Protein Science (1995), *4*:1118–1123. Cambridge University Press. Printed in the USA.
Copyright © 1995 The Protein Society

Interaction of SecB with intermediates along the folding pathway of maltose-binding protein

DEBORAH L. DIAMOND, SHARON STROBEL,[1] SANG-YEARN CHUN,[2] AND LINDA L. RANDALL

Department of Biochemistry and Biophysics, Washington State University, Pullman, Washington 99164-4660

(RECEIVED January 9, 1995; ACCEPTED March 24, 1995)

Abstract

SecB, a molecular chaperone involved in protein export in *Escherichia coli*, displays the remarkable ability to selectively bind many different polypeptide ligands whose only common feature is that of being nonnative. The selectivity is explained in part by a kinetic partitioning between the folding of a polypeptide and its association with SecB. SecB has no affinity for native, stably folded polypeptides but interacts tightly with polypeptides that are nonnative. In order to better understand the nature of the binding, we have examined the interaction of SecB with intermediates along the folding pathway of maltose-binding protein. Taking advantage of forms of maltose-binding protein that are altered in their folding properties, we show that the first intermediate in folding, represented by the collapsed state, binds to SecB, and that the polypeptide remains active as a ligand until it crosses the final energy barrier to attain the native state.

Keywords: chaperones; folding intermediates; SecB

Proteins that are synthesized in the cytoplasm and exported to noncytoplasmic locations must traverse a membrane barrier. Models that have been proposed to explain eukaryotic secretion and organelle biogenesis and export to the periplasmic space in gram-negative bacteria (Randall & Hardy, 1986; Rothman & Kornberg, 1986; Crooke & Wickner, 1987; Verner & Schatz, 1987) all incorporate the observation that to be transferred across biological membranes polypeptides must be in an unfolded or partially unfolded state (Eilers & Schatz, 1986; Maher & Singer, 1986; Randall & Hardy, 1986; Meyer, 1988). Folding to the native conformation occurs only after the protein arrives at its final destination. Thus, mechanisms that modulate protein folding are likely to exist in the cytoplasmic compartments of both prokaryotes and eukaryotes (Ellis & van der Vies, 1991).

In *Escherichia coli*, modulation of the folding of a subset of proteins that are exported to the periplasm or to the outer membrane is achieved by interaction with the chaperone SecB (Kumamoto & Beckwith, 1983, 1985). SecB binds its ligands prior to the acquisition of their native structures (Kumamoto & Gannon, 1988; Kumamoto, 1989; Kumamoto & Francetić, 1993) and maintains them in an export-competent state that is neither folded nor aggregated (Kumamoto & Beckwith, 1985; Randall & Hardy, 1986; Kumamoto & Gannon, 1988; Weiss et al., 1988; Kumamoto et al., 1989; Kusters et al., 1989; Lecker et al., 1989). Through its affinity for SecA, which is a component of the membrane-associated export apparatus, SecB mediates entry of polypeptides into the export pathway (Hartl et al., 1990).

The interaction of SecB with a subset of proteins that are to be exported is governed in part by a kinetic partitioning (Randall & Hardy, 1986; Hardy & Randall, 1991). SecB is precluded from binding to proteins that rapidly fold since it has no affinity for proteins in their native state; however, if the intrinsic rate of folding is slow relative to the rate of association with SecB, the polypeptide can interact with SecB and enter the export pathway. Because all physiologic ligands of SecB are precursor species that contain amino-terminal stretches of aminoacyl residues designated leader or signal sequences, it is not surprising that those leader sequences play a crucial role in mediating binding to SecB. However, the leader sequence does not bind directly to SecB, but rather it slows folding of the precursor and thereby promotes interaction of the non-native polypeptide with SecB (Park et al., 1988; Liu et al., 1989). Folding retardation by the leader sequence thus provides the cell with an exquisite mechanism whereby SecB can engage the precursor polypeptide before it folds into its mature form. An interesting question is: at which step along the folding pathway does SecB bind?

To address this question we have chosen to study the interaction of SecB with the exported, periplasmic, maltose-binding

Reprint requests to: Linda L. Randall, Department of Biochemistry and Biophysics, Washington State University, Pullman, Washington 99164-4660.

[1] Present address: Department of Molecular and Cellular Biology, University of Arizona, Tucson, Arizona 85721.

