JOURNAL OF BACTERIOLOGY, June 1995, p. 3277–3282 Vol. 177, No. 11
0021-9193/95/$04.00+0

Demonstration In Vivo that Interaction of Maltose-Binding Protein with SecB Is Determined by a Kinetic Partitioning

VIJAYA J. KHISTY AND LINDA L. RANDALL*

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

Received 7 November 1994/Accepted 23 March 1995

An early step in the export of maltose-binding protein to the periplasm is interaction with the molecular chaperone SecB. We demonstrate that binding to SecB in vivo is determined by a kinetic partitioning between the folding of maltose-binding protein to its native state and its association with SecB. A complex of SecB and a species of maltose-binding protein that folds slowly is shown to be longer-lived than a complex of the wild-type maltose-binding protein and SecB. In addition, we show that incomplete nascent chains, which are unable to fold, remain complexed with SecB.

SecB is one of a group of chaperone proteins, which includes GroEL, GroES, DnaK, and DnaJ, that are involved in enhancing the export of a subset of proteins to the periplasmic space or to the outer membrane in *Escherichia coli* (1, 4, 10, 12, 17, 28). SecB seems to be the only one of the chaperones that is dedicated to export. SecB mediates entry into the export pathway for the precursors it binds through its affinity for SecA, a component of the membrane-associated translocation apparatus (7). In addition, SecB has an equally important role in maintaining the precursors in a conformational state that is competent for transfer across the cytoplasmic membrane (19, 27). For the periplasmic maltose-binding protein, SecB binds before the polypeptide attains its final native conformation and blocks folding (14). For other precursors, such as the precursor of the outer membrane protein PhoE, formation of a complex with SecB prevents aggregation, which would preclude export (5).

It has been proposed previously, on the basis of studies of SecB and its ligands both in vivo and in vitro, that SecB distinguishes those proteins that it binds from those that it does not by virtue of their intrinsic folding rates (6, 14). The polypeptide ligand partitions between the folded state and the state associated with SecB in a manner that is dependent on the rate of folding relative to the rate of association with the chaperone. To demonstrate directly that a kinetic partitioning occurs in vivo, we compared the lifetimes of complexes between SecB and two species of maltose-binding protein that fold at different rates, the wild-type species and a species carrying an aminoacyl substitution that drastically reduces the rate of folding (13). We show that, in accord with the notion of a kinetic partitioning, the complex between SecB and the slowly folding maltose-binding protein is longer-lived than that between the wild-type maltose-binding protein and SecB. In addition, incomplete nascent polypeptide chains, which one would not expect to fold, remain complexed with SecB.

MATERIALS AND METHODS

Materials. [^{35}S]methionine (approximately 1,000 Ci/mmol) was purchased from New England Nuclear, and ImmunoPure immobilized protein A was obtained from Pierce Chemical Company. Goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase was purchased from Bio-Rad Laboratories. All other chemicals and enzymes were from Sigma Chemical Company.

Bacterial strains. The strains used are all derivatives of the *E. coli* K-12 strain MC4100 (F$^-$ *lacU169 araD139 rpsL150 thi flbB5301 deoC7 ptsF25 relA1*) (2). Both plasmids used in this study were constructed from plasmid pBAR43 which carries the *malE* allele under the control of the *lacUV5* promoter-operator (22). The *malE* allele on plasmid pGL1 encodes a slowly folding species of maltose-binding protein without the leader (MalE Δ2-26 Y283D) (14), and the *malE* allele on plasmid pUZ226 encodes the wild-type maltose-binding protein without the leader (MalE Δ2-26) (26). The plasmids were introduced into strain BAR1091 (F$^-$ *araD139 rpsL150 thi flbB5301 deoC7 ptsF25 relA1 malE* Δ*312 lacI*^{q}/F′ *lacI*^{q} Tn*5*), which carries a deletion in the chromosomal *malE* allele (22).

