Antifolding Activity of the SecB Chaperone Is Essential for Secretion of HasA, a Quickly Folding ABC Pathway Substrate

We have previously shown that SecB, the ATP-independent chaperone of the Sec pathway, is required for the secretion of the HasA homopentamer from *Serratia marcescens* via its type I secretion pathway, both in the reconstituted system in *Escherichia coli* and in the original host. The refolding of apo-HasA after denaturation with guanidine HCl was followed by stopped-flow measurements of fluorescence of its single tryptophan, both in the absence and presence of SecB. In the absence of SecB, HasA folds very quickly with a major phase (45 s\(^{-1}\)) accounting for 92% of the signal. SecB considerably slows down HasA folding. At stoichiometric amounts of SecB and HasA, a single phase (0.014 s\(^{-1}\)) of refolding is observed. Two double point mutants of HasA were made, abolishing two hydrogen bonds between N-terminal and C-terminal side chain residues. In both cases, the mutants essentially maintained the same secondary and tertiary structure as wild-type HasA and were fully functional. Refolding of both mutants was much slower than that of wild-type HasA and they were secreted essentially independently of SecB. We conclude that SecB has mainly an antifolding function in the HasA ABC secretion pathway.

SecB is a chaperone of the Sec pathway in Gram-negative bacteria. It was initially identified in *Escherichia coli* where it interacts with a subset of preproteins to be exported across the cytoplasmic membrane in such a way as to slow down their folding and target them to SecA, the ATPase of the Sec system (1–4) (5). Currently, this chaperone is restricted to Gram-negative bacteria, from the proteobacteria class. In the Sec pathway, the basis of the interaction of SecB with HasA, a large variety of denatured substrates whereas it is highly specific in vivo (6, 7).

The structure of SecB from *Haemophilus influenzae* is known at atomic resolution and has highlighted several important features of this chaperone (8). It is a tetramer composed of a dimer of dimers. Each monomer bears a putative peptide-binding site able to accommodate, in an extended conformation, hydrophobic peptides of 10–15 residues; on both sides the tetramer presents a patch of negatively charged residues that can interact with a SecA dimer. *In vitro* studies using peptide libraries have indicated that SecB binding site is around 9–10 residues long with a strong preference for aromatic and cationic amino acid residues (9). Furthermore, SecB is a highly negatively charged molecule; thus, negatively charged peptides bind to SecB only if the peptide contains enough aromatic residues in the peptide to overcome the electrostatic repulsion.

Maltose-binding protein (MBP), the best characterized SecB substrate in both *in vivo* and *in vitro* studies, interacts with SecB in kinetic partitioning: the competition between binding to the chaperone and folding reactions (10–13). At 25 °C MBP refolds by a complex pathway characterized by three phases. SecB slows down folding but does not block it; moreover, increasing amounts of SecB progressively slow down MBP refolding. At 5 °C MBP refolding is slower and SecB totally blocks refolding. This can be visualized by the existence of stable SecB-MBP complexes that can be isolated at 5 °C but not at 25 °C. MBP mutants have been isolated on the basis of their ability to translocate across the inner membrane without the presence of SecB. MBPY283D is a MBP mutant that is perfectly biologically active and folds more slowly than wild-type MBP making its translocation SecB-independent (2, 14). This variant associates with SecB blocking MBP folding even at 25 °C. A model has been proposed for the interaction of SecB and MBP: an unfolded form of MBP binds to SecB and is in equilibrium with unbound form. MBP does not fold on SecB and this explains the progressive blockade of MBP folding with increasing SecB amounts. Although the signal sequence of pre-LamB or pre-MBP does not interact with SecB by itself, it slows down the folding of the mature part of the protein (15, 16). SecB interacts with several parts of precursor molecules, protecting a large central portion of precursors to which it is bound (17–19).

Other substrates have been studied in great detail, like barnase of *Bacillus amylobacteriaces* (20) or R-BPTI (21). Studies with R-BPTI, which is a stable unfolded form of BPTI, have shown that association of a substrate with SecB is limited by diffusion and that the bound R-BPTI is easily exchanged with free R-BPTI. Such findings allowed the researchers to estimate a *K*\(_D\) of a few mM for the Sec-R-BPTI complex. In the case of barnase, which is a small secreted RNase with a standard signal peptide, SecB retards the folding of barnase but never blocks its folding in contrast to what has been observed with MBP. Barnase folds at least partially onto SecB which challenges the kinetics partition model.

