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Flagellin Domain That Affects H Antigenicity of *Escherichia coli* K-12

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***Escherichia coli* K-12 mutants with altered flagellum antigenicity were isolated by introducing random deletions into the flagellin gene. The deletions were identified in the central region of the gene. It is suggested that this region corresponds to the flagellin domain molecule which affects flagellum antigenicity.**

H antigen is the flagellum-specific bacterial antigen which, together with O antigen, is responsible for most of the antigenicity in bacteria (6). The H antigenicity is dependent on the amino acid sequence of the component protein, flagellin.

Several studies have been performed to examine the region involved in H antigenicity in the flagellin molecule. Parish et al. (16, 17) showed that one of the four cyanogen bromide-digested fragments of *Salmonella adelaide* flagellin, which is located in the central part of the intact molecule, was antigenically cross-reactive with the flagellar filament. Joys and Martin (8–10) reported that one peptide of 13 amino acid residues in the central region of *Salmonella typhimurium* phase 1 flagellin showed diversity in its sequence among several serological mutants and that this peptide responded to antiserum against phase 1 flagella. Genetic studies suggested that the H antigenic determinants were aligned in the central region of the flagellin gene, while nonflagellate mutants had mutations that were localized in both terminal regions (4, 5, 21, 22). Moreover, the amino acid sequences of the central regions of flagellins are very heterologous among bacteria, which also suggests that the central region of a flagellin affects the H antigenicity (2, 3, 7, 9, 12, 20).

In this study, I attempted to alter the H antigenicity of *Escherichia coli* K-12 by deleting a part of the flagellin gene

