 3–14, column fractions 1–12. Bands due to SecA and SecA64 are indicated by arrows. Bottom, ATPase activities of column fraction show that the highest activity co-purifies with SecA64.

E. coli receptor of LamB, the ϕ phage receptor of E. coli (32–35), and a second series from the signal sequence of the outer membrane protein OmpA (36–39). The interaction between protein and signal sequence was probed using a gel binding assay in which complex formation was detected by co-migration of a biotin-labeled signal peptide with SecA in a non-denaturing polyacrylamide gel (27). Due to its net positive charge, the free biotinylated peptide did not enter the gel to any appreciable extent, whereas SecA, a predominantly negative
protein, migrated into the gel, carrying bound peptide with it.

Binding of the signal peptide to SecA is relatively weak (Fig. 4), as expected from the low affinity interaction of SecA with pre-proteins in solution (12). In contrast, the SecA64 sample showed a clearly visible signal (Fig. 4), indicating that the peptide bound substantially more strongly to this fragment of SecA.

Functional, unlabeled signal sequences competed effectively for the binding of the biotin-labeled peptide to SecA64, whereas non-functional signal peptides did not (Fig. 5). A peptide corresponding to the wild-type LamB signal sequence effectively competed with the biotin-labeled peptide for binding, but the peptide containing the four-residue deletion in the hydrophobic core, LamBΔ78, competed poorly. A peptide that harbors a single proline to leucine change, which restores function to the deletion mutant in vivo, LamBΔ78r2 (32), competed for binding at near wild-type levels, indicating that effective competition requires a functional signal sequence.

The addition of LamBWT signal peptide inhibits the ATPase activity of SecA64 (Fig. 6). Under the same conditions, the ATPase activity of native SecA was affected only slightly by the signal peptide. For the family of LamB signal peptides, the capacity to inhibit SecA64 ATPase paralleled binding affinity as monitored by competition (Fig. 7A). At concentrations where the wild-type peptide clearly inhibits activity, the signal peptide corresponding to the nonfunctional deletion mutant, LamBΔ78, had no observable effect on the activity of SecA64. The signal peptide from the functional revertant strain, LamBΔ78, had no observable effect on the activity of SecA64.

To test the generality of the above observations, we examined the ability of a set of OmpA signal peptides (Fig. 3) (36–39) to inhibit SecA64 ATPase activity. Inhibition of the SecA64 ATPase by the OmpA peptides correlated with their in vivo function (Fig. 7B). The wild-type peptide is an effective inhibitor of activity at concentrations similar to those required for inhibition by the LamBWT peptide. The mutants OmpAΔS, OmpAΔ6–9, and OmpAΔSN are essentially without effect on the ATPase activity, consistent with their export defects. Puzzlingly, the OmpAΔS peptide, which functions at near wild-type levels in vivo, is able to inhibit SecA ATPase activity only slightly, decreasing the ATPase by 20% at the highest concentration tested.

Variants of the LamB signal peptide were tested for their ability to inhibit the SecA64 ATPase. A peptide with three additional basic residues in the N region of the LamB signal peptide (KRR-LamB, Fig. 3) potently inhibits SecA64 ATPase activity, with a 50% maximal inhibition near 5 μM (Fig. 8). In contrast, half-maximal inhibition by the LamBWT peptide required ~25 μM under similar conditions. In addition, the inhibition curve for the more highly water-soluble KRR-LamB signal peptide is quite clearly hyperbolic. Comparison of the ability of the L- and D-isomers of the LamBWT signal sequence to inhibit SecA64 ATPase activity showed that the L-peptide inhibits activity more strongly than the D-peptide (Fig. 8). Since the physical and chemical properties of these peptides are otherwise identical, we can conclude that the recognition of signal sequence by SecA is sensitive to the chirality of the peptide backbone.