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To whom correspondence should be addressed. Tel.: 33-1-40-61-32-76; Fax: 33-1-45-68-87-90; E-mail: pdelep@pasteur.fr.
Interaction of SecB Chaperone with an ABC Substrate

We have shown that, besides its function in the Sec pathway, SecB is also the chaperone of a sec-independent pathway, namely the secretion of the HasASM hemophore through its ABC pathway in *Serratia marcescens* (22, 23).

HasASM is a monomeric protein of 188 residues secreted by an ABC-type pathway under iron starvation conditions by *S. marcescens* (24) and other Gram-negative bacteria, *Pseudomonas aeruginosa, Pseudomonas fluorescens,* and *Yersinia pestis* (25, 26). HasA carries the heme from hemoproteins (e.g., hemoglobin) and delivers it to a specific receptor at the cell surface whereby the heme is internalized and used as an iron source (27). Both the secretion pathways and the heme acquisition pathways have been reconstituted in *E. coli*. We have shown that SecB, the chaperone of the Sec pathway, is required for HasASM secretion both in the original host and in the reconstituted system (22, 23). SecB does not affect the secretion apparatus but likely interacts with HasASM. We have proposed reconstituted system (22, 23). SecB does not affect the secretion that the N-terminal part of HasA SM interacts in a SecB-deicient of 11708M

defomed from absorbance at 277 nm using an extinction coefficient determined.

The equilibrium unfolding as a function of GdnHCl concentration were achieved using the following equation,

\[
G = -K T \ln (S)
\]

where \( G \) is the free energy of unfolding, \( K \) is the apparent equilibrium constant of unfolding at a concentration \( C \) of denaturant. The free energy of unfolding \( \Delta G^0 \) at zero denaturant concentration is given by,

\[
\Delta G^0 = RT \ln \left( \frac{S_m}{S_0} \right)
\]

where \( S_m \) and \( S_0 \) are the specific signals of the native and unfolded protein extrapolated to zero denaturant concentration, and \( K \) is the apparent equilibrium constant of unfolding at a concentration \( C \) of denaturant. The free energy of unfolding \( \Delta G^0 \) at zero denaturant concentration is given by,

\[
K = \frac{m \Delta C}{RT}
\]

where \( m \) is a constant characteristic of the protein and of the denaturant.

**Stopped-flow Measurements**—The measurements of HasASM refolding kinetics were carried out at 22 °C with a SFM300 mixing device from Bio-logic (Pont de Claix, France). The mixing device, equipped with a P15 (1.5 × 1.5-mm cross-section) fluorescence cell, was combined with the optical bench and detection module from Bio-logic. The excitation wavelength was 265 nm, and the emission wavelength was 320 or 350 nm was detected through a high pass filter. The kinetics were recorded by means of the Bio-Kine software package from Bio-logic. For refolding experiments, 10 or 20 μl of denatured HasASM (20 μM sodium phosphate, pH 7.0) was mixed with 390 or 380 μl of

To assess the secretion of wild-type and mutant HasA proteins, cultures of MC4100 or MC4100secBΔ harboring Has secretion functions from pSYC150 and wild-type or mutant hasA on a compatible plasmid were grown in LB medium at 30 °C with the appropriate antibiotics to late exponential phase. The culture was centrifuged at 4000 × *g* at 10 min and the supernatant precipitated with 20% trichloroacetic acid was analyzed by SDS-PAGE.

**Buffer**—All kinetic and equilibrium experiments were carried out in 20 mM sodium phosphate, pH 7.0. The protein concentrations were determined by absorbance spectrophotometry before use. Water was deionized and purified on a Millipore system. All solutions were filtered.

The equilibrium unfolding as a function of GdnHCl concentration were carried out by dilution of the proper amount of the 8 M denaturant stock containing leaving the samples at room temperature overnight prior to performing the titration experiments.

**Equilibrium Unfolding Experiments**—The equilibrium unfolding of HasASM and its mutants as a function of GdnHCl concentration was monitored by fluorescence and far-UV CD at 25 °C. Fluoromax (Spx Industries) photon counting spectrophotometer was used carry out the fluorescence studies. The samples were excited at 295 nm, and the resulting emission spectra were acquired between 310 nm and 410 nm. When working at a constant emission wavelength (i.e., the protein was excited at 295 nm and emission was monitored at 360 nm), fluorescence intensity was recorded for 30 s, with a sampling period and an integration time of 1 s, and then averaged. The slit width was 5 nm for the excitation light and 10 nm for the emission light. Measurements were made with a protein concentration of 3 μM and a 1.0-cm path length cuvette.