[2] Present address: Division of Oral Biology, University of California-San Francisco, San Francisco, California 94143-0512.

protein. We have compared the interaction of SecB and wild-type maltose-binding protein with that of SecB and each of two species of maltose-binding protein (MalE A276G, glycine substituted for alanine at position 276 and MalE W10A, alanine substituted for tryptophan at position 10) that have altered folding properties as a result of the single aminoacyl substitution. We conclude that SecB interacts tightly with intermediates along the folding pathway of maltose-binding protein, including the intermediate that immediately precedes the final step in folding. Maltose-binding protein loses the ability to interact with SecB only after it has proceeded through the final step in folding to acquire a stable tertiary structure.

Results

Folding studies of the various species of maltose-binding protein by fluorescence spectroscopy

The reversible folding transition of maltose-binding protein, which contains eight tryptophanyl residues, can be easily monitored in vitro by fluorescence spectroscopy since the fluorescence properties of tryptophan are sensitive to changes in the immediate environment. Refolding of denatured maltose-binding protein results in a progressive increase in the amplitude of the fluorescence signal that is attributed to the sequestering of tryptophanyl side chains in the interior of the native protein. We have previously used the intrinsic fluorescence of tryptophan to identify three kinetic phases during the refolding of maltose-binding protein (Liu et al., 1988; Park et al., 1988; Chun et al., 1993). There is an initial burst phase that cannot be resolved kinetically using stopped-flow fluorescence (dead time of the instrument was 8 ms; L. Randall, C. Mann, C.R. Matthews, unpubl. obs.). The remaining phases, one of which is attributed to isomerization of bonds in the peptide backbone and the other to folding, are resolved kinetically and have time constants on the order of seconds to minutes. It has been previously demonstrated that the polypeptide remains competent for binding SecB after the initial burst phase (Topping & Randall, 1994). In order to determine which of the other two kinetically resolved reactions renders the protein incapable of binding to SecB, we assessed the ability of SecB to interact with two species of maltose-binding protein (MalE A276G and MalE W10A) that each contain a single aminoacyl substitution in the mature region of the polypeptide that alters the folding properties. One of these species, MalE A276G, was obtained using a strategy to select for changes in the step that determines the rate of folding of maltose-binding protein in vivo based on our current understanding of the process of export of the protein to the periplasm. Polypeptides must be transported across the membrane before they acquire their final folded structure. Thus, it is possible to select for aminoacyl substitutions that slow folding by selection for suppressors of a mutational change in the leader peptide of precursor maltose-binding protein that results in inefficient export. A strain with low levels of maltose-binding protein will not grow on maltose since the binding protein is necessary for transport of the sugar. A decrease in the rate of folding of the polypeptide increases the time during which it is competent to enter the export pathway and results in an increased quantity of periplasmic maltose-binding protein, allowing growth on maltose. Using this selection strategy, four species of maltose-binding protein that fold slowly were obtained (Chun et al., 1993). However, with the exception of the substitution of glycine for alanine at residue 276, all substitutions decreased both of the kinetically resolved folding reactions. Examination of the three-dimensional structure of the protein shows that all four residues lie in one structural element of the native protein. Thus, it seemed likely that other substitutions in this area would affect the rate of folding. In a study of the fluorescent properties of maltose-binding protein for other purposes, Martineau and colleagues had changed the tryptophanyl residue at position 10 to an alanine (Martineau et al., 1990). Because position 10 lies within the structural element we had defined as crucial to folding, we further investigated MalE W10A and found that it was indeed altered in its folding.

Equilibrium studies of the guanidinium chloride-induced reversible unfolding of the proteins show sharp transitions between folded and unfolded states with midpoints at 1.0 M, 0.9 M, and 0.7 M guanidinium chloride for the wild-type maltose-binding protein, MalE A276G and MalE W10A, respectively (Fig. 1). Thus, the substitutions render the polypeptides less stable than the wild type. The change in the energy of stabilization caused by the substitution in MalE A276G was calculated to be a reduction of 1.9 kcal mol^{-1}, consistent with the previously published equilibrium studies carried out using urea as the denaturant, which showed a reduction in stability of 1.5 kcal mol^{-1} for this altered polypeptide (Chun et al., 1993). MalE W10A was shown to be the least stable of the three species, with the change in energy of stabilization relative to the wild type being a reduction of 4.3 kcal mol^{-1}. The previous study, carried out using urea gave a value of approximately -11 kcal mol^{-1} for the stability of the wild-type protein and the value calculated using the equilibrium data shown here is -12.8 kcal mol^{-1}.