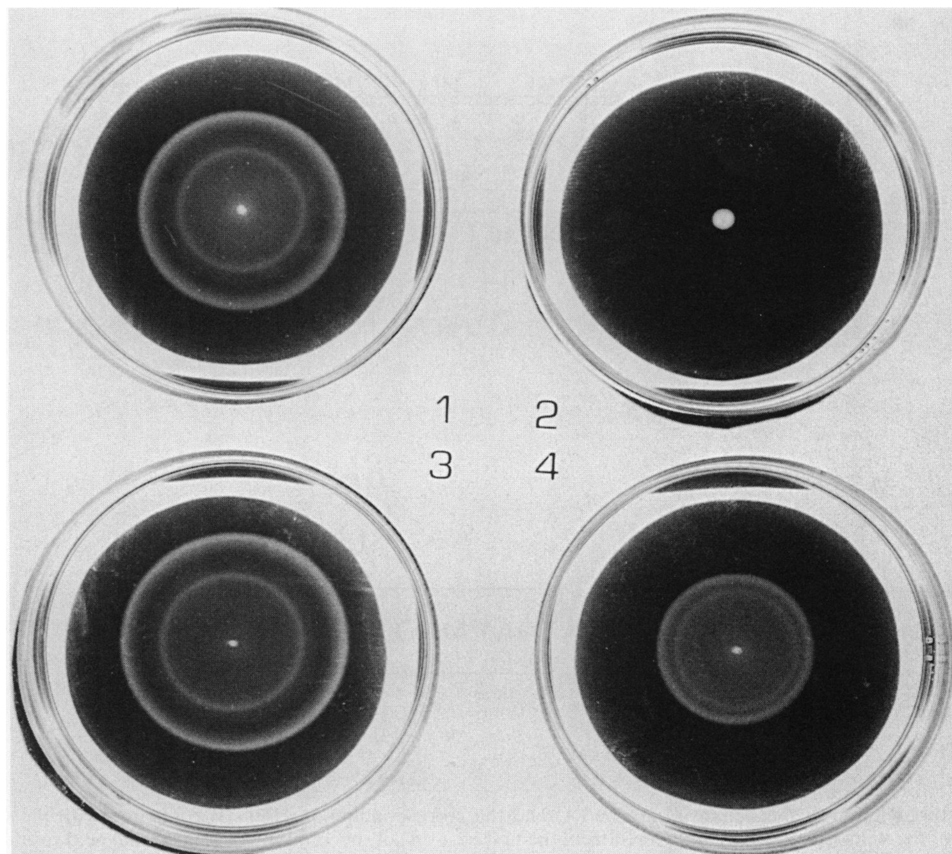

FIG. 1. Motility of C600 *hsm hsr hag*::Tn*10* carrying either pBR322/hag93 (plates 1 and 2) or pFD2 (plates 3 and 4) on lambda motility agar plates. In plates 2 and 4 the medium contained 0.0001 volume of rabbit antiserum against reconstituted flagellar filaments.

A

```
    10        20        30        40
ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAA - - - - - - -
M  A  Q  V  I  N  T  N  S  L  S  L  I  T  Q
1                          10
```

L pFD2

```
              680       690       700       710
- - - - - - - ACTGATAATGGTAATGATTACTATGCGAAAATCACCGGTGGTGAT
              T  D  N  G  N  D  Y  Y  A  K  I  T  G  G  D
                          230
```

L pFD1

pFD2 R

L pFD3

```
   730       740       750       760       770
AACGATGGGAAGTATTACGCAGTAACAGTTGCTAATGATGGTACAGTGACAATGGCGACT
N  D  G  K  Y  Y  A  V  T  V  A  N  D  G  T  V  T  M  A  T
240                           250
```

pFD3 R

```
   790       800       810       820       830       840
GGAGCAACGGCAAATGCAACTGTAACTGATGCAAATACTACTAAAGCTACAACTATCACT
G  A  T  A  N  A  T  V  T  D  A  N  T  T  K  A  T  T  I  T
260                           270
```

pFD1 R

```
   850       860       870       880
TCAGGCGGTACACCTGTTCAGATTGATAATACTGCAGGTTCCGCA - - - - - - -
S  G  G  T  P  V  Q  I  D  N  T  A  G  S  A
280                           290
```

```
                        1470      1480      1490
- - - - - - - - - - GTACCGCAGCAGGTTCTGTCTCTGCTGCAGGGTTAA
                    V  P  Q  Q  V  L  S  L  L  Q  G  *
                          490
```

B

NH2 1 239 284 497 COOH

ASL pFD2

QAC pFD3

239 254 259 278

GGDNDGKYYAVTVANDGTVTMATGATANATVTDANTTKATTITSGGTPVQ

251 284

pFD1 QAC

FIG. 2. Region of the deletion in the mutant *hag* gene (A) and the corresponding flagellin (B) compared with the wild-type gene and flagellin. Nucleotides of the wild-type gene are numbered from the first base (A) of the initiating codon. Amino acid residues of the wild-type flagellin are numbered from the NH_2 terminus; the one-letter code is used. In panel A the deleted region of the *hag* gene on each plasmid is indicated by an arrow. L and R, Left and right ends, respectively, of the deleted region. In panel B the substitution of three artificial residues for the deleted region in each flagellin is shown.

(*hag*) at random by in vitro DNA manipulation. The plasmid pBR322/hag93 (12), which carries the *hag* gene of *E. coli* K-12, was digested with DNase I in the presence of Mn^{2+} (14), and the linear form of pBR322/hag93 DNA was prepared by using 0.8% agarose gel electrophoresis and further digested with *Bal* 31 exonuclease at various concentrations to remove up to several hundred base pairs of nucleotides from both ends (13). Next, the DNA was treated with Klenow fragment and ligated in the presence of the unphosphorylated 8-mer *Hin*dIII linker (dCAAGCTTG; Takara Shuzo Co., Ltd.) (13). This ligated DNA was introduced into a nonmotile mutant, *E. coli* C600 *hsm hsr hag*::Tn*10*, which was obtained by the insertion of the transposon Tn*10* into the *hag* gene of C600 *hsm hsr* (1) from *E. coli* K-12 YK4516 (a gift from Y. Komeda).