CD measurements were acquired in a Jobin-Yvon CD6 spectropolarimeter. Far-UV CD spectra were obtained by averaging five individual scans with a constant 0.5 nm step and with 5-s integration time from 180 to 200 nm and 2 s from 200 to 260 nm. A cell with a path length of 0.2 cm was used with a protein concentration of 3 μM. Near-UV CD spectra were acquired between 350 nm and 450 nm using 30 μM of protein in a 0.1-cm path length cell. For GdnHCl titrations, the absorbances were measured at 222 nm with a sampling period and time constant of 1 s during 60 s. The final ellipticity was calculated by averaging the 60 recorded values. Measurements were made with a protein concentration of 30 μM in a 0.02-cm path length cell. All spectra were corrected by subtracting the appropriate baseline.

**Thermal Denaturation**—Thermal denaturation was followed by monitoring mean residue ellipticities at 222 nm (far-UV) using a Jobin-Yvon CD6 dichrograph interfaced with a Haske water bath. The signal was acquired with a 0.02 cm pathlength cell and a protein concentration of 30 μM with a sampling period of 60 s and an integration time of 1 s. The results were then averaged. The heating rate was 0.8 °C/min with a stabilization delay of 3 min before each recording.

**Data Analysis**—The measured fluorescence intensities and the mean residue ellipticity were analyzed in terms of a two-state transition to obtain the fraction of unfolded protein and free energy of folding as a function of denaturant. Non-linear least square fittings of the equilibrium transitions were achieved using the following equation,

\[
S = S_m + nC \left( \frac{S_m - S_0}{K} \right) \frac{S_m}{S_0}
\]

where \( S \) is the signal (CD or fluorescence) measured, \( n \) and \( u \) the slopes of the native and unfolded baseline, \( S_m \) and \( S_0 \) the specific signals of the native and unfolded protein extrapolated to zero denaturant concentration, and \( K \) the apparent equilibrium constant of unfolding at a concentration \( C \) of denaturant. The free energy of unfolding \( \Delta G^0 \) at zero denaturant concentration is given by,

\[
\Delta G^0 = RT \ln \left( \frac{S_m}{S_0} \right)
\]
buffer (20 mM sodium phosphate, pH 7). The duration of the injections was 30 ms yielding a 4 ms dead time. Refolding kinetics were performed at various protein concentrations. For SecB-dependent refolding, SecB was added to the refolding buffer (from 7.5 \times 10^{-3} \mu M to 1.5 \mu M SecB tetramer) with a final concentration of HasASM of 0.375 and 0.75 \mu M. At either SecB concentration, kinetic traces were fitted using a multiexponential decay,

\[ I = \sum_{i} A_i e^{-k_i t} + A_n \]  

(Eq. 3)

where \( A_i \) is the amplitude of each phase, \( k_i \) its rate constant, and \( A_n \) is the plateau value.

RESULTS

Apo-HasASM Unfolding and Refolding in Vitro HasASM Refolds Spontaneously with Fast Kinetics—HasASM is a 188-amino acid monomeric protein with a unique domain of \( \alpha/\beta \)-fold (31). Fig. 1, A and B, show the chemical (Fig. 1A, left) and thermal (Fig. 1B, right) denaturations of WT HasASM and of two mutants (see below for the description of the mutants), followed by monitoring the disappearance of the secondary structures using far-UV CD. During chemical denaturation of WT HasA, an increase in the fluorescence of the unique tryptophan was observed together with a shift in the maximum fluorescence emission wavelength (from 322 to 353 nm) (data not shown). Both fluorescence and far-UV CD (Fig. 1A) gave superimposable equilibrium denaturation curves using GdnHCl as denaturant. The data could be satisfactorily fitted to a two-state model \( F \leftrightarrow U \) for unfolding. The GdnHCl concentration of half unfolding is given by 

\[ \Delta G / \Delta C_m \]  

where \( C_m \) is the midpoint of the thermal transition \( T_m \) at 59.5 \pm 0.5 °C is similar to the value reported by differential scanning calorimetry microcalorimetry. Because this thermal transition of HasASM is not reversible regardless of the heating rate chosen between 20 and 60 °C/h, we could not extract the thermodynamic parameters from the thermal denaturation data.