To ensure the physiological relevance of the interactions between SecA64 and synthetic signal peptides, we have examined the effects of several of the same signal peptides on translocation ATPase and in vitro protein translocation, both of which were previously shown to be inhibited by the addition of signal peptides (13–15). Strikingly, translocation ATPase (Fig. 9A) and in vitro translocation activities (Fig. 10A) are inhibited by the family of OmpA signal peptides to an extent that closely parallels their interaction with SecA64. Moreover, the order of effectiveness of the peptides is as expected from their in vivo export activities with the exception of an unexpectedly strong inhibition of translocation ATPase by the Δ8 mutant. It is notable that significantly more signal peptide is required to inhibit the SecA translocation ATPase than in vitro translocation of proOmpA. This difference can be attributed to the substantially lower quantity of precursor protein present in the latter assay and, hence, the much higher ratio of signal peptide to precursor. Also, signal peptide may inhibit the in vitro translocation assay at more than one site, for example the SecYEG complex, as well as SecA.

A similar result was obtained when we compared the effect of the two enantiomers of the LamBWT signal peptide. The natural, L-enantiomer inhibited the SecA translocation ATPase (Fig. 9B) and in vitro protein translocation (Fig. 10B) to a greater extent than did the D-enantiomer, as had been seen for SecA64 ATPase inhibition. In all cases, the D-enantiomer does inhibit but requires a higher concentration. Again, these results point to a fundamental parallel between the behavior of the “stripped down” proteolytic domain, SecA64, and SecA when engaged with the export machinery in the process of protein translocation.

**DISCUSSION**

Removal of approximately one-third of the SecA molecule by limited chymotrypsinolysis provided us with SecA64, a soluble, activated 64-kDa form of this large molecule with which to examine its function as a signal sequence receptor. SecA64 is predicted to contain the signal sequence/pre-protein binding region of SecA based on the location of PrlD mutations, which rescue maltose-binding protein signal sequence mutants (40), and on biochemical evidence, which places the pre-protein-binding site between residues 267 and 340 of SecA (41). The pre-protein-binding site has a low affinity for pre-proteins in free SecA, but the binding affinity increases when SecA becomes bound to a lipid bilayer (12). The increase in binding affinity is triggered by a conformational change in SecA, which also activates its ATPase activity (13, 42, 43).

Our data argue that removal of the C-terminal domain from SecA functionally mimics its binding to the cytoplasmic membrane. Proteolytic removal of the C-terminal domain leads to increased signal sequence binding as well as activation of ATPase to near translocation ATPase levels. The limited chymotryptic digestion conditions we employed resulted in removal of ~10 residues from the N terminus as well, but we have also...
shown that such an N-terminally trimmed SecA is fully functional. In an extension of earlier work showing that a comparable C-terminal truncation of SecA to that observed here activated its ATPase activity (18), Economou and co-workers (16) recently identified a segment of the C-terminal domain that they postulate acts as an intramolecular switch. They dubbed this region the intramolecular inhibitor of ATP hydrolysis or IRA. These researchers further hypothesized that this region mediates binding between the N- and C-terminal portions of SecA and that its removal causes a conformational change in the N-terminal region of SecA. Our data are entirely consistent with this model and add the important functional dimension that the conformational change accompanying activation of ATP hydrolysis also remodels the binding site for signal peptide in a manner that enhances affinity. Thus the C terminus of SecA must regulate the accessibility or structure of the signal sequence-binding site either directly by obstructing the binding site or indirectly by allosteric effects on the tertiary structure of the N-terminal region.