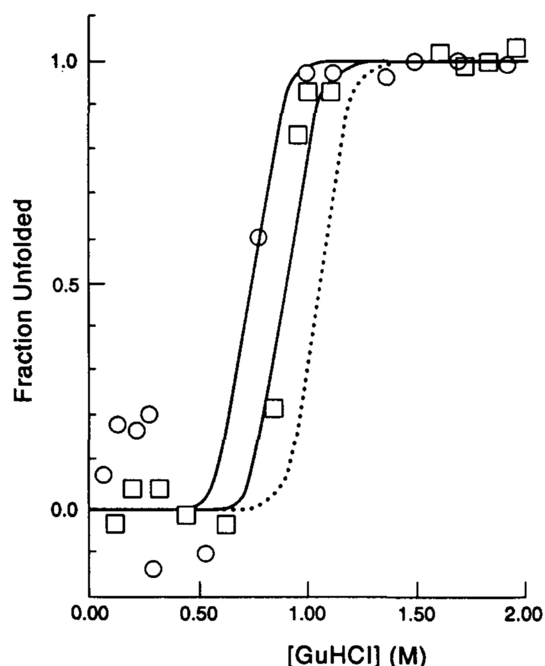

Fig. 1. Equilibrium transition curves for unfolding of maltose-binding protein species. Experimental details are described in the Materials and methods. ○, MalE W10A; □, MalE A276G. Dotted line without symbols represents results for the wild-type maltose-binding protein at 25 °C (Liu et al., 1988).

Because the object of our study was to determine which of the steps in folding were affected, we next carried out complete kinetic analyses of the folding transitions of the altered species of maltose-binding protein to determine precisely how each of the folding reactions was affected by the aminoacyl substitutions. Each of the purified mature proteins, either in the native (no denaturant present) or unfolded (2 M guanidinium chloride present) state, was subjected to a rapid change in the concentration of denaturant and the relaxation time to establish a new equilibrium mixture of the native and unfolded states was determined by monitoring the change in the intrinsic fluorescence of tryptophan with time. The results of the kinetic studies of the reversible folding transitions are displayed in Figure 2. The relaxation times for unfolding and refolding as a function of the final concentration of guanidinium chloride connect smoothly at the inflection point for each species of maltose-binding protein, indicating that the refolding reaction is the reverse of the rate-limiting step in unfolding. Since the unfolding reaction has as its starting point the native state, it is reasonable to consider the endpoint of refolding to be the native folded form (Matthews, 1991). Interpretation of the data is therefore based on the assumption that all the species of maltose-binding protein studied refold to the native conformation.

As determined in previous studies of wild-type maltose-binding protein (Liu et al., 1988; Fig. 2), the step that determines the rate of refolding changes at approximately 0.5 M guanidinium chloride. Below that concentration the relaxation time shows no dependence on the final concentration of guanidinium chloride, whereas above 0.5 M the relaxation time increases with the concentration of denaturant. The denaturant-independent folding reaction most likely corresponds to *cis-trans* prolyl isomerization (Kim & Baldwin, 1982; Beasty et al., 1987) whereas that for the denaturant-dependent phase is attributed to steps involving folding of the polypeptide and acquisition of tertiary structural contacts. Extrapolation to 0 M denaturant shows that the rates of the denaturant-dependent folding reaction for the altered proteins are slower by approximately 7- and 40-fold relative to that for the wild-type (rate constants: MalE A276G, 0.24 s^{-1}; MalE W10A, 0.04 s^{-1}; wild-type, 1.7 s^{-1}). In contrast, the rate of the denaturant-independent reaction is not significantly affected (Fig. 2).