The refolding kinetics of WT HasASM were also monitored by the fluorescence changes of its single tryptophan residue detected by a stopped-flow spectrophotometer (Fig. 2A, trace a). HasASM is largely unfolded in 5.5 \mu M GdnHCl and reversibly refolded upon dilution of the chaotropic agent leading to a large decrease of fluorescence. Upon dilution of apo-HasASM from 5.5 \mu M GdnHCl to 0.14 and 0.28 \mu M GdnHCl, about 50% of the fluorescence intensity decreased within the dead time; nevertheless, this signal decrease is not due to a burst phase (phase faster than 4 ms) but rather to a dilution effect of GdnHCl. Indeed, extrapolation of fluorescence at 0.14 \mu M GdnHCl from the curve obtained between 3.0 and 5.5 \mu M GdnHCl gives the same intensity as this from the first fluorescence measurement in the denaturation kinetics (not shown). The time course of refolding as followed by Trp fluorescence, was fitted to a double exponential decay. The first phase (45 s \(^{-1}\)) corresponds to 92% of the relative amplitude, and the second (1.8 s \(^{-1}\)) accounts for the remaining 8% (see Fig. 3). These rate constants and amplitudes are only slightly temperature-dependent between 4 and 22 °C (data not shown).

SecB Slows Down in Vitro HasASM Refolding—We have previously shown that HasASM secretion is SecB-dependent in the original host and in the reconstituted system in E. coli in vivo (22, 23). This prompted us to study the in vitro interaction of SecB with HasASM. We could first show that no stable complexes exist between SecB and folded apo-HasASM detected either by coimmunoprecipitation or by gel electrophoresis under non-denaturing conditions (data not shown). Apo-HasASM refolding was also studied in the presence of various amounts of SecB in the renaturation buffer at 22 °C (Figs. 2B and 3). We used a high pass filter to collect emitted light above 350 nm to minimize the fluorescence contribution of SecB, which displays a maximum fluorescence emission around 320 nm in 0.14 \mu M GdnHCl. Analysis of the refolding kinetics showed that SecB affects HasASM folding (Fig. 2B, traces a–c). It is clear that SecB slows down HasA folding. This is a direct evidence for interaction, despite the very fast HasASM folding kinetics. The folding of HasASM is strongly retarded when more SecB is added to the refolding buffer (Fig. 2B, traces a–c). The HasASM–SecB interaction takes place very early, before or during the fast HasASM folding phase (45 s \(^{-1}\)). Analysis of both amplitude and rate constant of the different phases observed in the presence of increasing amounts of SecB (Fig. 3) show the progressive appearance of a slow folding phase not observed in the absence of SecB. The amplitude of the major fast folding phase progressively decreases with higher concentrations of SecB, although the rate constant does not change at around 50 s \(^{-1}\). A slower phase progressively appears with an apparent rate constant of 0.013 s \(^{-1}\), at a 2:1 ratio of SecB tetramer to HasA; there is only a slight decrease of the rate constant with higher SecB concentrations. At the same time, the intermediate phase of 1.8 s \(^{-1}\) stays at the same small amplitude with a substoichiometric

\(^2\) A. Lecroisy, personal communication.
amount of SecB. Calculation of the stoichiometry of bound HasASM to SecB, as extrapolated from the fluorescence data, indicated that this might vary from two HasA SM molecules bound per SecB tetramer at low SecB concentration to one HasA SM molecule bound per SecB tetramer at higher SecB concentration. As found for barnase in the presence of GroEl or SecB, addition of more SecB up to a 4:1 SecB:HasASM ratio never blocked the folding of HasASM. Altogether our experiments indicate that HasASM binds SecB tetramer with a 1:1 stoichiometry, and this considerably slows down the folding of HasASM, 3200-fold. The very fast formation of HasA SM-SecB complex suggests that the chaperone binds unfolded HasASM or a very early folding intermediate. These experiments suggested that a function of SecB in HasA secretion would be antifolding and that slowly folding variants of HasA might be secreted independently of SecB.

