Identification and Nucleotide Sequence of the Gene Determining the Adhesion Capacity of Serratia marcescens

YOSHIMITSU MIZUNOE,1,* TETSURO MATSUMOTO,1 KAZUNOBU AMAKO,2 MUTSUO SEKIGUCHI,3 AND JOICHI KUMAZAWA1

Department of Urology,1 Department of Bacteriology,2 and Department of Biochemistry,3 Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

Received 16 November 1990/Accepted 11 March 1991

Three open reading frames, designated smfE, smfF, and smfG, within the mannose-resistant fimbriae gene cluster of Serratia marcescens were identified. smfG, which is responsible for determining the receptor binding of S. marcescens, encodes a 280-amino-acid polypeptide with a typical prokaryotic signal sequence.

Serratia marcescens US46, isolated from a patient with a urinary tract infection, possesses two types of fimbriae: one is mannose sensitive, and the other is mannose resistant (MR) (6). By introducing a cosmid library, prepared from the chromosomal DNA of this strain, into a nonfimbriated Escherichia coli strain, we isolated two types of fimbrial gene clusters, each encoding MR and mannose-sensitive fimbriae (13). Subcloning of the MR fimbriae gene cluster revealed that formation of MR fimbriae and the capacity to adhere to chicken erythrocytes are controlled by a 12-kb DNA fragment.

Analyses of the Pap pilis and the type 1 pilus gene clusters derived from E. coli indicated that these adhesion properties could be ascribed to a protein coded by a gene other than the major pilin gene (7, 10–12, 16). To identify the gene for the adhesion capacity of S. marcescens, transposon insertion analysis was performed with plasmid pYM141 (13), which carries the sequence required for the synthesis of functional MR fimbriae of this organism.

Transposon Tn3 was inserted into pYM141, and about 70 isolated insertion mutants were examined for their abilities to exhibit hemagglutination and to form fimbriae by the procedures we reported elsewhere (13). E. coli P678-54 cells harboring pYM1085, one of the insertional mutants, did not exhibit MR hemagglutination, although they did possess fimbriae (Table 1). The cells were aggregated by anti-MR fimbria monoclonal antibody (6).

A 2,251-bp PstI-HaeII DNA fragment containing the region which was thought to control the MR hemagglutination capacity (Fig. 1) was subjected to DNA sequencing. We identified three open reading frames, designated smfE, smfF, and smfG (Fig. 2). The smfG gene appears to code for an adhesin, since the transposon insertion into this gene led to formation of a hemagglutination-negative fimbriated mutant (Fig. 1). The smfG gene consists of an 840-nucleotide coding region with a presumed initiation codon (ATG), preceded by a consensus ribosome-binding sequence (15), at nucleotide position 1348. The sequence of the smfG gene, initiated at this position and terminated at the TAG triplet at position 2187, would code for a polypeptide of 280 amino acid residues. In the amino-terminal region, a typical prokaryotic signal peptide sequence (5) was detected.

To obtain further support for the notion that the smfG gene encodes an adhesin, a small deletion was introduced at the SacI site located within this prospective coding sequence. Plasmid pYM821, carrying the 12-kb DNA fragment at the DraI site of pACYC184 (3), was partially digested by SacI and treated with T4 DNA polymerase in the absence of nucleotide substrate to remove the 3' protruding ends. A plasmid clone with a deletion at the SacI site within the smfG sequence was selected and designated pYM822 (Fig. 1). E. coli P678-54 cells harboring pYM822 did not exhibit MR hemagglutination; however, they did possess fimbriae, as revealed by electron microscopic observations as well as by aggregation with anti-MR fimbria monoclonal antibody (Table 1). Therefore, it is apparent that the smfG gene codes for a protein responsible for the adhesion capacity of MR fimbriae of S. marcescens.

The primary structure of the SmfG protein is homologous to the structure of the PapG protein of E. coli (10), especially in the carboxyl-terminal regions (Fig. 3). Even though the adhesins proteins (SmfG and PapG) are about twice the size of most other fimbrial proteins, there are certain similarities between the adhesins and the other fimbrial subunits.

* Corresponding author.