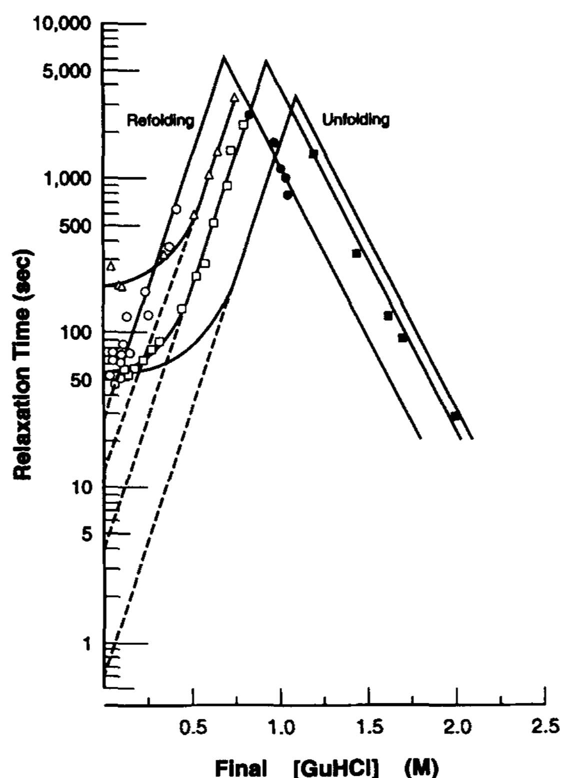

Fig. 2. Relaxation times for unfolding and refolding of maltose-binding protein. Relaxation times for folding (open symbols) and unfolding (closed symbols) were obtained from kinetic studies in the Materials and methods. △, Mature wild-type MalE at 5 °C; ○, mature MalE W10A at 25 °C; □, mature MalE A276G at 25 °C. Line without symbols represents results for the wild-type maltose-binding protein at 25 °C (Liu et al., 1988). Dashed lines represent extrapolation to 0 M guanidinium chloride.

Ability of SecB to block folding of the various species of maltose-binding protein

We evaluated the interaction of SecB with the wild-type and altered species of maltose-binding protein by monitoring the ability of SecB to block the folding of each species (Fig. 3). Consistent with previous observations (Liu et al., 1989), at 21 °C SecB decreased the rate of folding of wild-type, mature, maltose-binding protein, but did not exert a blockage of folding (Fig. 3). When the reaction was carried out at reduced temperature (5 °C) so that both the denaturant-independent and denaturant-dependent folding reactions of the wild-type protein were slowed, SecB was able to exert a blockage in folding (Fig. 3A). In contrast to the situation for the wild-type protein, SecB blocked the folding of MalE W10A at 21 °C. The extent of blockage by SecB observed with MalE W10A at 21 °C was comparable to that observed with the wild-type protein at 5 °C. In the case of MalE A276G, which at 21 °C folds more rapidly than either MalE W10A at 21 °C or wild-type at 5 °C, SecB could not exert a tight blockage, but the rate of folding was drastically reduced (Fig. 3B). It should be noted that to assess the extent of blockage of folding for the wild-type maltose-binding protein and MalE W10A, we considered the folding reaction to be over when the change in fluorescence over 30 min was less than 10% of the total change in fluorescence expected for complete folding. At true equilibrium, one would expect the entire population of each species of maltose-binding protein to be folded because the energy of stabilization of each folded species (varying from −12.8 kcal mol^{-1} for wild type to −8.5 kcal mol^{-1} for MalE W10A) exceeds the energy of stabilization of the complex under the conditions used (calculated using −1.37 kcal mol^{-1} for each order of magnitude above the dissociation constant [K_d = 1 nM], maltose-binding protein at 25 nM, and the highest concentration of SecB tetramer used being 75 nM). However, the effect on the rate of folding is so severe that except in the case of MalE A276G it was not possible to assess the reaction at true equilibrium.

Discussion

The selectivity of SecB for its ligands can be explained in part by a kinetic partitioning (Randall & Hardy, 1986; Hardy & Randall, 1991). SecB has no affinity for native, stably folded polypeptides; thus, polypeptides that fold slowly are favored to bind. Studies of interaction between purified proteins showed that at

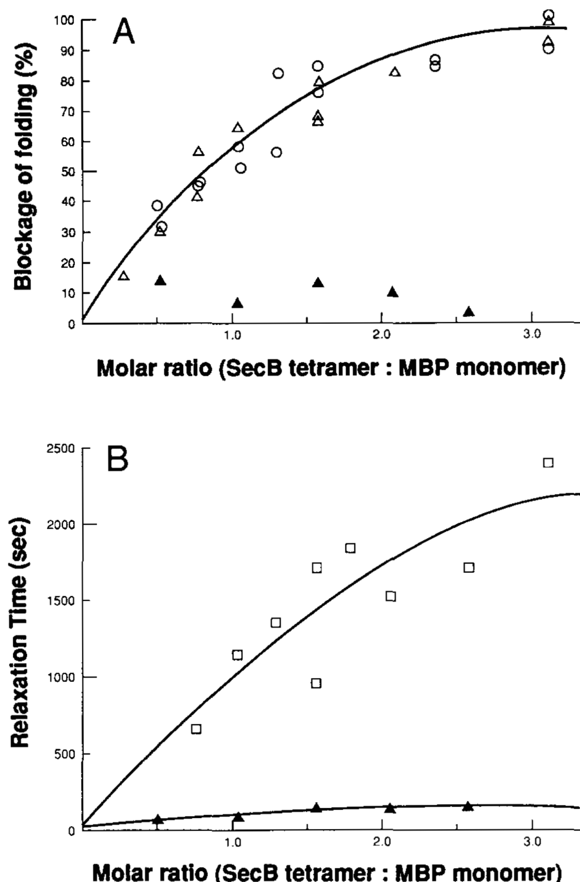

Fig. 3. Effect of SecB on folding of maltose-binding protein. Detailed description of the experiment is given in the Materials and methods. **A:** Blockage of folding. Folding of wild-type maltose-binding protein was assessed at 21 °C and at 5 °C (▲ and △). Blockage of folding of MalE W10A (○) was carried out at 21 °C. **B:** Retardation of rate of folding. Effect of SecB on the rate of folding was carried out at 21 °C. Wild-type maltose-binding protein (▲); MalE A276G (□).

