Spontaneous Missense Mutations in the rplX Gene for Ribosomal Protein L24 from Escherichia coli

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Ribosomal protein L24 from Escherichia coli initiates assembly of the 50S ribosomal subunit (10). The protein binds to the 5' end of 23S rRNA and protects about 480 nucleotides from RNase digestion (1, 13). Temperature-sensitive mutants in the gene rplX for protein L24 have been described, and they show an impairment in ribosome assembly at the nonpermissive temperature (2, 3). One of them lacks protein L24 completely, due to a nonsense mutation at the beginning of the structural gene (8; Fig. 1, position 20). Another mutant protein has a change from the amino acid Gly to Asp at position 84 in the protein (2). This mutant L24 protein shows a reduced affinity to the 23S rRNA.

Recently, we described the temperature-sensitive mutant KNS19 with an altered protein L24 (9). By analyzing the DNA sequence, we found a mutation leading to the same amino acid alteration at position 84 as described by Cabezón et al. (2). From this mutant, we could isolate suppressor mutants in a plasmid-coded 23S rRNA gene which proved the interaction of protein L24 and 23S rRNA in vivo (9). The above results suggested that the amino acid Gly at position 84 in protein L24 is important for the interaction of the protein with 23S rRNA. We were interested in whether (and which other) amino acids may substitute for the amino acid Gly at position 84. Therefore, we isolated temperature-resistant pseudorevertants of mutant KNS19. Their amino acid sequences were compared with the sequences of the original mutant and other mutants isolated independently which also showed an altered protein L24, and the substitutions were determined. We expected that this approach might reveal some information about functionally important amino acids and positions in protein L24.

The temperature-resistant revertants from mutant KNS19 showed various growth rates. The mutations of all slow-growing revertants mapped at chromosomal locations different from rplX. These studies will be described elsewhere. The fast-growing revertants appeared after the incubation of agar plates at 42°C overnight. We then analyzed the ribosomal proteins of these revertants by two-dimensional gel electrophoresis by the method of Geyl et al. (5) to see whether the position at which protein L24 migrated was different from that at which the mutant KNS19 protein migrated. Protein L24 from KNS201 and KNS203 migrated at the same position as did the wild-type protein L24, whereas protein L24 from strain KNS191 migrated at the same altered position as the mutant protein from KNS19. This indicated that at least KNS201 and KNS203 were temperature-resistant because of a mutation within the gene for protein L24. The mutation in strain KNS191 was, however, also located close to or within the gene for protein L24 by PI transduction.

We analyzed the mutations in the various rplX revertant genes. For this purpose, we cloned all the mutant genes by a positive-selection procedure similar to the one previously described (9). As a recipient strain for cloning, we used the temperature-sensitive mutant KNS19. Clones were isolated which complemented the rplX19 mutation. We sequenced the DNA fragments containing the genes for protein L24 and identified the mutations (Fig. 1 and Table 1). In all cases, a mutation had occurred that altered the mutant codon GAC for the amino acid Asp at position 84 in the protein. KNS201 had a GCC codon; i.e., it had reverted to the wild-type codon. KNS203, which also showed a wild-type protein in the two-dimensional gel electrophoresis, had a TAC codon for the amino acid Tyr. KNS191 had a GAG codon for Glu, which explained why the electrophoretic properties of this L24 protein were similar to those of the original mutant protein. This result indicated that at least two other amino acids could replace the Gly residue at position 84. However, the mutants with the amino acid alterations had slower growth rates than that of the wild-type strain CP78 at the nonpermissive temperature, i.e., at 42°C, at which the mutant KNS19 was unable to grow. KNS201 (Gly) had a generation time of 30 min at 42°C, which was similar to that of the wild-type CP78, whereas KNS191 (Glu) with 55 min and KNS203 (Tyr) with 70 min grew significantly more slowly than the other strains did (Table 1).

There were two other mutants obtained by a selection from streptomycin dependence to independence which showed altered L24 proteins. The mutants were derivatives of the same parental strain VT from which the rplX19 mutation in KNS19 was derived (4). Both mutants harbored additional mutations in other ribosomal proteins which we
found to be responsible for the suppression of streptomycin dependence (K. Nishi and J. Schnier, unpublished results).

We also cloned these mutant rplX genes by the same positive-selection procedure and sequenced them (Fig. 1 and Table 1). Mutant LL3 had a change from GGC (Gly at position 56) to the codon GAC for the amino acid Asp. Mutant AC4 had an alteration of GAA (Glu at position 62) to AAA coding for Lys. In both cases, the charge alterations caused the expected altered electrophoretic mobility of mutant L24 proteins (data not shown). By P1 transduction, we could eliminate all other additional ribosomal mutations in mutants LL3 and AC4, and the mutated genes were transduced into strain CP78. The resulting transductants with the single mutations in the rplX gene, MK301 (derived from LL3) and MK401 (derived from AC4), were tested for growth at various temperatures. Both mutants had growth characteristics similar to those of the parental strain CP78, which suggested that the mutations did not essentially alter the functional properties of protein L24.

All amino acids in the protein which were altered by mutations were found in the regions conserved in protein L24 from Bacillus stearothermophilus (6). This includes position 20 in the protein, where we found at least three different amino acid replacements (8). This indicates that these positions might be of importance for the functional properties of the protein. However, it is interesting that so far only one missense mutation, namely, the one at position 84 of the protein, caused a conditional lethal phenotype (temperature-sensitive growth), whereas mutations at other positions did not cause any impairment in growth. It could be that either the amino acids at positions 56 and 62 are not as important for the function of the protein as the one at position 84 is or they are important but the particular amino acid replacements do not essentially alter the general properties of the protein. One explanation could be that whether the introduction of a charged or differently charged amino acid causes an altered property of the protein depends on the position of the amino acid in the protein. However, we could not attribute the alteration of the protein property to the charge change, since the amino acid Glu, which like Asp is also negatively charged, could replace the wild-type amino acid Gly in one of the temperature-resistant revertants.

It is known that Gly is important for turn structures (14). Therefore, it could be that the two amino acids at positions 56 and 84 are involved in hairpin loops. The primary sequence at positions 56 and 57 is Gly-Gly. The presence of two consecutive Gly residues in the protein indicates a type 1 β-hairpin loop (12). Instead of the Gly in the first position, either Asp or Asn was found to allow this type of loop as well (12). This could explain why a mutation which alters the Gly to Asp at position 56 has no apparent effect on the properties of protein L24. On the other hand, there is evidence that the peptide around position 84 forms another loop. It was reported that a peptide from γ-chymotrypsin A (LQDQKFGHF) forms a β-hairpin with a five-residue loop (12). This peptide shows a striking sequence homology to the part of protein L24 around position 84 (KADRVGFRF). From our mutation analysis, we conclude that Asp instead of Gly would disturb the correct loop formation. The revertant analysis suggests that Glu and to some extent Tyr can recover the original loop, although mutants having Glu and Tyr at position 84 in protein L24 showed slower growth rates. This means that Gly was the preferential amino acid to maintain the correct protein structure. We could not find any reasonable explanation for the third mutational point at position 62. It is interesting that this amino acid (Glu) is close to the Gly at position 56. It may indicate that this region within protein L24 is also important for the function, although the mutations did not cause any apparent altered property of the protein.

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LITERATURE CITED