The motility test was done for approximately 10,000 transformants on lambda motility agar plates (11) containing rabbit antiserum against wild-type flagella. The antiserum was prepared by the immunization of a female New Zealand White rabbit with reconstituted flagellum fraction (12, 19). Most of the motile transformants showed very small swarming zones in the presence of the antiserum. However, 13 strains showed distinctly larger swarming zones. From these I selected four strains whose plasmids had *Hin*dIII sites that are not found in pBR322/hag93. These plasmids were designated pFD1, pFD2, pFD3, and pFD4. The *hag* mutant strains possessing these plasmids showed good motility even in the presence of the antiserum, although the strain possessing pBR322/hag93, whose *hag* gene encodes the wild-type flagellin, showed limited motility (Fig. 1). A 10-fold or higher concentration of the antiserum was required for C600 *hsm hsr hag*::Tn*10* carrying the selected plasmids to attain the same extent of motility inhibition as observed in the strain carrying pBR322/hag93. However, control serum from an unimmunized rabbit had no effect on any strains (data not shown). This fact indicates that the flagellins encoded by the *hag* genes on pFD1, pFD2, pFD3, and pFD4 produce flagellar filaments which exhibit an antigenicity significantly different from that of the wild type.

DNA sequences of more than 100 nucleotides around the *Hin*dIII site of each plasmid were determined by the dideoxy method (18) with M13mp18 phage (15, 23). A comparison of these sequences with that of the wild-type *hag* gene (12) is shown in Fig. 2A. The *hag* genes on pFD1, pFD2, and pFD3 had 101, 47, and 59 base pairs, respectively, deleted from the wild-type *hag* gene. The same deletion occurred in the *hag* genes on plasmids pFD3 and pFD4. In all cases, the sequence of the 8-mer *Hin*dIII linker dCAAGCTTG was inserted into the gap. The codon frames of the remaining parts were not shifted from that of the wild-type *hag* gene. Thus, the mutant *hag* genes on pFD1, pFD2, and pFD3 encode the mutant flagellins, in which are deleted 34 amino acid residues from Asn-251 to Pro-284, 16 residues from Asp-239 to Thr-254, and 20 residues from Thr-259 to Ile-278 of the wild-type flagellin, respectively (Fig. 2B). An insertion of three amino acid residues was present in each deficient region as a result of the presence of *Hin*dIII linker DNA in the gene.

These deletions occurred in the small region of 46 amino acid residues from Asp-239 to Pro-284 of the wild-type flagellin, which accounts for less than 10% of the wild-type molecule. Furthermore, the deficient region of the pFD1-encoded flagellin includes that of the pFD3-encoded flagellin and partially overlaps that of the pFD2-encoded flagellin. From these results, I conclude that the region from residue 239 to residue 284 is the domain which strongly affects the H antigenicity of *E. coli* K-12. This region might contain some of the epitopes for the H antigen, since the deletion of it led to a drastic change of the H antigenicity. This result is consistent with those of others, which suggested the direct participation of the central region of bacterial flagellin in the exhibition of H antigenicity (8–10, 16, 17).

Another interesting point is that even the abnormal flagellin with a small deletion in the central region can result in flagella which confer good motility to bacteria. On the other hand, it has been reported that both terminal regions of a flagellin molecule show high amino acid sequence homology among various bacteria and seem to be important for flagellum construction (2, 3, 7, 9, 12, 20). Also, genetic studies have suggested that both ends of flagellin play a role in flagellum construction (4, 5, 21, 22). Use of plasmid pFD1, pFD2, or pFD3 should enable us to increase the extent of the deletion in the central region of the *E. coli* flagellin very easily and allow us to examine the relationship between the structure and the function of the flagellin molecule.