25 °C, rapid folding of the mature species of maltose-binding protein precludes SecB binding; however, the retardation of folding effected by the presence of the leader is sufficient to allow binding of SecB to the precursor under the same conditions (Liu et al., 1989). SecB can also bind the mature protein, which does not carry a leader, if folding is slowed by other means. For example, SecB will bind to wild-type mature maltose-binding protein if the folding reaction is slowed by decreasing the temperature from 25 °C to 5 °C (Liu et al., 1989; Hardy & Randall, 1991; see Fig. 3). It has also been shown that an aminoacyl substitution in the mature portion of maltose-binding protein (MalE Y283D) slows the folding of that polypeptide sufficiently so that SecB can bind at 25 °C (Liu et al., 1989; Hardy & Randall, 1991). It is clear from these examples that slowing folding allows SecB to bind; however, because in each case the rate constants for both of the folding reactions that are resolved kinetically are decreased, we could not determine which of the folding reactions precluded binding of SecB. Here we have addressed this question using the two species of maltose-binding protein, MalE A276G and MalE W10A, that have the rate of only one of the steps decreased relative to that of the wild-type species.

The initial step in folding of maltose-binding protein from the denatured state is a rapid collapse to a compact intermediate that contains a substantial amount of secondary structure as determined by stopped-flow CD (Chun et al., 1993; Topping & Randall, 1994). It has been shown previously that SecB can bind the protein after it has undergone this collapse (Topping & Randall, 1994). However, it is not clear whether the ligand must collapse to allow binding, since when the denaturant is diluted to initiate refolding in the presence of SecB, one cannot determine whether binding precedes or follows the collapse. Of the two remaining phases, which are kinetically resolved, the rate of one is independent of the concentration of denaturant present during refolding, whereas the rate of the other is denaturant dependent. The reaction reflected by the denaturant-independent rate constant is most likely *cis–trans* isomerization of the polypeptide backbone (Kim & Baldwin, 1982; Beasty et al., 1987). The denaturant-dependent folding reaction is believed to involve folding of the polypeptide chain and the acquisition of tertiary contacts. Each of the altered species of maltose-binding protein studied here contains a single amino acid substitution (MalE A276G or MalE W10A) that slows the denaturant-dependent but not the isomerization phase (Fig. 2). At 21 °C, SecB does not exert a blockage of the folding of wild-type maltose-binding protein and has only a small effect on the rate of folding. Under the same conditions, SecB does block the folding of MalE W10A and drastically decreases the rate of folding of MalE A276G (Fig. 3). Therefore, we conclude that the denaturant-dependent reaction is the folding reaction that is relevant for precluding binding of SecB to maltose-binding protein. Once the protein has formed the tertiary contacts characteristic of this reaction, even if the folding intermediate contains an isomer of the backbone not found in the native state, SecB cannot bind. We can eliminate the possibility that the altered amino acids directly affect the binding energy of the complexes since each of the changes lies outside of the binding site for SecB on the polypeptide ligand (Topping & Randall, 1994). We can also be certain that the effects we observe result from the differences in the kinetics of folding and not from the differences in stability among the proteins. Each of the altered proteins retains the ability to bind maltose (K_d 4 μM for MalE W10A, the least stable; Martineau et al., 1990) and the binding of maltose drastically stabilizes the folded state; thus, if the binding to SecB were thermodynamically favored over folding, then the inclusion of maltose in the assay would shift the equilibrium toward the free, folded state. However, there was no effect of maltose on the ability of SecB to block the appearance of folded protein for even the least stable species, MalE W10A (data not shown).

It was previously shown in vivo that a physiologic function of the leader in SecB binding was the retardation of folding of the precursor. In vitro studies revealed that both the isomerization and denaturant-dependent folding reactions were affected by the presence of the leader. We can now specifically attribute the physiologic function of the leader in binding SecB to the interference with the denaturation-dependent reaction. This denaturant-dependent folding reaction, as shown previously by complete kinetic analyses of folding of the wild-type and four altered species of maltose-binding protein, lies on the pathway to the native state in vivo as well as in vitro (Chun et al., 1993). The reaction occurs late in the folding pathway, proceeding through a transition state with structure that is near native (Chun et al., 1993). The structural element associated with this transi-

tion state lies near the amino-terminus of the mature protein (Spurlino et al., 1991). Taken together, these observations suggest that the leader, which retards folding to allow SecB to bind, may do so by interfering with the formation of this element of structure.