FIG. 1. Restriction maps of plasmid pYM141 and its smfG mutant derivatives. Genes belonging to the smf gene cluster are indicated by black boxes below the restriction map. The PstI-HaeII region (the DNA sequence of which is shown in Fig. 2) is enlarged, and detailed restriction sites are given in the middle of the figure. Plasmids pYM141 and pYM821 carry the same wild-type DNA fragment at different sites on the pACYC184 plasmid. Plasmid pYM1085 carries the Tn3 transposon (V), whereas pYM822 possesses a deletion at the SacI site of the smfG gene (V'). MRHA, MR hemagglutination; Fim., fimbria formation.
distance between two carboxyl-terminal cysteine residues of the SmfG and the PapG proteins is 31 residues, which is the same for the two cysteine residues in the major and the minor fimbrial proteins (2, 9, 13, 14). SmfG and PapG have a glycine residue 14 amino acid positions from the carboxyl terminus, as was found in the major and the minor E. coli (Pap and type 1, respectively) (2, 9, 14) and S. marcescens fimbrial proteins (Fig. 3 and 4).

The adhesin gene (papG) for E. coli Pap pili is the furthest downstream of the pap gene cluster (10). The smfG gene, coding for the adhesin of MR fimbriae of S. marcescens, is also located the most promoter distal of the smf gene cluster. Upstream of the smfG gene there are two open reading frames, designated smfE and smfF (Fig. 2). Both of the coding sequences are preceded by the consensus sequence for the ribosomal binding site (15) and are probably actively

FIG. 2. Nucleotide sequence of the smfE, smfF, and smfG genes and the primary structures of the SmfE, SmfF, and SmfG proteins. Putative ribosome-binding sites for smfE, smfF, and smfG are indicated by horizontal lines. Predicted amino acid sequences are shown, and potential processing positions of the mature proteins are indicated by triangles. Numbers refer to nucleotide positions.
TABLE 1. Agglutination properties and fimbrae of bacterial strains

| Strain | MRHA | Agglutination by anti-MR antibody | Fimbrae |
|--------|------|----------------------------------|---------|
| P678-54 | -    | -                                |         |
| P678-54(pYM141) | +    | +                                |         |
| P678-54(pYM1005) | -    | -                                |         |
| P678-54(pYM821) | +    | +                                |         |
| P678-54(pYM822) | -    | -                                |         |

* E. coli P678-54 cells harboring various plasmids were grown overnight on L broth agar at 37°C and suspended in phosphate-buffered saline (PBS).

Kume, MR hemagglutination. To examine MRHA, chicken erythrocytes were suspended at a concentration of 2% in PBS containing 1% d-mannose and mixed with the bacterial suspension on a glass slide.

1-2 Fimbriae present on the bacterial surface were identified by electron microscopy.

expressed. The prospective sizes of the SmfE and SmfF proteins, calculated from the nucleotide sequence, were 163 and 180 amino acids, respectively, and the proteins also carried the typical prokaryotic signal peptide sequence (5) at the amino-terminal regions.

The primary structures of the SmfE and SmfF proteins are homologous to the structure of the SmfA protein (13), which is the major fimbrial subunit of S. marcescens (Fig. 4). It is most likely that the SmfE and SmfF proteins are minor fimbrial subunits, as is the case with the PapE and PapF proteins of Pap fimbrae (8, 9).

The sizes and arrangements of the six genes within the smf gene cluster of S. marcescens are similar to those of the E. coli pap fimbra gene cluster. The primary structures of major and minor fimbrial subunits of S. marcescens show a high homology to those of the Pap fimbral subunits. As for adhesin, there is a homology between MR fimbrae of S. marcescens and E. coli Pap fimbrae, especially in the carboxyl-terminal half. The carboxyl-terminal region of PapG was found to be required for incorporation into the pilus (4).

These results imply that genes encoding the S. marcescens MR fimbrae and the E. coli Pap fimbrae may have evolved from a common ancestor. While genes for the fimbrial structure and the region of the adhesin gene necessary for pilus assembly may have been conserved, the unidentified region related to the binding specificity in the adhesin gene may have changed. Differences in binding specificities of the adhesins may explain the difference in pathogenesis between S. marcescens and E. coli.