Labeling with [^{35}S]methionine. Cell cultures were grown at 30°C in M9 salts (15) supplemented with thiamine hydrochloride (2 μg/ml) and glycerol (0.4%). When the culture reached an optical density at 560 nm of approximately 0.5 (2.5 × 10^8 cells per ml), isopropylthiogalactoside (IPTG) was added to 5 μM for expression of MalE Δ2-26 or to 10 μM for expression of MalE Δ2-26 Y283D. Growth was continued for 30 min before labeling to allow the plasmid-encoded species of maltose-binding protein to reach levels of expression equivalent to that of the chromosomally encoded allele in MC4100 when it is induced by maltose. In other experiments in which maximal expression of maltose-binding protein was desired, 30 min before labeling, IPTG was added to 0.5 mM. The cells were labeled with 60 μCi of [^{35}S]methionine per ml of culture at a specific activity of 0.75 Ci/mol. Fifteen seconds after the addition of the isotope, the specific activity was reduced 625-fold by the addition of nonradioactive methionine. To label cells for analysis of nascent polypeptide chains, 15 s after the addition of the isotope, chloramphenicol was added to a final concentration of 100 μg/ml. In both experimental designs, at specified times following dilution of the isotope or termination of protein synthesis, 1 ml of the culture was transferred to a thin-walled glass tube held in an ice-water bath. The temperature dropped to 5°C within 10 s. The chilled cells were harvested by centrifugation and converted to spheroplasts as described previously (19). The spheroplasts were lysed by suspension in a mixture of 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 150 mM potassium acetate, and 0.5% Tween 20 (pH 7.6). The samples were subjected to sonication on ice for 1.5 min in a cuphorn sonicator (Tekmar) to shear the DNA.

Immunoprecipitation. To determine the total amount of maltose-binding protein in each extract, a portion of the sample was brought to 0.5% sodium dodecyl sulfate (SDS) in 10 mM Tris-acetate (pH 7.6) and heated for 10 min at 60°C. One milliliter of 10 mM Tris-acetate–1% Triton X-100 (pH 7.6) was added, and the sample was centrifuged for 15 min at 16,000 × *g* in an Eppendorf centrifuge. A saturating amount of antiserum to maltose-binding protein bound to ImmunoPure immobilized protein A was added to the supernatant, and the mixture was incubated on ice for 2 to 4 h with occasional shaking. The sample was centrifuged for 4 s at 16,000 × *g* in an Eppendorf centrifuge, and the supernatant was discarded. The agarose beads were collected by centrifugation as before and washed twice: first with 1 ml of a solution containing 10 mM Tris-acetate, 0.15 M NaCl, 0.1% SDS, 0.5% Triton X-100, and 5 mM EDTA, pH 7.6, and second with 1 ml of 10 mM Tris-acetate, pH 7.6. The beads were suspended in 30 μl of sample buffer for SDS-polyacrylamide gel electrophoresis (18). To determine the amount of maltose-binding protein in complex with SecB, immunoprecipitation was carried out under nondenaturing conditions. A portion of the lysate was diluted to 1 ml with the buffer used for lysis, and antiserum to SecB bound to ImmunoPure immobilized protein A was added. The mixture was incubated on ice and processed as described above except that the beads were washed twice with 1 ml of the buffer used for lysis. Polypeptides were removed from the

* Corresponding author. Mailing address: Department of Biochemistry and Biophysics, Washington State University, Pullman, WA 99164-4660. Phone: (509) 335-6398. Fax: (509) 335-9688.

protein A-agarose beads by incubation in the sample buffer for SDS-polyacrylamide gel electrophoresis for 5 min at 100°C.

Analyses of complexes formed in vitro. Mature wild-type MalE, mature MalE Y283D (the slowly folding species), and SecB were purified as described previously (3, 21). The two species of maltose-binding protein were unfolded by incubation in 10 mM Tris-HCl, pH 7.6, containing 2 M guanidinium chloride (GuHCl) at room temperature for 1 h. Complexes between SecB and the two species of maltose-binding protein were formed by diluting the denaturant from the unfolded maltose-binding protein into a tube containing SecB held on ice so that the final concentrations were 1.5 μM SecB tetramer in 20 mM HEPES–150 mM potassium acetate–0.1 M GuHCl, pH 7.0. Each species of maltose-binding protein was present at 3 μM. When the two different species of maltose-binding protein were present in the complex, the order of addition was varied. Between 10 and 12 s elapsed between additions in any one experiment. Immediately following formation of the complexes, 200 μl of the solution was analyzed by size exclusion high-performance liquid chromatography (HPLC) with a TSK3000.SW column in 20 mM HEPES–150 mM potassium acetate, pH 7.0, at a flow rate of 1 ml/min. Chromatography was carried out at 10°C to retard the folding of the ligands. The protein was precipitated in successive 1-ml fractions, starting after the elution of 11 ml, by the addition of trichloroacetic acid to 8%; the precipitates were collected by centrifugation, washed with acetone, suspended in sample buffer, and analyzed by electrophoresis on SDS-polyacrylamide gels. The gels were stained with Coomassie brilliant blue.