We could not determine whether binding of SecB precedes but does not block the rapid collapse of the polypeptide into a compact folding intermediate or whether the binding follows the collapse. However, it is clear that the polypeptide is competent for binding after the initial collapse and remains competent as a ligand until it crosses the final energy barrier to reach the native state. We cannot further define the preferred intermediate if in fact there is one. It is not clear how many intermediates lie along the folding pathway, but none of the intermediates are sufficiently stable to be populated at equilibrium. Because each intermediate would be in rapid equilibrium with all others, even if one intermediate contained elements of structure that favored binding to SecB, binding of that intermediate would pull the equilibrium to repopulate that state. Continued binding by SecB of that intermediate would thereby effect a complete blockage of folding. What is certain is that binding to SecB is not precluded until the polypeptide has crossed the final energy barrier to achieve the native state.

The folding intermediates recognized by some chaperones may lie off the productive pathway as has been proposed for GroEL and GroES by King and colleagues based on their studies of the interaction of the chaperonin with structural proteins of phage P22 (Gordon et al., 1994). Temperature-sensitive folding mutations in the polypeptides were shown to populate intermediates that lie off the productive pathway and were recognized by the chaperonin. The ability to interact with abnormal folding intermediates may reflect the in vivo function of GroEL, which is a heat-shock induced chaperone that facilitates folding by blocking entry into pathways such as aggregation that may occur at elevated temperature or under conditions of stress. In contrast, SecB appears to interact with intermediates that lie along the normal pathway of folding. This conclusion is supported by the ability of SecB to bind the wild-type maltose-binding protein if its folding is slowed by decreasing the temperature of the reaction, which is not expected to favor off-pathway intermediates. In addition, the amino acid substitutions that slow folding to allow binding do not appear to populate intermediates that lie off the productive pathway, but rather change the rate constant of the folding reaction from a near-native intermediate to the final folded state (Chun et al., 1993). That SecB binds intermediates that lie along the normal pathway of folding is consistent with its role in vivo to capture polypeptides before they fold and direct them into the export pathway.

Materials and methods

Materials

Guanidinium chloride (ultrapure) was purchased from Schwarz/Mann and HEPES from Sigma. Amylose resin was purchased from New England Biolabs.

Protein purification

The matured forms of wild-type maltose-binding protein, MalE A276G and MalE W10A, were purified by affinity chromatography using an amylose resin as described (Liu et al., 1988). The *E. coli* K12 strain used as the source of wild-type MalE protein was MC4100 (*F⁻ΔlacU169 araD139 rpsL150 thi flbB5301 deoC ptsF25 relA1*) (Casadaban, 1976). The plasmid carrying *malE A276G* is a derivative of pBAR43 (Rasmussen et. al, 1985) and the plasmid carrying *malE W10A* is a derivative of pPD386 (Martineau et al., 1990). The host strain for the plasmid carrying *malE A276G* is BAR1091, a derivative of MC4100 that carries a deletion in the *malE* gene (*ΔmalE312*; Fikes & Bassford, 1987). The host strain for the plasmid carrying *malE W10A* is ED9, which is a derivative of MC4100 that carries a deletion in the chromosomal *malE* (*ΔmalE 444 rec*A1 *Srl*::Tn10; Martineau et al., 1990). SecB was purified using the cell strain and procedure described by Weiss et al. (1988), with the following modification. The second gel-filtration chromatography was replaced by ion-exchange chromatography with a Pharmacia Mono Q HR 5/5 column in 10 mM Tris-HCl, pH 7.6, with a linear 150-mL gradient of 0–0.6 M NaCl.

Fluorescence spectroscopy

The parameter used to monitor the denaturant-induced unfolding or refolding of the maltose-binding protein species was the change in intrinsic fluorescence of tryptophan. Fluorescence measurements were made using a Shimadzu RF-540 fluorescence spectrophotometer with an excitation wavelength of either 280 nm (for maximal absorption by tryptophan) or 295 nm (to minimize the contribution of tyrosine fluorescence to the emission spectra) and an emission wavelength of 344 nm. The excitation and emission bandwidths were 2 nm and 5 nm, respectively. All fluorescence measurements were made at 25 °C unless otherwise noted.