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LITERATURE CITED

1. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36:**525–557.
2. **DeLange, R. J., J. Y. Chang, J. H. Shaper, and A. N. Glazer.** 1976. Amino acid sequence of flagellin of *Bacillus subtilis* 168. III. Tryptic peptides, *N*-bromosuccinimide peptides, and the complete amino acid sequence. J. Biol. Chem. **251:**705–711.
3. **Gill, P. R., and N. Agabian.** 1983. The nucleotide sequence of the M_r=28,500 flagellin gene of *Caulobacter crescentus*. J. Biol. Chem. **258:**7395–7401.
4. **Homma, M., H. Fujita, S. Yamaguchi, and T. Iino.** 1987. Regions of *Salmonella typhimurium* flagellin essential for its polymerization and excretion. J. Bacteriol. **169:**291–296.
5. **Horiguchi, T., S. Yamaguchi, K. Yao, T. Taira, and T. Iino.** 1975. Genetic analysis of *H1*, the structural gene for phase-1 flagellin in *Salmonella*. J. Gen. Microbiol. **91:**139–149.
6. **Iino, T.** 1969. Genetics and chemistry of bacterial flagella. Bacteriol. Rev. **33:**454–475.
7. **Iino, T.** 1977. Genetics of structure and function of bacterial flagella. Annu. Rev. Genet. **11:**161–182.
8. **Joys, T. M.** 1976. Identification of an antibody binding site in the phase-1 flagellar protein of *Salmonella typhimurium*. Microbios **15:**221–228.
9. **Joys, T. M.** 1985. The covalent structure of the phase-1 flagellar filament protein of *Salmonella typhimurium* and its comparison with other flagellins. J. Biol. Chem. **260:**15758–15761.
10. **Joys, T. M., and J. F. Martin.** 1973. Identification of amino acid changes in serological mutants of the *i* flagellar antigen of *Salmonella typhimurium*. Microbios **7:**71–73.
11. **Kondoh, H., and H. Ozeki.** 1976. Deletion and amber mutants of *fla* loci in *Escherichia coli* K-12. Genetics **84:**403–421.
12. **Kuwajima, G., J. Asaka, T. Fujiwara, T. Fujiwara, K. Node, and E. Kondoh.** 1986. Nucleotide sequence of the *hag* gene encoding flagellin of *Eschericha coli*. J. Bacteriol. **168:**1479–1483.
13. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 98–148. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Melgar, E., and D. A. Goldthwait.** 1968. Deoxyribonucleic acid nucleases. II. The effects of metals on the mechanism of action of deoxyribonuclease I. J. Biol. Chem. **243:**4409–4416.
15. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101:**20–78.
16. **Parish, C. R., and G. L. Ada.** 1969. Cleavage of bacterial flagellin with cyanogen bromide. Chemical and physical properties of the protein fragments. Biochem. J. **113:**489–499.
17. **Parish, C. R., R. Wistar, and G. L. Ada.** 1969. Cleavage of bacterial flagellin with cyanogen bromide. Antigenic properties

of the protein fragments. Biochem. J. **113**:501–506.

18. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
19. **Wakabayashi, K., H. Hotani, and S. Asakura.** 1969. Polymerization of salmonella flagellin in the presence of high concentrations of salts. Biochim. Biophys. Acta **175**:195–203.
20. **Wei, L.-N., and T. M. Joys.** 1985. Covalent structure of three phase-1 flagellar filament proteins of *Salmonella*. J. Mol. Biol. **186**:791–803.
21. **Yamaguchi, S., H. Fujita, K. Sugita, T. Taira, and T. Iino.** 1984. Genetic analysis of *H2*, the structural gene for phase-2 flagellin in *Salmonella*. J. Gen. Microbiol. **130**:255–265.
22. **Yamaguchi, S., and T. Iino.** 1969. Genetic determination of the antigenic specificity of flagellar protein in salmonella. J. Gen. Microbiol. **55**:59–74.
23. **Yanish-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.