Polyacrylamide gel electrophoresis and limited proteolysis. Electrophoresis on 14% polyacrylamide gels was carried out as described previously (18). For autoradiography, Kodak XAR-5 film was applied to the stained, dried gels. The bands corresponding to MalE polypeptides on either the stained gels or the autoradiograms were quantified by scanning densitometry with a Helena Laboratories Quick Scan R&D. To detect nascent chains, the lane containing polypeptides derived either from immunoprecipitation of maltose-binding protein or from immunoprecipitation of the complex was cut out and placed lengthwise across the top of a second SDS–15% polyacrylamide gel and subjected to limited proteolysis followed by electrophoresis and fluorography as described by Josefsson and Randall (8).

Quantification. The efficiency of immunoprecipitation of the specific ligand by an antiserum either to maltose-binding protein or to SecB under both denaturing and nondenaturing conditions was determined as follows. The amount of each protein species in the total sample and in the immunoprecipitate was determined by immunoblotting. The immunoprecipitated proteins and a portion of the total sample were transferred from SDS-polyacrylamide gels to nitrocellulose. The specific proteins were detected by treating the nitrocellulose with the appropriate antiserum (either to maltose-binding protein or to SecB) followed by goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase and 4-chloro-1-naphthol (16). The efficiencies of immunoprecipitation were shown to be 10% for all cases.

Since the efficiencies of immunoprecipitation were the same, the proportion of radiolabeled maltose-binding protein in the extracts that was initially present in the complex with SecB could be estimated by a direct comparison of the autoradiograms for immunoprecipitation under native conditions by using antiserum to SecB (the amount in the complex) with the autoradiograms for immunoprecipitation under denaturing conditions by using antiserum to maltose-binding protein (the total amount in the extract). All quantifications were done with autoradiograms having exposures such that the intensities of the bands or spots were in the linear range.

RESULTS AND DISCUSSION

Our working model describes the localization of maltose-binding protein as a partitioning of the unfolded precursor between folding in the cytosol and export to the periplasm, where it folds to the matured, native state (Fig. 1A). The chaperone SecB binds the newly synthesized precursor, thereby keeping it competent for transfer across the cytoplasmic membrane and favoring the export pathway over the folding pathway. It has been shown in vitro that binding of purified maltose-binding protein to SecB involves a partitioning that depends on the rate of folding ($[P_u]k_f$) relative to the rate of binding to SecB ($[P_u][B]k_{on}$), thus, a kinetic partitioning. We do not observe this initial partitioning in normally functioning cells because there is negligible flux through the folding pathway: partitioning is poised to favor the formation of the complex, and the precursor is rapidly exported. To demonstrate that the amount of precursor in complex with SecB in vivo is a function of the rate constant of folding (k_f) relative to the pseudo first-order rate constant of binding to SecB ($[B]k_{on}$), it is necessary to eliminate the complication of the disappearance of the complex through the export pathway, so that we can

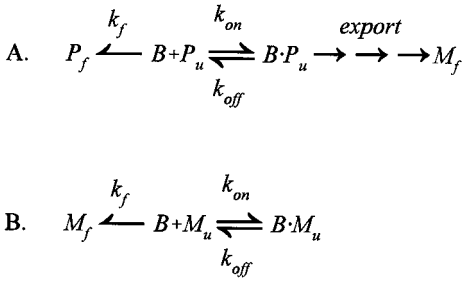

FIG. 1. Kinetic partitioning of maltose-binding protein. (A) During normal export, the unfolded precursor of maltose-binding protein (P_u) partitions between the folded state in the cytosol (P_f) and the matured, folded state in the periplasm (M_f). (B) Removal of the leader peptide blocks the export pathway, and the mature, unfolded maltose-binding protein (M_u) partitions in the cytosol between the folded state (M_f) and the state complexed with SecB ($B \cdot M_u$). The relevant rate constants are the rate constant of folding (k_f), the rate constant of association (k_{on}), and the rate of constant of dissociation (k_{off}).