Several previous reports have shown signal peptide modulation of SecA lipid and translocation ATPase activities. Synthetic signal peptides inhibited SecA translocation ATPase in in vitro systems (13, 14). Signal peptides also inhibit translocation of proOmpA into E. coli membrane vesicles (15). In all of these cases, the signal peptide apparently acts by competing with the signal sequence of the protein to be translocated from the signal sequence-binding site on SecA. By preventing access of the pre-protein to the signal sequence recognition site, export is blocked. There is also evidence that the binding of the signal sequence is coupled to the ATPase activity of SecA during

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2 J. P. You and P. C. Tai, unpublished results.
translocation. Activation of SecA-dependent translocation ATPase requires the presence of a functional signal sequence at the N terminus of the secretory protein (13). This suggests that productive binding of the signal sequence directly triggers changes in the conformation of SecA, since the translocation ATPase is not observed in the absence of a functional signal sequence even when all the other required molecules are present. The signal sequence need not be covalently linked to the secretory protein to stimulate ATPase activity; a synthetic signal sequence and the mature portion of pre-OmpA together can stimulate the translocation ATPase of SecA nearly as well as the addition of the intact pre-protein, but either one alone has no effect (13). This observation suggests that there are probably binding sites on SecA for both the signal sequence and the mature part of the secreted protein and, further, that both sites must be occupied to result in a productive change in SecA conformation. It is also clear that the interaction of SecA with lipid and with the SecYEG complex modulates the conformational change to its translocation-active state and the ATPase activities (44).

From past work, we had expected that signal sequence binding would also have an effect on the interaction of SecA64 with ATP. Moreover, we had anticipated that signal peptides would inhibit the ATPase activity of SecA64 as we observed them to do by analogy to their effect on the translocation ATPase activity of SecA (11–13), which SecA64 appears to mimic. The exact mechanism of this inhibition is not clear. In studies where synthetic signal peptides were shown to act as competitive inhibitors of translocation ATPase, the inhibition was postulated to arise from competition at the pre-sequence-binding site, preventing functional binding of proOmpA (13). In the case of SecA64, this cannot be the mechanism of inhibition, since this is a purified system, and no functional coupling between SecA64 and another polypeptide is taking place that the signal sequence can block. Binding of the signal sequence to its binding site must therefore cause a conformational change in SecA64, which alters either ATP binding, ADP release, or the rate of hydrolysis. It was noted previously that increasing the ATP concentration could reverse the inhibitory effect of signal peptides (13). Although we did not explore this effect systematically, we also observe a weaker inhibition at higher ATP concentration (compare Fig. 6 and 7A); both these results
suggest that the functional coupling between these two sites is bi-directional. The basis for this inhibition is likely to reflect the relationship between ATP binding/hydrolisis and pre-secretory protein binding/release by SecA during export. It is intriguing that Kendall and co-workers observe a stimulation of SecA ATPase by signal peptides in the presence of lipid vesicles (11) and that this stimulatory effect also correlates with the functionalities of the signal peptides (45). Their observations most likely reflect an earlier step in precursor recognition before the conformational change that accompanies activation of SecA translocation ATPase. In our simplified system where SecA64 behaves like SecA after activation, the inhibition could be due to the inability of the protein to reach the next step of the cycle due to the absence of the other components of the translocase. A similar observation is seen in the case of the signal recognition particle (SRP); signal peptides inhibit the GTPase activity seen when SRP is combined in a simple *in vitro* system with its detergent-solubilized receptor (46, 47). We also show that the ATPase inhibition is reduced by signal sequence mutations that block export *in vivo*, suggesting that at least one of the effects of these mutations is to disrupt the interaction between SecA and the pre-secretory protein. Since evidence suggests that the pre-protein encounters SecA early in the translocation process (1–4), these mutations probably block export at the SecA recognition step rather than a later step in secretion.

All of our inhibition plots show a complex curve shape that is not consistent with simple inhibition as a result of peptide binding. If binding of the signal peptides is taking place at a single binding site and if the ATPase activity is diminished as a result of peptide binding, then the inhibition plot would be hyperbolic. Instead the curves are sigmoidal; at low peptide concentrations the effect on ATPase is negligible, but as the peptide concentration increases, the onset of inhibition occurs rapidly. The reason for this apparently cooperative behavior is not known. If one assumes a two-binding-site model, then the apparent cooperativity could arise from an interaction between these two sites. Binding of a peptide to the “mature protein” site, for example, might be weak if the signal sequence-binding site is unoccupied; binding could be strengthened by placing a functional signal sequence in the signal sequence-binding site. If both sites need to be occupied to inhibit the ATPase activity, then the apparent cooperativity could arise as the binding of signal sequences to the signal sequence site affects the binding affinity for binding to the mature site (or vice versa). Additionally, these peptides are highly amphiphilic and of low water solubility. The apparent cooperativity may arise from signal peptide self-association. Supporting this interpretation is the hyperbolic inhibition curve of the more soluble KRR-LamB signal peptide.