Equilibrium studies of folding

Native proteins (4–15 μg) were added to varying concentrations of guanidinium chloride in 3 mL of 10 mM HEPES (pH 7.6–7.8 adjusted with KOH), and the fluorescence intensity of each sample was measured after equilibration at 25 °C. The final concentration of guanidinium chloride in each sample was confirmed by measuring the index of refraction.

Kinetic studies of folding

The concentration of guanidinium chloride as indicated for each experiment was confirmed by measuring the index of refraction. For unfolding reactions, the native proteins (final concentration of 1–5 μg/mL) were added to varying concentrations of guanidinium chloride in 10 mM HEPES, pH 7.5–7.8, held in a stirred cuvette in the chamber of the spectrophotometer. The addition of protein was made manually with a plastic plunger and the time that lapsed between addition of the protein to the cuvette and the first reading of fluorescence was approximately 5–10 s. For refolding transitions, each MalE species was unfolded by incubation for at least 2 h at room temperature in 2 M guanidinium chloride buffered with 10 mM HEPES (pH 7.5–7.8). Refolding was initiated by dilution of the denatured protein and buffered guanidinium chloride such that the final protein concentration would be 1–5 μg/mL and the final guanidinium chloride concentration would be as indicated for each experiment.

The relaxation time to achieve the new equilibrium was extracted from a plot of the log of the change in fluorescence versus time.

Blockage of folding of maltose-binding protein by SecB

Denatured mature maltose-binding protein species were refolded by dilution into a volume of 10 mM HEPES (pH 7.5–7.8) held in a cuvette in the chamber of the spectrophotometer so that the final concentrations were: maltose-binding protein, 25 nM, and guanidinium chloride, 0.03 M. When SecB was present, it was added to the solution held in the cuvette before the maltose-binding protein was added. For determination of the molar ratio of the SecB tetramer to maltose-binding protein, the protein concentrations were determined using an extinction coefficient of 11,900 M^{-1} cm^{-1} for SecB monomer and the method of Lowry et al. (1951) for maltose-binding protein. The magnitude of the blockage of folding caused by SecB was calculated from a comparison of the change in fluorescence in the presence of SecB with the same parameter determined in the absence of SecB.

Acknowledgments

We thank Virginia F. Smith for assistance in the purification of SecB and Traci B. Topping for purification of MalE A276G, determination of the extinction coefficient for SecB and for critically reading the manuscript. We are grateful to K. Dane Wittrup for making the initial observations that led to this study and Maurice Hofnung for providing the strain producing MalE W10A. This work was supported by grant GM29798 to L.L.R. from the National Institutes of Health.

