JOURNAL OF BACTERIOLOGY, Apr. 1995, p. 1906–1907 Vol. 177, No. 7
0021-9193/95/$04.00+0

Export of Periplasmic Galactose-Binding Protein in *Escherichia coli* Depends on the Chaperone SecB

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Received 12 October 1994/Accepted 25 January 1995

The efficient export of galactose-binding protein to the periplasm of *Escherichia coli* is shown to be dependent on the presence of the cytosolic chaperone SecB.

SecB is a molecular chaperone that facilitates the export of proteins to the periplasmic space and to the outer membrane of *Escherichia coli*. SecB binds precursor polypeptides in the cytosol and delivers them to SecA, a component of the membrane-associated translocation apparatus. In addition, SecB maintains the precursors in a state compatible with the transfer across the cytoplasmic membrane by preventing their folding and/or aggregation (2). Not all exported proteins require the function of SecB, and even for those that do depend on SecB, the requirement is not absolute. In the complete absence of the chaperone, some proportion of the protein is properly localized, but the process is slow, and often a substantial amount of the precursors accumulates in the cytosol in a folded or aggregated state (8).

SecB, like all proteins classified as chaperones, is characterized by its remarkable ability to recognize a protein as nonnative, having no affinity for its polypeptide ligands after they have attained their final, stable tertiary structure (5). There is no apparent consensus in sequence among the ligands for SecB. The selectivity that SecB displays in recognizing polypeptides as ligands can be explained in part by a kinetic partitioning of the potential ligand between the folded state and the state complexed with SecB. Since the binding site for SecB is inaccessible in native proteins, precursors that fold rapidly have a low probability of being bound. Binding studies carried out in vitro with pure proteins have shown that if polypeptides are maintained in a nonnative state, SecB binds with a wide range of affinities reflecting differences in the energy of binding in the various complexes (5). To better understand the molecular basis of these interactions, it is important to identify as many physiologic ligands of SecB as possible. SecB has been shown to enhance the export of the outer membrane proteins LamB (8), OmpA (3), OmpF (8), and PhoE (4), whereas export of the lipoprotein of the outer membrane shows no requirement for SecB (3, 6). Periplasmic proteins that are efficiently exported in the absence of SecB include TEM β-lactamase (3) and ribose-binding protein (8). Alkaline phosphatase is exported efficiently without SecB if cultures are grown at 42°C, but at 30°C a dependence on SecB can be demonstrated (3, 8, 9). The periplasmic proteins that require SecB for efficient export are maltose-binding protein (8) and oligopeptide-binding protein (3). Here we show that galactose-binding protein also requires SecB.

The export of galactose-binding protein was examined in *E. coli* K-12 strain MC4100 (F$^-$ *lacU169 araD139 rpsL150 thi flbB5301 deoC7 ptsF25 relA1*) (1), which has a complete, functional export apparatus, and in an isogenic derivative, CK1953, which carries the *secB*::Tn*5* mutation (8) and therefore is lacking the chaperone SecB. The strains were grown at 30°C in M9 minimal salts medium (10) supplemented with thiamine hydrochloride (2 μg/ml) and galactose (0.4%). Exponentially growing cultures were labeled at a density of 2.5×10^8 cells per ml by the addition of 60 μCi of [^{35}S]methionine per ml. To ensure that incorporation of the isotope was linear for the duration of the labeling period, nonradioactive methionine was included to give a final concentration of 90 nM methionine. Fifteen seconds after addition of the isotope, nonradioactive methionine was added to a final concentration of 40 μM, and growth was continued. The cultures were sampled at the times indicated in Fig. 1 by pipetting 0.5 ml of the cell suspension directly into an equal volume of 10% trichloroacetic acid. The samples were processed for immunoprecipitation and analysis by sodium dodecyl sulfate–11% polyacrylamide gel electrophoresis followed by autoradiography as described previously (12, 13). The radioactivity in the bands on the autoradiograms corresponding to precursor galactose-binding protein and to the mature form (identified by coelectrophoresis with authentic mature galactose-binding protein detected by staining the gel with Coomassie blue) was quantified by densitometric scanning with a Helena Laboratories Quick Scan R + D. Since the radiolabel was [^{35}S]methionine and three of the nine methionines are in the leader sequence (7), a factor of 1.5 was used to convert the radioactivity recovered in the mature form to the amount of precursor polypeptide from which it was derived, and these values were used to calculate the percentage of the total population of precursor that was processed.

In the strain containing the normal export apparatus including the chaperone SecB, the precursor galactose-binding protein was rapidly and completely exported to the periplasm and cleaved to the mature form (Fig. 1). The half time for complete export at 30°C was less than 15 s. In the strain that lacks SecB, export of galactose-binding protein proceeded at a drastically reduced rate (Fig. 1). The half time for export was approximately 2 min. Clearly the presence of SecB expedites export of galactose-binding protein.

It has been shown previously that the export of ribose-binding protein is independent of the function of SecB, whereas that of maltose-binding protein is dependent on SecB (8). In accord with these observations it can be shown in vitro that SecB binds maltose-binding protein with 50-fold-higher affinity (K_d, 1 nM) than it does ribose-binding protein (K_d, 50 nM) (5). Galactose-binding protein is more closely related to ribose-binding protein than to maltose-binding protein, as assessed by

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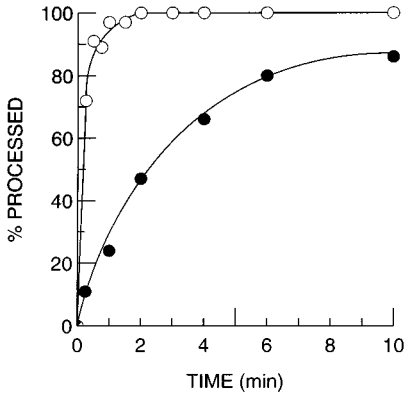

FIG. 1. Kinetics of export. Exponentially growing cultures were labeled as described in the text. Samples were withdrawn at the times indicated and processed for immunoprecipitation of maltose-binding protein. The strains were MC4100 (○), a strain normal for export, and CK1953 (●), which carries *secB*::Tn*5* and does not produce the SecB protein. The data are the averages from three experiments for MC4100 and two experiments for CK1953.

sequence similarity and by the three-dimensional structure (11); however, perhaps surprisingly, the affinity of galactose-binding protein for SecB is similar to that of maltose-binding protein for the chaperone. We have been able to isolate a complex between purified galactose-binding protein and SecB by size exclusion chromatography, and by using titration calorimetry, we have shown that the dissociation constant is in the range of 20 nM (14). Comparative studies of the three binding proteins in complex with SecB in vitro may help to elucidate the molecular basis of the binding energy.

We thank Vijaya Khisty for critically reading the manuscript.

This work was supported by Public Health Service grant GM 29798 to L. L. Randall from the National Institutes of Health. E. L. Powers was supported by Biotechnology Training Grant T32 GM08336 from the National Institutes of Health.

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