Distinct Mutation Sites in prlA Suppressor Mutant Strains of Escherichia coli Respond Either to Suppression of Signal Peptide Mutations or to Blockage of Staphylokinase Processing

TOMOYUKI SAKO* AND TOHRU IINO
Yakult Central Institute for Microbiological Research, Kunitachi, Tokyo 186, Japan
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We have cloned and sequenced some prlA mutant alleles of the Escherichia coli secY gene. From the mutation sites determined, it is strongly suggested that distinct regions in the SecY (PrIA) protein are involved in the recognition of different structural features of a signal peptide as it functions.

Protein export across membranes is achieved through the interaction of the exported protein with various cellular factors. Several genes which are directly involved in the export process have been identified in Escherichia coli (3, 7, 9, 12, 13, 16, 17, 24). Among those, secY (prlA) is a well-characterized gene, and its product has been shown to be localized in the cytoplasmic membrane by traversing the membrane many times (1, 2). The interaction between the SecY (PrIA) protein and the signal peptide of an exported protein was first proposed as a result of the isolation of suppressor mutations in this gene against signal peptide mutations in lamB and male (6, 7). However, since a prlA mutant allele which has been isolated as a suppressor of a particular signal peptide mutation can usually suppress many other signal peptide mutations in the same gene and in the other genes at the same time (3, 6, 23), it is uncertain that the SecY protein directly interacts with a signal peptide. We recently found that export of the staphylokinase (19) from Staphylococcus aureus is blocked in two of the prlA mutant strains examined (the prlA4 and prlA401 strains; Iino and Sako, submitted for publication), although staphylokinase is efficiently exported to the periplasmic space in the wild-type strain (18). Two causes can be proposed for this blockage: (i) the structure of the signal peptide and (ii) an alteration of the SecY protein by the particular prlA mutation. We have already shown that single amino acid substitutions in the staphylokinase signal peptide cause resumption of its processing in the mutant cells (Iino and Sako, submitted). In the present study we attempted to determine the mutation sites of some prlA mutant alleles which affect or do not affect the processing of staphylokinase.

We cloned the 2.8-kilobase-pair (kb) PstI fragments (5, 21) from the chromosomes of E. coli MCR60 prlA4 (7), MCR60 prlA3 (6), and RL401 prlA401 (3), as well as from the chromosomes of IQ85 secY24 (21) and MCA4100 secY+ (11), by shotgun cloning followed by Southern hybridization (22) to the secY-specific probe obtained from pKY6 (21), and we sequenced the entire secY regions by the dyeoxy-chain termination method (20) with a set of synthetic primers and pUC119 vector (25). The results are summarized in Fig. 1. We confirmed that the sequence of the secY+ allele was consistent with that reported previously (5). The prlA3 and prlA401 alleles had single transversions causing amino acid exchanges from Phe-67 (TTC) to Cys (TGC) and from Ser-282 (AGT) to Arg (CGT), respectively. On the other hand, the prlA4 allele had two transversions causing amino acid exchanges from Phe-286 (TTC) to Tyr (TAC) and from Ile-408 (ATC) to Asn (AAC). These two mutations in prlA4 were not artificially introduced during cloning and sequencing, because two additional, independently isolated primary clones also had the same mutations. We have named the former and latter mutations prlA4-1 and prlA4-2, respectively.

The next question was which mutation in the prlA4 allele is effective for the suppression of signal peptide mutations and for the blockage of staphylokinase processing. To answer this, we separated the two mutations by cutting the 2.8-kb fragment with ClaI into 2.2-kb amino-terminal prlA4-1-containing and 0.6-kb carboxyl-terminal prlA4-2-containing fragments (5) and then rejoined each half with another half obtained from the secY+ fragment to make 2.8-kb fragments carrying each of the two mutations. We first analyzed the processing of staphylokinase by using a system in which the plasmid-coded secY function can be determined. The sak moiety, which also contained the temperature-sensitive cI857 repressor gene and early rightward promoter pr of bacteriophage lambda, was isolated from pTI10 (10) as a 1.9-kb EcoRI-BgIII fragment and inserted into pTS143, which was derived from pBR322 and carried the chloramphenicol resistance gene as a selective marker (Sako, unpublished), to make pTS391. Each of the 2.8-kb PstI fragments was next inserted into pTS391. By introducing these plasmids into IQ85 secY24, we could analyze the effects of various plasmid-coded secY alleles on staphylokinase processing at 42°C, because the chromosomal secY24 allele was inactive at this high temperature. The secY+ allele

FIG. 1. Hydrophathy of the SecY protein and mutation sites found in prlA mutant strains. Hydrophathy analysis was carried out by the method of Kyte and Doolittle (14). Filled bars in the SecY protein diagram are the proposed transmembrane segments (2).
fully supported staphylokinase processing, whereas the parental prlA4 allele could not (Fig. 2). This inhibitory effect was kept by the prlA4-1 allele. In contrast, the prlA4-2 allele did not block staphylokinase processing at all. Thus, the blockage of staphylokinase processing was caused by the prlA4-1 mutation.

Next, the suppression activities against signal peptide mutations were analyzed. We constructed recombinant plasmids which carried each of the 2.8-kb PsI fragments on pTS143 and introduced them into TS1094 ΔmalE12-18 lanBS60 Tn10, which was derived from RL401. On minimal agar plates supplemented with maltose as the sole carbon source, the parental prlA401 strain (TS1096) and TS1094 merodiploids carrying each of the prlA401, prlA4, and prlA4-2 alleles grew, suggesting that the both signal peptide mutations in malE and lanB were suppressed. When the processing of the mutant malE product was analyzed in TS1094 merodiploids, the prlA401, prlA4, and prlA4-2 alleles had the expected suppression activities (Fig. 3). Therefore, the mutation in the prlA4 allele active in the suppression of signal peptide mutations was prlA4-2.

Note that both the mutations effective for the blockage of staphylokinase processing (prlA4-1 and prlA401) are localized within transmembrane segment 7 of the proposed structure of SecY (Fig. 1; 21). Thus, it is probable that the SecY protein interacts directly with the staphylokinase signal peptide, at least at transmembrane segment 7. Since some mutations in sak which strengthen the β-turn probability of the signal peptide suppressed the prlA4-specific blockage of staphylokinase processing (Iino and Sako, submitted), the segment may recognize a certain secondary structural feature of the signal peptide. In contrast, the mutations effective for the suppression of signal peptide mutations are widely distributed, although they appear to be localized within the hydrophobic domains (Fig. 1). This suggests that the suppression of signal peptide mutations which lower the hydrophobicity (4) and/or α-helix probability (8) is achieved by an overall conformational change of SecY. Thus, distinct regions or domains in the SecY protein may be involved in recognition of different structural features of a signal peptide.

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LITERATURE CITED