References

Beasty AM, Hurle M, Manz JT, Stackhouse T, Matthews CR. 1987. Mutagenesis as a probe of protein folding and stability. In: Oxender D, Fox CF, eds. *Protein engineering*. New York: Alan R Liss, Inc. pp 103–108.
Casadaban MJ. 1976. Transposition and fusion of the lac genes to selected promoters in *Escherichia coli* using bacteriophage lambda and mu. *J Mol Biol 104*:541–555.
Chun SY, Strobel S, Bassford PJ Jr, Randall LL. 1993. Folding of maltose-binding protein. Evidence for the identity of the rate-determining step in vivo and in vitro. *J Biol Chem 268*:20855–20862.
Crooke E, Wickner W. 1987. Trigger factor: A soluble protein that folds pro-OmpA into a membrane-assembly-competent form. *Proc Natl Acad Sci USA 84*:5216–5220.
Eilers M, Schatz G. 1986. Binding of a specific ligand inhibits import of a purified precursor protein into mitochondria. *Nature 322*:228–232.
Ellis RJ, van der Vies SM. 1991. Molecular chaperones. *Annu Rev Biochem 60*:321–347.
Fikes JD, Bassford PJ Jr. 1987. Export of unprocessed precursor maltose-binding protein to the periplasm of *Escherichia coli* cells. *J Bacteriol 169*:2352–2359.
Gordon CL, Sather SK, Casjens S, King J. 1994. Selective in vivo rescue by GroEL/ES of thermolabile folding intermediates to phage P22 structural proteins. *J Biol Chem 269*:27941–27951.
Hardy SJS, Randall LL. 1991. A kinetic partitioning model of selective binding of non-native proteins by the bacterial chaperone SecB. *Science 251*: 439–443.
Hartl FU, Lecker S, Schiebel E, Hendrick JP, Wickner W. 1990. The binding cascade of SecB to SecA to SecY/E mediates preprotein targeting to the *E. coli* plasma membrane. *Cell 63*:269–279.
Kim PS, Baldwin RL. 1982. Specific intermediates in the folding reactions of small proteins and the mechanism of protein folding. *Annu Rev Biochem 51*:459–489.
Kumamoto CA. 1989. *Escherichia coli* SecB protein associates with exported protein precursors in vivo. *Proc Natl Acad Sci USA 86*:5320–5324.
Kumamoto CA, Beckwith J. 1983. Mutations in a new gene, SecB, cause defective protein localization in *Escherichia coli*. *J Bacteriol 154*:253–260.
Kumamoto CA, Beckwith J. 1985. Evidence for specificity at an early step in protein export in *Escherichia coli*. *J Bacteriol 163*:267–274.
Kumamoto CA, Chen L, Fandl J, Tai PC. 1989. Purification of the *Escherichia coli* SecB gene product and demonstration of its activity in an in vitro protein translocation system. *J Biol Chem 264*:2242–2249.
Kumamoto CA, Francetić O. 1993. Highly selective binding of nascent polypeptides by an *Escherichia coli* chaperone protein in vivo. *J Bacteriol 175*: 2184–2188.
Kumamoto CA, Gannon PM. 1988. Effects of *Escherichia coli* SecB mutations on pre-maltose binding protein conformation and export kinetics. *J Biol Chem 263*:11554–11558.
Kusters R, de Vrije T, Breukink E, de Kruijff B. 1989. SecB protein stabilizes a translocation-competent state of purified prePhoE protein. *J Biol Chem 264*:20827–20830.
Lecker S, Lill R, Ziegelhoffer T, Georgopoulous C, Bassford PJ Jr, Kumamoto CA, Wickner W. 1989. Three pure chaperone proteins of *Escherichia coli*-SecB, trigger factor and GroEL-form soluble complexes with precursor proteins in vitro. *EMBO J 8*:2703–2709.
Liu G, Topping TB, Cover WH, Randall LL. 1988. Retardation of folding as a possible means of suppression of a mutation in the leader sequence of an exported protein. *J Biol Chem 263*:14790–14793.
Liu G, Topping TB, Randall LL. 1989. Physiological role during export for the retardation of folding by the leader peptide of maltose-binding protein. *Proc Natl Acad Sci USA 86*:9213–9217.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. *J Biol Chem 193*:265–275.
Maher PA, Singer SJ. 1986. Disulfide bonds and the translocation of proteins across membranes. *Proc Natl Acad Sci USA 83*:9001–9005.
Martineau P, Szmelcman S, Spurlino JC, Quiocho FA, Hofnung M. 1990. Genetic approach to the role of tryptophan residues in the activities and fluorescence of a bacterial periplasmic maltose-binding protein. *J Mol Biol 214*:337–352.
Matthews CR. 1991. The mechanism of protein folding. *Curr Opin Struct Biol 1*:28–35.
Meyer DI. 1988. Preprotein conformation: The year's major theme in translocation studies. *Trends Biochem Sci 13*:471–474.
Park S, Liu G, Topping TB, Cover WH, Randall LL. 1988. Modulation of folding pathways of exported proteins by the leader sequence. *Science 239*:1033–1035.
Randall LL, Hardy SJS. 1986. Correlation of competence for export with lack of tertiary structure of the mature species: A study in vivo of maltose-binding protein in *E. coli*. *Cell 46*:921–928.
Rasmussen BA, MacGregor CH, Ray PH, Bassford PJ Jr. 1985. In vivo and in vitro synthesis of *Escherichia coli* maltose-binding protein under regulatory control of the lacUV5 promoter-operator. *J Bacteriol 164*: 665–673.
Rothman JE, Kornberg RD. 1986. An unfolding story of protein translocation. *Nature 322*:209–210.
Spurlino JC, Lu GY, Quiocho FA. 1991. The 2.3-Å resolution structure of the maltose- or maltodextrin-binding protein, a primary receptor of bacterial active transport and chemotaxis. *J Biol Chem 266*:5202–5219.
Topping TB, Randall LL. 1994. Determination of the binding frame within a physiological ligand for the chaperone SecB. *Protein Sci 3*:730–736.
Verner K, Schatz G. 1987. Import of an incompletely folded precursor protein into isolated mitochondria requires an energized inner membrane, but no added ATP. *EMBO J 6*:2449–2456.
Weiss JB, Ray PH, Bassford PJ Jr. 1988. Purified SecB protein of *E. coli* retards folding and promotes membrane translocation of maltose-binding protein in vitro. *Proc Natl Acad Sci USA 85*:8978–8982.