observe partitioning between the folding and the formation of a complex in isolation (Fig. 1B). It was possible to achieve this by removing the leader sequence from maltose-binding protein, since although the leader is essential for translocation (23), it has only an indirect role in binding of the precursor to SecB. The leader retards the folding of the polypeptide, thereby increasing the probability that SecB can bind (14, 21).

To demonstrate that the rate constant of folding comes into play during a kinetic partitioning, we compared the lifetimes of complexes containing SecB and two species of mature maltose-binding protein that fold at different rates: the wild type and a species carrying an aminoacyl change, tyrosine substituted for an aspartate at position 283, that drastically slows the folding of the protein (13). The complexes were isolated from extracts of cells that had been labeled for 15 s with [^{35}S]methionine by immunoprecipitation under nondenaturing conditions with an antiserum to SecB. Analysis by SDS-polyacrylamide gel electrophoresis and autoradiography showed that maltose-binding protein was the predominant species among those polypeptides isolated in complex with SecB (Fig. 2), which is in agreement with previous observations by Kumamoto and Francetić (11). As expected, the preimmune serum did not precipitate either SecB or maltose-binding protein. Initially, a large proportion of each species of maltose-binding protein that was radiolabeled during the 15-s incubation was found bound to SecB, whether it was the wild type (ranging from 30 to 100%) or the species that folds slowly (ranging from 50 to 70%) (see Materials and Methods for details). The radiolabeled maltose-binding protein disappeared from the complex, with a half-life of 9 min for the slowly folding species compared with a half-life of 2 min for the wild-type species (Fig. 3A). The half-lives of the complexes did not vary with the cellular concentrations of the components. The same half-lives were observed whether the two species of maltose-binding protein were expressed at levels corresponding to the amount of the wild-type protein expressed from the chromosomal gene or at maximally induced levels. Under the conditions of full induction, which corresponded to between four- and eightfold more maltose-binding protein relative to expression from the chromosomal gene (Fig. 3B), SecB appeared to be saturated, since 10% or less of the newly synthesized maltose-binding protein was bound. The variation in the amount of SecB in all experiments and among all strains never exceeded threefold. The lack of effect of variation in the amount of the proteins on the lifetimes of the complexes is as expected, since one would see an effect only if the cellular concentrations of the components of the complex

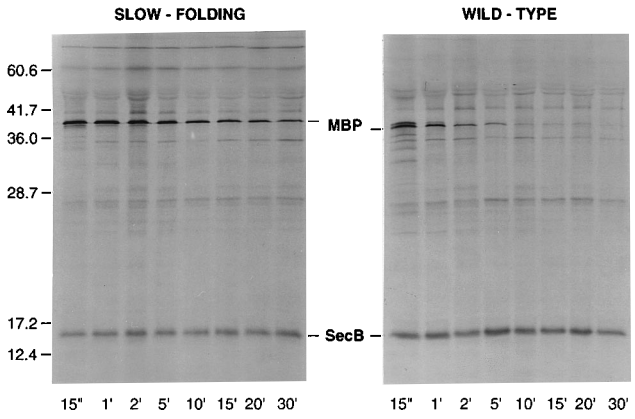

FIG. 2. Disappearance of MalE polypeptides from the complex with SecB. Exponentially growing cultures were radiolabeled, samples were taken at the times indicated, complexes were isolated, and polypeptides were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography as described in Materials and Methods. The positions of the MalE polypeptides (MBP) and SecB are indicated. Numbers at the left are molecular masses (in kilodaltons) of the following standards: α-amylase (60.6 kDa), actin (41.7 kDa), glyceraldehyde-3-phosphate-dehydrogenase (36.0 kDa), carbonic anhydrase (28.7 kDa), myoglobin (17.2 kDa), and cytochrome *c* (12.4 kDa).

were poised near the value of the dissociation constant. The concentrations of SecB and the two species of maltose-binding protein in the cell are in the micromolar range (9, 25), whereas the dissociation constant of the complex is in the nanomolar range (6).

