Temperature-Sensitive sec Mutants of Escherichia coli: Inhibition of Protein Export at the Permissive Temperature

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Phenotypes of secY and secA temperature-sensitive mutants at permissive (low) temperature have been examined. The secY24 mutant was found to be extremely susceptible to export inhibition by a basal-level synthesis of the MalE-LacZ 72-47 hybrid protein or to overproduction of a normal secretory protein such as maltose-binding protein or β-lactamase. Comparison of this phenotype of secY24 with those of the secY100 and secA51 mutants under similar conditions suggested that MalE-LacZ protein and overproduced secretory protein do not nonspecifically enhance the partial secretion defect but act synergistically with secY24 to inhibit protein export.

The secY (prtA) gene product of Escherichia coli is embedded in the cytoplasmic membrane by its 10 transmembrane segments (1). Its essentiality for translocation of envelope proteins across the membrane has been demonstrated both in vivo (4, 8, 12) and in vitro (2, 5). It was proposed that an integral membrane component such as SecY might form a channel for protein translocation (1). Since temperature-sensitive mutations produce altered proteins that not only lose activity at high temperatures but also could function abnormally at low temperatures, their phenotypes under the latter conditions might be useful in studying the gene function within the cell. As an approach to analyzing the secY function in vivo, we studied phenotypes of temperature-sensitive sec mutants at the permissive low temperature.

Three temperature-sensitive mutations, secY24 (12), secY100 (see below), and secA51 (11), have been examined. The secY100 mutant (K. Ito, Y. Hirota, and C. Ueguchi, manuscript in preparation) has been identified among the collection of E. coli temperature-sensitive mutants obtained from PA3092 (6). It contains three mutations within secY which should result in amino acid changes: Pro-40 to Ser, Ala-40 to Val, and Gly-167 to Glu. All the sec mutations used were in the background of strain MC4100 (13).

Protein secretion in these temperature-sensitive mutants is retarded to various degrees at 30°C. Cells growing at 30°C were pulse-labeled with [35S]methionine for 0.5, 1, or 2 min (Table 1). Labeling was terminated by trichloroacetic acid, and proteins were solubilized and subjected to immunoprecipitation with antiserum against the outer membrane OmpA protein by previously published procedures (7). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (9) and autoradiography, precursor and mature forms of OmpA were quantitated by densitometer tracings with a Biomed laser scanning densitometer. Export of OmpA at 30°C was affected most profoundly by the secA51 mutation (in strain MM52), followed by secY100 (in strain K1330) and secY24 (in strain IQ85), in this order. Similar results were obtained for another outer membrane protein, OmpF, and a periplasmic protein, MalE (a maltose-binding protein) (data not shown).

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for normal growth. After a full induction of the lac promoter with cyclic AMP, Bla synthesis increased several-fold, and progressively larger fractions of OmpA were labeled as the precursor form (data not shown). The secY100 and secA51 mutants were affected only transiently under such conditions (not shown).

pKY57 is a derivative of pNO1575, deleted for the Smal (multiplying site)-AarI fragment such that the lac promoter has been placed closer to bla and most of the lacZ' sequence has been eliminated. In addition, the HindIII site has been subjected to digestion, filling in by T4 DNA polymerase, and ligation, generating an in-frame TAG codon which stops the lacZ' translation at codon 8. Cells bearing pKY57 synthesized Bla at a level about 10% of total protein synthesis in the absence of lacI and in the presence of both glucose and cyclic AMP, whereas Bla synthesis was repressed slightly (to about 8%) in the absence of cyclic AMP. Plasmid pKY57 was found to be almost incompatible with the secY24 mutation, unless the lac repressor-overproducing mutation (lacP') coexisted. In the absence of the repressor, pKY57 transformants of the secY24 mutant (IQ85) could be obtained if glucose was added, although the growth was very slow. Such transformants were severely inhibited in protein secretion (Table 1), and the pattern of total protein synthesis was markedly altered. The alteration includes a marked induction of the heat shock proteins such as GroE and DnaK (data not shown). The secY100 and the secA51 mutants were relatively less affected by pKY57, in both growth and protein secretion (Table 1).

The results reported above show that protein export in the secY24 mutant at its permissive temperature (30°C) is extremely susceptible to the low-level synthesis of MalE-LacZ hybrid protein and to an excessive synthesis of an exportable protein. The sensitivities in different mutant strains to MalE-LacZ or a secretory protein did not correlate with the intrinsic partial defects of the strains expressed at 30°C.

It is possible that the secY24 mutation decreases the quantity of the functional SecY-containing export machinery and makes the cell unable to cope with the increased load of export. Our studies of the time course of secretion inhibition after different levels of induction of Bla (data not shown) suggest that continuous synthesis of Bla above certain levels and over certain periods may be required to cause the severe export block characteristically observed in the secY24 mutant cells. Such observations may be consistent with another possibility: that the secY24 mutation alters the quality of the SecY protein so that it is extremely sensitive to jamming by the hybrid protein or by a continuous inflow of protein molecules to be exported. In this context, it seems possible that SecY is a component of the hypothetical translocation channel and that secY24 slows down a reaction process in which the translocating protein moves through or out of the channel. Under such circumstances, a continuous influx of translocating molecules above certain critical levels could cause irreversible jamming of the altered channel. Obviously, further studies, including those in vitro, are needed to determine whether there is a specific point in the translocation pathway at which combinations of secY24 and other MalE-LacZ, MalE, or Bla synergistically exert the inhibitory effect.

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