**N-terminal-C-terminal Interactions in HasASM Affect Folding Rate but Not SecB Binding**—We have proposed in previous studies that both the N- and C-terminal parts of HasASM might be involved in the HasASM secretion process (28, 29). Exami-
The concentration of SecB (not represented on Fig. 4). The HasA mutants, observed in the absence of SecB decreased, for WT HasA. The amplitudes of the two refolding phases of fluorescence data for the mutants in a similar way as that done instead of three for the WT. The Fig. 4 shows the analysis of the data gave midpoints ($C_m$) of 1.99 ± 0.2 M for protein 7–17 and 2.1 ± 0.2 M for protein 148–167. The free energies of denaturation ($\Delta G$) is 8.4 ± 1.9 kcal mol$^{-1}$ for 7–17 and and 10.6 ± 2.8 kcal mol$^{-1}$ for 148–167. Differences of $\Delta G$ between mutant and wild-type HasA$^\text{SM}$ are compatible with

\begin{align*}
\text{Fig. 4. Phases of refolding of 7–17 and 148–167 mutants in presence of increasing concentrations of SecB tetramer.} & \quad A, \text{amplitudes of the slow and fast refolding phases: 7–17 slow phase (●), 148–167 slow phase (○), 7–17 fast phase (□), 148–167 fast phase (△). B, rate constants of the slow and fast refolding phases: 7–17 slow phase (●), 148–167 slow phase (○), 7–17 fast phase (□), 148–167 fast phase (△). Stopped-flow fluorescence was measured at 22 °C with a final concentration of HasA$^\text{SM}$ at 0.75 μM in 20 mM sodium phosphate, pH 7.0. Each data point corresponds to the average of four independent kinetic series, each including nine successive individual traces. The error bars represent the S.D. values. Each experiment was performed twice.}
\end{align*}
the loss of two hydrogen bonds (about 4 kcal mol\(^{-1}\)) as a result of the mutations. Although the HasASM mutants are destabilized with respect to the wild-type protein, their fractions of unfolded molecules at 0 M GdnHCl extracted from the free energy of denaturation remain very low: 7.8 \times 10^{-7} for the 7–17 mutant and 1.8 \times 10^{-8} for the 148–167 mutant versus 3.9 \times 10^{-2} for the wild-type protein. Thermal unfolding also indicated a lower stability of both mutant proteins (Fig. 1B). The midpoint values of the thermal transition \(T_m\) are 52.6 \pm 0.5 °C for 7–17 and 55.0 \pm 0.5 °C for 148–167.

As a control we used another double mutant of HasA, H32A,Y75A, affecting heme binding (32). This mutant had a somewhat reduced stability (half-denaturation at 2.2 \pm 0.2 M), refolded with the same kinetics as that of the wild-type protein, and was SecB-dependent for its secretion at the same extent as wild-type protein (data not shown).

**DISCUSSION**

Here we have studied HasASM folding and its interaction with SecB in vitro together with the effect of mutations disrupting hydrogen bonds between the C and N terminus of HasASM on its folding and their secretion dependence upon SecB. HasASM folding is simple with no burst-phase intermediate and with a fast and major folding phase, making it an attractive model to study interactions with SecB. This is the first time a natural SecB-dependent substrate that is not a Sec substrate has been studied in interaction with SecB in vitro.

The interaction between HasASM and SecB differs in several characteristics of the interaction of SecB with Sec substrates. Since HasASM and the mutants do not contain any arginine or lysine and are mostly negatively charged, the basis of interaction between HasA SM and SecB cannot rely on electrostatic interaction between residues but through hydrophobic interactions (33, 34). Since HasASM is a natural substrate of SecB, the absence of positive charge in HasASM proves that positive charges are not a prerequisite for interaction with SecB. As seen with other substrates, SecB slows down HasA folding by more than three orders of magnitude. However, in contrast to the MBP or galactose-binding protein refolding, which is totally blocked at high SecB concentrations, which allows the isolation of permanent stable complexes (12, 18), this does not appear to be the case for HasASM. Instead, there appears to be a slow folding phase of 0.014 s\(^{-1}\) whose apparent rate constant does not significantly change with varying SecB amounts.

\[
\text{HasASM} + \text{SecB} \rightleftharpoons \text{HasASM} \cdot \text{SecB} \rightleftharpoons \text{HasASM} \cdot \text{SecB} \rightleftharpoons \text{HasASM} \cdot \text{SecB} + \text{HasASM}
\]

**REACTION 1**

This is reminiscent of the behavior of barnase (a synthetic SecB substrate) in the presence of SecB, which has been interpreted as at least partial refolding when bound to this chaperone (20). This is in contrast to the model of kinetic partitioning like for MBP in which the unfolded protein is bound to SecB, released, and folded only when free in solution. It is likely what happens with barnase occurs with HasASM.

