Possession of Flagellar Hooks by Some Non-flagellate Mutants of *Salmonella abortusequi*

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SUMMARY

The possession of flagellar hooks by non-flagellate mutants derived from phase 1 stable strains of *Salmonella abortusequi* was determined by electron microscopic examination of material detached from these bacteria by mechanical means. Hooks were found in all the preparations from *H1* mutants but none in those from *fla* mutants representative of fourteen different complementation groups. The hooks from *H1* mutants were morphologically indistinguishable from those of wild-type. These results show that all the *fla* genes are involved in hook formation and that the flagellin structural gene *H1* is not.

INTRODUCTION

A bacterial flagellum is composed of a filament, hook, and basal body (Abram, Vatter & Koffler, 1965, 1966; Cohen-Bazire & London, 1967). The filament is the distal helical part of the flagellum, and is a polymer of flagellin. The basal body is the proximal structure, bound to the cell wall and membrane (DePamphilis & Adler, 1971). The hook connects the filament to the basal body. Both filaments and hooks can be detached from flagellated cells by mechanical shaking (Abram, Mitchen, Koffler & Vatter, 1970).

In *Salmonella*, the non-flagellate phenotype results from mutation in either the *H* or *fla* genes (Iino, 1969*a*). The *H* genes, *H1* and *H2*, are the structural genes for the phase 1 and the phase 2 flagellins, respectively. The precise functions of *fla* genes are not known. Fourteen *fla* complementation groups have been recognized (Joys & Stocker, 1965; Iino & Enomoto, 1966; Yamaguchi & Iino, 1969; Yamaguchi, Iino, Horiguchi & Ohta, 1972). So-called non-flagellate mutants lack the filament, but it is not known if they possess other parts of the flagellum. In this study the possession of hooks by non-flagellate mutants, including both those representative of almost all *fla* complementation groups and *H1* mutants derived from phase 1 of stable strains *Salmonella abortusequi* SL23, was determined by electron microscope observation of material mechanically detached from bacteria.

METHODS

Bacteria. SJ241 (*H1-a*) is a phase 1 stable derivative of *Salmonella abortusequi* SL23. Non-flagellate strains, SJW412, SJW414 and SJW417, are *H1* mutants of SJ241. The *fla* strains SJW310 (*flaAI*), SJW297 (*flaAII*), SJW315 (*flaAIII*), SJW272 (*flaB*), SJW227 (*flaD*), SJW413 (*flaL*),

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SJW291 (*flaN*), SJW337 (*flaP*) and SJW246 (*flaQ*) are mutants of SJ241. Other *fla* strains, SJW141 (*flaC*), SJW162 (*flaE*), SJW164 (*flaF*), SJW159 (*flaK*) and SJW467 (*flaM*) are mutants of SJ925 (*Hr-gt*), a derivative of SJ241, in which *Hr-a* of SJ241 was replaced by *Hr-gt* of *Salmonella budapest* strain SJ6 by transduction.

Media. Nutrient broth was 1% (w/v) peptone and 1% (w/v) meat extract in 0.05 M-phosphate buffer (pH 7.2), to which 1.5% (w/v) agar was added to give nutrient agar.

Isolation of hooks from non-flagellate mutants. Ten ml quantities of overnight broth cultures of non-flagellate strains were spread on to nutrient agar in trays (20 × 30 × 1.8 cm). After incubation for 24 h at 37°, bacteria were harvested in 0.85% saline, and centrifuged at 10,000g. Approximately 10 g bacteria (wet weight) were suspended in 100 ml saline, and 10 g fine glass beads added. This suspension was shaken at 750 strokes/min (25 mm amplitude) for 30 min and centrifuged at 10,000g to remove bacteria. The supernatant was centrifuged at 98,000g for 1 h and the pellet resuspended in 0.1 M-tris-hydrochloride (pH 7.8). This suspension was then given two cycles of differential centrifugation first at 10,000g for 10 min to sediment bacterial debris and then at 98,000g for 1 h to sediment hooks. A small portion of this pellet was resuspended in distilled water and observed with the electron microscope. If hooks were seen the remainder of the pellet was resuspended in 1 ml 0.1 M-tris-hydrochloride (pH 7.8), to which 0.1 ml 0.2% lysozyme in 0.1 M-tris-hydrochloride (pH 7.8) and 1 ml 2% Brij-58 in the same buffer were added to lyse remaining cell debris. After incubation at 30° for 30 min, dilution to 30 ml with distilled water and centrifugation at 98,000g for 1 h, the final pellet was resuspended in 0.5 ml distilled water and observed with the electron microscope.