We thank A. Takade for assistance with the electron microscopic studies and M. Ohara for reading the manuscript.

REFERENCES

1. Adler, H. L., W. D. Fisher, and A. A. Hardigree. 1966. Miniature Escherichia coli cells deficient in DNA. Proc. Natl. Acad. Sci. USA 57:321–326.

2. Baga, M., S. Normark, J. Hardy, P. O’Hanley, D. Lark, O. Olsson, G. Schoonik, and S. Falkow. 1984. Nucleotide sequence of the papA gene encoding the Pap pilus subunit of human uropathogenic Escherichia coli. J. Bacteriol. 157:330–333.

3. Chang, A. C. Y., and S. N. Cohen. 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the pA5A cryptic miniplasmid. J. Bacteriol. 134: 1141–1156.

4. Hultgren, S. J., F. Lindberg, G. Magnusson, J. Kihlgren, J. M. Tennent, and S. Normark. 1989. The PapG adhesin of uropathogenic Escherichia coli contains separate regions for receptor binding and for the incorporation into the pilus. Proc. Natl. Acad. Sci. USA 86:4357–4361.

5. Inouye, M., and S. Haglebog. 1980. Secretion and membrane localization of proteins in Escherichia coli. Crit. Rev. Biochem. 7:339–371.

6. Jingushi, S., M. Mitsuyma, T. Moriya, and K. Amako. 1987. Antigenic analysis of Serratia marcescens fimbrae with monoclonal antibodies. Infect. Immun. 55:1600–1606.

7. Klemm, P., and G. Christiansen. 1987. Three fim genes required

FIG. 3. Comparison of the structures of the SmfG and PapG proteins. The amino acid composition of the E. coli adhesin PapG (10) is aligned with the SmfG sequence. Vertical lines indicate amino acid identity or conservative amino acid replacement. Amino acid positions are numbered relative to the SmfG protein sequence.
for the regulation of length and mediation of adhesion of *Escherichia coli* type 1 fimbiae. Mol. Gen. Genet. 208:439–445.

8. Lindberg, F., B. Lund, L. Johansson, and S. Normark. 1987. Localization of the receptor-binding protein adhesin at tip of the bacterial pilus. Nature (London) 328:84–87.

9. Lindberg, F., B. Lund, and S. Normark. 1986. Gene products specifying adhesion of uropathogenic *Escherichia coli* are minor components of pili. Proc. Natl. Acad. Sci. USA 83:1891–1895.

10. Lund, B., F. Lindberg, B. Marklund, and S. Normark. 1987. The papG protein is the α-D-galactopyranosyl-(1→4)-β-D-galactopyranose-binding adhesin of uropathogenic *Escherichia coli*. Proc. Natl. Acad. Sci. USA 84:5898–5902.

11. Maurer, L., and P. E. Orndorff. 1987. Identification and characterization of genes determining receptor binding and pilus length of *Escherichia coli* type 1 pili. J. Bacteriol. 169:640–645.

12. Minion, F. C., S. N. Abraham, E. H. Beachey, and J. D. Goguen. 1986. The genetic determinant of adhesive function in type 1 fimbiae of *Escherichia coli* is distinct from the gene encoding the fibrial subunit. J. Bacteriol. 165:1033–1036.

13. Mizunoe, Y., Y. Nakabeppu, M. Sekiguchi, S. Kawabata, T. Moriya, and K. Amako. 1988. Cloning and sequence of the gene encoding the major structural component of mannose-resistant fimbiae of *Serratia marcescens*. J. Bacteriol. 170:3567–3574.

14. Orndorff, P. E., and S. Falkow. 1985. Nucleotide sequence of *pilA*, the gene encoding the structural component of type 1 pili in *Escherichia coli*. J. Bacteriol. 162:454–457.

15. Shine, J., and L. Dalgarno. 1975. Determinant of cistron specificity in bacterial ribosomes. Nature (London) 254:34–38.

16. Uhlin, B. E., M. Norgren, M. Baga, and S. Normark. 1985. Adhesion to human cells by *Escherichia coli* lacking the major subunit of a digalactoside-specific pilus-adhesin. Proc. Natl. Acad. Sci. USA 82:1800–1804.