The major folding phase of HasASM has a rate constant inferior to 45 s\(^{-1}\), which is much faster than the rate constants observed for characterized substrates of the Sec system. Fur-
thermore, in the case of Sec substrates, the presence of the signal sequence slows down folding. This has been proposed as the basis of the in vivo selectivity of SecB for its substrates (10, 16); this clearly does not apply to the case of HasASM. SecB binds to HasASM in millisecond time scale. At 1:1 HasASM:SecB stoichiometry, we estimated the second-order binding rate constant at $3 \times 10^8 \text{ M}^{-1} \text{s}^{-1}$, suggesting that the rate of HasASM association to SecB is diffusion-limited (21). So, a slow folding kinetics is not a prerequisite for in vivo implication of SecB in this case. It is very likely that this explains that secretion competence of HasASM cannot be observed for a long period of time, since uncoupling of HasASM synthesis from its secretion apparatus leads to no secretion even when SecB is overexpressed (29). Moreover this is consistent with the observed absence of blockage of folding of HasASM by SecB.

In the Sec system, SecB fulfills two separate functions of slowing down precursor folding and of targeting to SecA, the ATPase of the Sec system (35). In the ABC system of HasASM secretion, the precise functions of SecB are not known. Here, we have shown that SecB is able to strongly slow down the folding of wild-type HasASM and that the secretion of the slower folding variants of HasASM is much less SecB-dependent than the secretion of wild-type HasASM. Given the fact that folded HasASM is no longer secretion competent, it, thus, seems that the main role of SecB in the ABC system is slowing down HasASM folding. The behavior of the two mutants we have constructed also supports the importance of slowing down of folding function of SecB in this system. Although it is difficult to extrapolate from the in vitro folding kinetics to in vivo, it is clear that both mutants fold more slowly than wild-type HasASM. We conclude that interactions between the N terminus and the C terminus of the protein are at least involved in the rapid folding kinetics of HasASM. The mutants were secreted to a significant extent in the absence of SecB, they were fully functional, and they were indistinguishable from wild-type in terms of global fold. Although both mutants display a reduced stability as observed with the thermal and chemical denaturations, the very low fraction of unfolded proteins in “native” conditions seems insufficient to explain their efficient secretion in the absence of SecB, especially in light of the fact that HasA22–75, which has the same reduced stability, is as SecB-dependent as the wild-type. SecB interacts with wild-type and mutant HasASM so as to slow down their folding. Although able to interact with HasASM mutants, SecB has little effect on their secretion; this is also what was observed for the SecB independent mutant of MBP. In the Sec system, a cascade of increasing binding affinities allows the transfer of the precursor from SecB to SecA and to the translocon (35). This might not occur in the case of HasASM if folding still occurs on SecB; efficient secretion would result from competition between folding and secretion. In the Sec system SecB has an essential targeting function to the SecA ATPase and an antifolding function. In the HasA ABC secretion system, SecB has an antifolding function. We have previously shown that the N-terminal part of HasA is involved in efficient secretion and in an early interaction with the transporter. It remains to be determined whether SecB plays a role in this interaction.

We had proposed that both the C terminus of HasASM bearing the secretion signal and the N terminus part of HasASM via a SecB-dependent recognition of the transporter were involved in efficient HasASM secretion (28). The behavior of the mutants might give clues on how this could happen; one of the functions of SecB, upon binding to HasASM, would keep both ends of HasASM freely available to the transporter. If one assumes that the SecB binding sites defined by the peptide library technique are relevant to the in vivo situation, there appears to be only one such site in HasASM fulfilling the criteria defined (9). This site is at the N terminus of HasASM between residues 18–28, YLGQWASTFG, with three aromatic residues and no negative charge. The N-terminal position of the putative binding site of SecB and the very fast folding in vitro of HasASM suggest that the chaperone would bind the nascent chain of HasASM. This is in agreement with our model of secretion in which HasASM is targeted in a cotranslational fashion to the ABC transporter (29).

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