The FhuA Protein Is Involved in Microcin 25 Uptake

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Received 18 August 1993/Accepted 22 September 1993

A chromosomal Tn5 insertion resulting in complete resistance to the peptide antibiotic microcin 25 was mapped to the min 4 region of the *Escherichia coli* genetic map. Additional experiments showed that the insertion disrupted the *fhuA* gene, which encodes the multifunctional outer membrane receptor for ferrichrome, the antibiotic albomycin, colicin M, and bacteriophages T5, T1, and φ80. Thus, microcin 25 and all of these agents share the same receptor.

Microcins are low-molecular-weight peptide antibiotics produced by members of the family *Enterobacteriaceae* (1). We have recently described the isolation and characterization of microcin 25 (Mcc25), a plasmid-encoded peptide of approximately 2,000 Da excreted by a fecal strain of *Escherichia coli* (8).

As an approach to the study of the mechanism of action of Mcc25, we have isolated Mcc25-resistant mutants of *E. coli* K-12. In this study, we identified a gene, *fhuA*, whose product seems to be required for microcin action.

Mcc25 sensitivity was determined by a cross-streaking assay as described previously (8). Sensitivity to colicin M was determined by stabbing colicin M-producing strain TO4 into a Luria broth plate which had been previously seeded with the test strain. After overnight incubation, growth inhibition zones were examined. Phage φ80φvir sensitivity was determined by the cross-streak method. Transposon Tn5 mutagenesis was done with λ::Tn5 by the method of Berg (2). Conjugation and P1 transduction were done as described by Miller (7).

As the first step in this work, a Tn5-mutagenized culture of *E. coli* K-12 strain AB259 (thi r o reA HfrH) was plated onto Luria broth agar containing kanamycin (30 μg/ml) and Mcc25. A few Mcc' Km' clones were obtained. They were purified and tested for sensitivity to Mcc25. One of these clones, designated SBG103, was selected for further analysis. Absolute linkage of Tn5-encoded Km' and Mcc25' was tested by P1 transduction. Loss of sensitivity to Mcc25 was always 100% cotransduced with Km'. This confirmed that the Tn5 insertion was actually responsible for the microcin resistance phenotype. Strain SBG103 was then conjugated with *E. coli* K-12 strain AB1133 (thi argE his proA thr leu ara met il y galK lacY supE rpsL λ' F', cysH lacY' rfdD glpR thi supE) and samples were drawn at various times and plated on Luria broth supplemented with streptomycin (100 μg/ml) and kanamycin. This experiment allowed us to map the Tn5 insertion to the 0- to 10-min region of the *E. coli* K-12 map. A bacterial cross was then performed between SBG103 and AB1133 by using the method of DeHaan et al. (4) and selecting for Thr+ Sm'. Recombinants were scored for inheritance of Leu+', ProA', and Kan'. The gradient of transmission indicated that the locus mutated was located at approximately 4 min on the *E. coli* K-12 genetic map. The location of the insert was then more precisely determined by P1 transduction. Bacteriophage P1 propagated on SBG103 was used to transduce YA139 (Hfr *pB reA spoT λ' ) to Pan+. Sixty-six transducants selected on M63 minimal plates were scored for Km'. Of these, 38 (58%) were Km'. Using the formula of Wu (10) to convert cotransduction frequency into map distance placed the transposon 0.32 min away from the *panB* gene. Inspection of the linkage map of *E. coli* showed *panB* to lie near a well-known genetic locus designated *fhuA* (formerly *tonA*). The FhuA receptor protein in the outer membrane of *E. coli* is required for uptake of the iron carrier ferrichrome; the antibiotic albomycin, a structural analog of ferrichrome; and colicin M. Also, it serves as a receptor for bacteriophages T5, T1, φ80, and UC-1 (3, 6, 9). A mutation at this site is known to impart resistance to these phages and to colicin M. Thus, the product of the *fhuA* gene could play a similar role in Mcc25 uptake.

When strain H1024 (W3110 *fhuA*::Tnl0), a well-characterized *fhuA* mutant, was available, we tested its sensitivity to Mcc25 and found that it was resistant. To rule out the possibility that another mutation was responsible for this phenotype, a transduction was performed with H1024 as the donor and BM21 (F· gyrA λ+) as the recipient. Tc' transductants were selected. Twenty-four transductants were tested for sensitivity to phage φ80 and Mcc25, and all became resistant to both agents, thus confirming that *fhuA* inactivation led to microcin resistance. *E. coli* K-12 strain C600 (thr leuB *fhuA lacY λ- rfdD glpR thi supE) was also completely insensitive to Mcc25.

On the other hand, SBG103 was insensitive to phage φ80 and colicin M, whereas its parental strain, AB259, was fully susceptible to these agents.

In addition to *E. coli*, several *Salmonella* species were found to be sensitive to Mcc25 (8). However, all of the *Salmonella typhimurium* strains tested were resistant to Mcc25. This species is also insensitive to colicin M, but Graham and Stocker reported that the resistance could be overcome by provision of *fhuA* of *E. coli* in an F' episome (5). Thus, resistance of *S. typhimurium* to Mcc25 might also be explained by the lack of an appropriate receptor. *E. coli* AY25, the wild-type producer of Mcc25, was susceptible to colicin M, but not to the extent of K-12 strains. This might reflect a cell surface difference between AY25 and K-12 strains. The latter are rough (i.e., they did not synthesize O antigen), while AY25 is able to grow in minimal medium containing valine and thus presumably is not K-12. Along these lines, Graham and Stocker have noticed that rough *Salmonella* strains are much more sensitive to colicin M than are smooth strains (5).
We are grateful to B. Bachmann (E. coli Genetic Stock Center), F. Moreno, and V. Braun for gifts of bacterial strains.

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) grant PID 3-013800, Fundación Antorches grant 12576/1-000065, and funds from the Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT). R.A.S. was the recipient of a CONICET fellowship. R.N.F. is a career investigator of CONICET.

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