The basis for the recognition of signal sequences by SecA is not well understood but is thought to involve the overall physical and chemical properties of the signal sequence rather than sequence-specific interactions. SecA64 provides a system to explore directly the requirements for signal sequence interaction with SecA by following either binding (competition for binding to biotinylated LamBWT) or ATPase inhibition or both. Previous work has established that the essential features of *E. coli* signal sequences include both positive charges in the N-terminal region and a hydrophobic core of minimum length approximately seven residues and average residue hydrophobicity between Ala and Leu (5, 48, 49). To distinguish the relative importance of hydrophobicity of the central h-region from possible requirements for specific secondary structure, we have compared enantiomers of LamBWT. These peptides have identical amino acid sequences and, thus, identical hydrophobicities, but they differ in the handedness of any secondary structure they adopt. In interfacial environments, under conditions where the LamBWT adopts a right-handed α-helical conformation (33–35), the D-enantiomer forms a left-handed α-helix, as indicated by a circular dichroism spectrum of opposite sign (data not shown). If the binding of the signal sequence to SecA64 depends on stereochemically specific contacts and not merely on the overall hydrophobicity of the peptide, then the D-peptide should bind and thus inhibit less well than the natural ligand, the L-peptide, as was observed.

The N-terminal positive charge has been proposed to play an important role in SecAsignal peptide interaction (9), although recent work suggests that the hydrophobic core may compensate for lack of positive charges (8, 10). The increased charge of the KRR-LamB signal peptide indeed led to enhanced binding to SecA64. This result is consistent with the published reports that a greater positive charge in the N region of the signal sequence enhanced cross-linking of the pre-protein to SecA (9).

We found that the signal peptides had largely the same relative effectiveness in inhibition of SecA64 ATPase and in inhibition of translocation ATPase *in vitro*, with one exception (OmpAΔ8). This result argues strongly that their actions on SecA64 have the same origin as their effect on
intact translocation-active SecA. Moreover, their inhibitory effect on SecA provides the most likely mechanism for their inhibition of translocation in the more complex system. However, the potency of the signal peptides as inhibitors is lower in the SecA64 system than in either the translocation ATPase or in vitro translocation assays. Biophysical data have previously shown that there is a tight correlation between the hydrophobicity of the hydrophobic cores of these sequences and their in vivo function (38, 39). It is likely that the signal peptides that are most effective in vivo partition into the membrane in either the translocation ATPase or translocation assays and, thus, may be acting at a higher effective concentration in the region of membrane-bound SecA. We conclude that SecA64 is providing a simplified system devoid of membranes to analyze the nature of the interaction of the signal sequence with the export apparatus and, furthermore, that a key interaction in the complex translocation apparatus occurs between SecA and the signal sequence as expected.

In the present study, we have taken advantage of our identification of a proteolytically stable domain of SecA, SecA64, to develop a system for characterization of functionally relevant binding of signal peptides to SecA. We have shown that the effects of signal peptides on SecA64 closely parallel their effects on SecA translocation ATPase and SecA-mediated in vitro translocation. We are now in a position to develop biophysical approaches such as NMR and fluorescence to explore in greater detail the structural origins of signal peptide recognition by activated SecA. These studies will complement the emerging structural picture of the Bacillus subtilis SecA from x-ray crystallography (50).

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Functional Signal Peptides Bind a Soluble N-terminal Fragment of SecA and Inhibit Its ATPase Activity

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