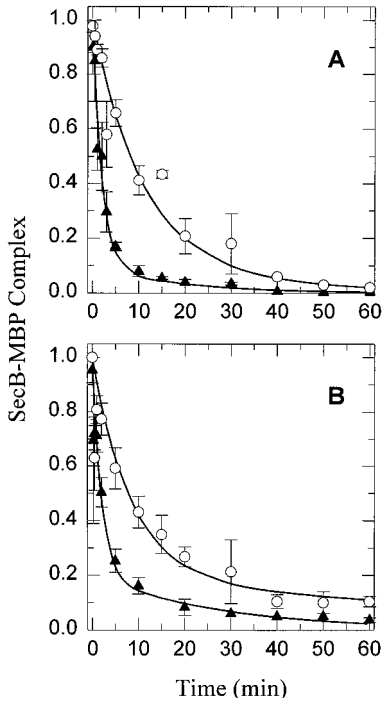

FIG. 3. Half-lives of complexes comprising SecB and two species of maltose-binding protein (MBP). Data from experiments similar to those whose results are shown in Fig. 2 were quantified by scanning densitometry as described in Materials and Methods. (A) Expression of MalE polypeptides at levels corresponding to that of the wild-type protein expressed from the chromosome. (B) Maximal expression of the MalE polypeptides. ○, slowly folding species of maltose-binding protein; ▲, wild-type maltose-binding protein. Standard errors are shown by error bars.

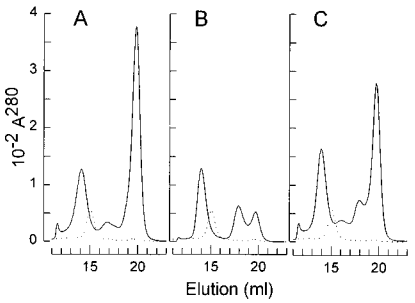

FIG. 4. Absorbance profile of proteins resolved by size exclusion chromatography. HPLC of protein mixtures was carried out as described in Materials and Methods. (A) Complex with wild-type maltose-binding protein. (B) Complex with slowly folding maltose-binding protein. (C) Complex with wild-type and slowly folding maltose-binding protein. In each case, the dotted line represents SecB only (20 μg applied) and the solid line represents the mixture of SecB and unfolded maltose-binding protein at a ratio of 24 μg of maltose-binding protein per species to 20 μg of SecB. When both species of maltose-binding protein were present, they were added simultaneously.

The observed correlation of the half-lives of the complexes with the rate of folding of the ligand is consistent with the hypothesis that binding to SecB is determined by a kinetic partitioning. Other models that could provide a rationale for the correlation between the rate of folding and the rate of disappearance of the maltose-binding protein from the complex with SecB might be envisioned. For example, the polypeptides might fold at different rates while bound to SecB and be released only when they have attained their native state. In this type of model, the differences in the lifetimes of the complexes in vivo would reflect different rate constants for dissociation. Once dissociated, the polypeptide would be unable to rebind since it would be folded. Such a model and the model based on a kinetic partitioning of the ligand between binding and folding are easily distinguished in vitro by examining the recovery of the two species of maltose-binding protein in complex with SecB when they are added to the SecB in different orders. If, as proposed in the model that evokes a kinetic partitioning, the polypeptides bound to SecB are in equilibrium with a pool of unfolded, free polypeptides, then the same ratio of the two species will be recovered in complex with SecB, regardless of the order of addition. The actual ratio that is observed will depend on the relative rates at which the polypeptides exit the pool of free ligands, which are in turn determined by the rates of folding. In the alternative model, the lifetime of the complex is determined only by the rate of dissociation, and the ligand does not occur in the free state. Thus, if one species were added to SecB before the other, it would be the predominant species recovered in complex with SecB. The results of an analysis in which the wild-type polypeptide and the species which folds slowly were added to SecB either at the same time or one before the other support the model of kinetic partitioning. When unfolded, wild-type maltose-binding protein was added in a twofold molar excess over the SecB tetramer and the mixture was analyzed by size exclusion HPLC, the profile of the A_{280}s of the proteins eluted from the column (Fig. 4A) showed that SecB was saturated and well resolved from the free maltose-binding protein. The addition of mature unfolded MalE Y283D at a twofold excess also resulted in saturation of SecB (Fig. 4B). It should be noted that whereas the great majority of the uncomplexed wild-type maltose-binding protein refolded during the time of elution of the column and was recovered as a single peak at the position characteristic of folded maltose-binding protein, the uncomplexed slowly fold-

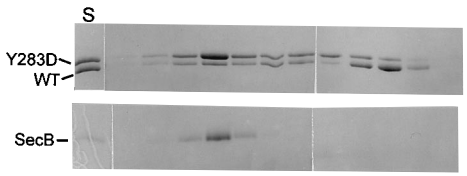

FIG. 5. Binding of wild-type and slowly folding maltose-binding protein to SecB. Equal amounts of the two species of maltose-binding protein were unfolded and added simultaneously to a limiting quantity of SecB. The mixture, which contained 20 μg of SecB and 24 μg of each species of maltose-binding protein, was subjected to size exclusion chromatography (see Fig. 4C for absorbance profile). SDS–14% polyacrylamide gel electrophoresis of trichloroacetic acid precipitates of successive 1-ml fractions, starting after the elution of 11 ml, was carried out. Shown are only the regions of the Coomassie blue-stained gel containing SecB and the two species of mature maltose-binding protein: slowly folding (Y283D) and wild type (WT). Lane S contains 10% of the quantity of the sample applied to the column. One-quarter of the proteins precipitated from each of the first seven fractions shown was applied to the gel, whereas one-eighth of the proteins precipitated from the fractions thereafter was applied.

ing species was recovered in two peaks, with the unfolded species eluting earlier than the fully folded form. When the two unfolded species were mixed and added simultaneously to SecB, the amount of material recovered in complex with SecB, as determined by the A_{280}, was the same as when either species was added alone (Fig. 4C), which is consistent with the conclusion that SecB is saturated. Analysis of the protein content of each fraction indicated that mature MalE Y283D was recovered preferentially (Fig. 5). That this preferential recovery of the slowly folding species is the result of a partitioning between bound and free forms of the ligand and does not result from differences in the rate constants of dissociation is shown by the fact that the slowly folding species and the wild-type species were recovered in complex with SecB in the same ratio whether the ligands were added simultaneously (ratio of 3.5 for mature MalE Y283D to mature wild-type MalE [Fig. 6, lane 5]) or the slowly folding species was added before (ratio of 3.4 [Fig. 6, lane 4]) or after (ratio of 3.2 [Fig. 6, lane 3]) the wild-type protein. Thus, in vitro the polypeptides are in rapid equilibrium between the free and bound forms. In vivo SecB can form complexes with polypeptides while they are still in the process of elongation on the ribosome. Although we cannot directly demonstrate that binding of nascent polypeptide chains is also in rapid equilibrium, this seems to be likely. At least when the chain is fully elongated in vivo, the binding should be reflected by the in vitro study described here. Thus, we conclude that the difference in the lifetimes of the complexes observed in vivo does reflect a kinetic partitioning. The unfolded maltose-binding protein in vivo is in rapid equilibrium between the bound and the free state. Upon each dissociation of the complex, the polypeptide either rebinds or folds with a probability that is proportional to the rate constant for binding relative to the rate constant for folding. The partitioning continues until the entire population has folded, since the free energy of stabilization of the folded protein (approximately −10 kcal/mol (1 cal = 4.184 J) for wild-type maltose-binding protein and −7 kcal/mol for the slowly folding species) (3) exceeds the energy of stabilization of the complex, which is estimated to be approximately −4 kcal/mol (assuming that the cellular concentrations of the components are 3 orders of magnitude above the dissociation constant and each order of magnitude provides −1.37 kcal/mol). The folding reaction is essentially irreversible, and the concentrations of the components are well above the dissociation constant for binding to SecB; thus, if kinetic partitioning determines binding, the decay of the complex should be proportional to the rate constant of folding of the ligand, as was observed. The interpretation that the observed differences in the half-lives of the complex reflect the differences in the rates of folding assumes that the affinities of the species for SecB are the same. This is likely to be the case, because the mutational change responsible for the altered folding rate (Y283D) lies outside the region on the maltose-binding protein identified as the binding site for SecB (24).

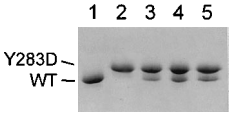

FIG. 6. Mature wild-type and slowly folding maltose-binding protein in complex with SecB. The wild-type (WT) and/or the slowly folding (Y283D) maltose-binding protein was unfolded and added to a limiting amount of SecB. The mixtures were subjected to size exclusion chromatography, and 1-ml fractions were collected and analyzed as described in the legend to Fig. 5. Shown here are the fractions from each column which contained the majority of the complex. One quarter of the protein present in each of the fractions was subjected to SDS gel electrophoresis so that the Coomassie blue-stained bands would be in the linear range of sensitivity of the densitometer used for quantification. The unfolded mature maltose-binding protein was added to SecB to form the complexes as follows: lane 1, wild-type MalE only; lane 2, MalE Y283D only; lane 3, wild-type MalE followed by MalE Y283D; lane 4, MalE Y283D followed by wild-type MalE; lane 5, simultaneous addition of wild-type MalE and MalE Y283D. Only the region of the Coomassie blue-stained 10% polyacrylamide gel that contains the two species of maltose-binding protein is shown.

To obtain further support for the notion that the lifetime of the complex reflects the rate of folding of the ligands, we examined complexes comprising SecB and incomplete nascent polypeptides, which are not expected to fold and which were shown previously to bind (11). To trap the polypeptides while nascent, chloramphenicol was added to the growing cell cultures 15 s after the addition of [^{35}S]methionine and the complexes were isolated by immunoprecipitation with antiserum to SecB under nondenaturing conditions. Nascent polypeptides cannot be detected after separation on the basis of molecular weight by SDS-gel electrophoresis since the population is heterogeneous with respect to length. Accordingly, we made use of a technique that includes a second step of electrophoresis combined with limited proteolysis (8). The second-dimensional gel simultaneously displays peptides derived from the fully elongated protein and peptides derived from the incomplete, nascent polypeptides that migrated in the first-dimensional gel at positions of molecular weights lower than those of the completed polypeptides. Using this analysis, we could detect the presence of nascent chains in the complex (Fig. 7). The nascent polypeptides remained bound to SecB, as indicated by the persistence over the duration of the experiment of peptides that appear as horizontal or diagonal streaks and are characteristic of nascent species. In contrast, the fully elongated protein disappeared from the complex, as indicated by the disappearance of the peptides that appeared as spots in a vertical array in the second-dimensional gel directly below the position of the fully elongated protein in the first dimension of electrophoresis. It is interesting that the presence of chloramphenicol increased the apparent half-life of the complex comprising the fully elongated wild-type maltose-binding protein and SecB from 2 to 7 min (compare Fig. 3 and 8). Among the possible explanations of this effect are the following. The band on the gel that was quantified as being representative of fully elongated polypeptides may contain a significant quantity of chains that were very near completion but still nascent. Alternatively, if, as we propose, the maltose-binding protein in complex with SecB is in equilibrium with a free pool and if SecB is limiting, as seems to be the case when maltose-binding protein is max-

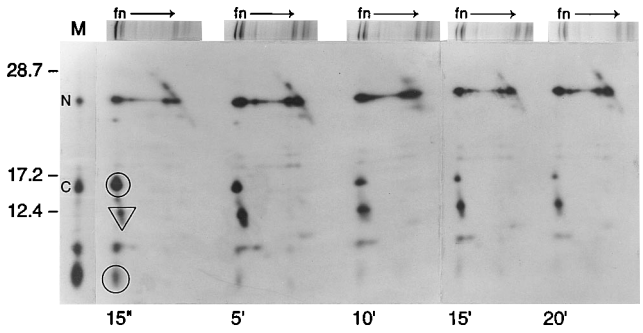

FIG. 7. Analysis of complexes of SecB with nascent and fully elongated species of maltose-binding protein. An exponentially growing culture expressing the wild-type maltose-binding protein without the leader (MalE Δ2-26) at maximally induced levels was radiolabeled, protein synthesis was stopped, samples were taken at the times indicated, complexes were isolated, and polypeptides were analyzed by two-dimensional polyacrylamide gel electrophoresis combined with limited proteolysis and fluorography as described in Materials and Methods. Peptides derived from nascent chains appear as horizontal or diagonal streaks below the main diagonal, which represents material that has not been proteolyzed and thus migrates at the same apparent molecular weight in the first and second electrophoresis. Peptides derived from the fully elongated maltose-binding protein appear as spots in a vertical array in the second-dimensional gel directly below the position of the fully elongated protein in the first dimension of electrophoresis. The first-dimensional gel (direction of migration is indicated by arrows) is shown at the top of the second-dimensional gel. M, reference peptide pattern; f, fully elongated maltose-binding protein; n, nascent species; N, amino-terminal peptide; C, carboxy-terminal peptide. The peptides designated by the circles and the triangle were quantified, and the data are presented in Fig. 8. Numbers at the left are molecular masses (in kilodaltons), and the standards were the same as for Fig. 2.

imally produced, then the decrease in specific activity of the maltose-binding protein with time that would occur if protein synthesis were to continue after the labeling period would have the effect of making the half-life of the radioactive species in complex with SecB appear to be shorter than it would be if the specific activity were constant, as would be the case in the presence of chloramphenicol. Even though the cause of the effect is obscure, it is clear that the fully elongated species did exit the complex whereas the nascent species did so only very slowly. Since the rate-determining step in the acquisition of the stable tertiary fold of the maltose-binding protein involves formation of a structural element that comprises stretches of aminoacyl residues from both the amino-terminal and carboxy-terminal regions (3), one would not expect polypeptides to acquire a structure with sufficient stability to preclude binding to SecB until synthesis is complete. Since the binding frame on the maltose-binding protein is contained within the region from aminoacyl residue 75 to 250 in the primary sequence (24), once the nascent polypeptides reach the length which contains the entire binding frame, the rapidly folding, wild-type species and the slowly folding species would be equally competent to bind SecB. This would explain why such a large proportion of both species is in complex with SecB initially.

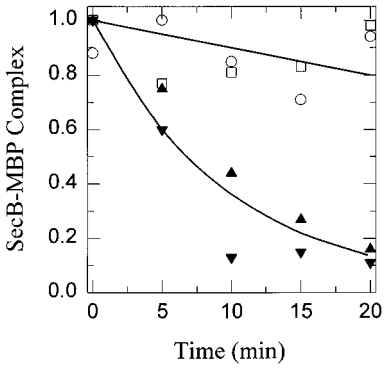

FIG. 8. Half-lives of complexes of SecB with fully elongated and nascent species of wild-type maltose-binding protein (MBP). Those peptides used for quantification are indicated in the legend to Fig. 7. The amount of each peptide derived from the complex was normalized to the total amount of the corresponding peptide. The circled spots in Fig. 7 were quantified as representative of the fully elongated maltose-binding protein. ▲, peptide with higher molecular mass; ▼, peptide with lower molecular mass. The spot within the triangle in Fig. 7 was quantified as representative of nascent species (○). A nascent species (n) previously characterized (20) and which appears as a band in the first dimension of electrophoresis was also quantified (□).

In summary, we have demonstrated that the complex between SecB and a species of maltose-binding protein that folds more slowly than does the wild-type species is longer-lived than the corresponding complex with the wild-type protein. We also showed that nascent polypeptides remain complexed with SecB when their elongation is stopped. These observations support the notion that the earliest step in the export of maltose-binding protein includes a kinetic partitioning between premature folding in the cytosol and association with SecB, which in turn directs the polypeptides into the export pathway.

ACKNOWLEDGMENTS

We thank Traci Topping for purifying SecB and the two species of maltose-binding protein and for critically reading the manuscript.

This work was supported by Public Health Service grant GM 29798 to L.L.R. from the National Institutes of Health.

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