Isolation of hooks from flagellate bacteria. Flagella were isolated and partially purified as described by Yamaguchi & Iino (1969), and crude preparations of hooks obtained from them as follows. Flagella were suspended in distilled water to a final concentration of 10 mg flagella/ml. One ml of the suspension was heated at 60° for 10 min, cooled to 20°, and centrifuged at 98,000g for 1 h, and the pellet resuspended in 0.5 ml distilled water and observed with the electron microscope. Such preparations contained many isolated hooks and a few filaments, most of which had a hook at one end.

Electron microscopy. Samples were negatively stained with 1% uranyl acetate (pH 4.0) and then examined with a JEM 7A electron microscope.

Chemical. Egg white lysozyme (three times crystallized) was obtained from Seikagaku Kogyo Co., Tokyo, Japan. Brij-58 was obtained from Wako Pure Chemical Industries Co., Osaka, Japan.

RESULTS

Hooks were isolated from all the *Hr* mutants, but not from any of the *fla* mutants examined.

After the two cycles of differential centrifugation, the preparations from *Hr* mutants contained both hooks and many membraneous particles; the majority of the latter were removed by treatment with lysozyme and Brij-58. In the preparation from SJW337 (*flaP*) a few flagella were observed and as this strain is known to be leaky, the flagella observed were likely to have been those detached from the few flagellate bacteria in the culture.

Hooks obtained from *Hr* mutants were of almost uniform length (72 ± 8 nm) and diameter (18 nm) (Fig. 1). Their size and shape were indistinguishable from those of the parent strain SJ241 (Fig. 2). One end of these hooks appeared to be frayed or split and the other end blunt (Fig. 1, 2 and 3).

From the observation that *Escherichia coli* hooks showed either a cross-hatched surface pattern or one consisting of parallel lines oblique to the axis of the hook, DePamphilis &

Fig. 1. Hooks isolated from sjw417, an *H1* mutant of phase 1 stable *Salmonella abortusequi* strain sj241.

Fig. 2. Hooks and a flagellum isolated from *Salmonella abortusequi* strain sj241. Round bodies are vesicular fragments of the cell envelope.

Fig. 3. A hook isolated from sjw417.

All the samples were stained with 1 % uranyl acetate.

Adler (1971) proposed a structure comprising several helical coils. They suggested that the first pattern would be the superimposed images of the top and bottom surfaces of the hook and the second a view of one side. Many of the hooks we observed seemed to be more complex; each consisting of a part with a cross-hatched pattern, another showing parallel oblique lines and another appearing hazy (Fig. 3). Such patterns suggest a structure composed of several fine coils and twisted to form a short helix of relatively small pitch.

DISCUSSION

Hook and filament are not only distinguishable morphologically, they also differ in serological specificity (Lawn, 1967) and solubility in acid and/or alcohol (Abram *et al.* 1970). However, it is still possible that they are both composed of flagellin molecules in different conformational states. The presence of hooks in *Hr* mutants showed that hook formation is not under the control of the flagellin structural gene. A similar result was also obtained with *Salmonella typhimurium* (R. J. Martinez, personal communication).

It has been proposed that *fla* either produces an internal inducer for flagellin synthesis, or controls the production of a component of the whole flagellum-forming apparatus (Iino, 1969*a*). The absence of hooks as well as filaments from the *fla* mutants favours the second hypothesis. In *Escherichia coli* eight different structures have been distinguished in the hook-basal body complex (DePamphilis & Adler, 1971), and this could imply the involvement of as many as eight different *fla* genes. If this is so, how does the *fla* region control the formation of the hook and/or the filament besides being involved in the formation of some specific structure? An examination of *fla* mutants for basal body defects could aid understanding of the function of *fla* genes.

It has been shown that flagellar filaments have a structural polarity, i.e. only the distal ends of the fragments of the filaments appear to be frayed, and that they are formed by a sequential polymerization of flagellin molecules at the distal end of each filament (Asakura, Eguchi & Iino, 1968; Iino, 1969*b*). The structural similarity of the distal frayed end of the hook (Fig. 3) to that of the filament may also indicate formation of the hook by sequential polymerization of subunit molecules. As hooks are about the same length irrespective of the presence or absence of the filaments it is suggested that their maximum length is limited by some factor, and that the possibility of the addition of flagellin to these distal ends being the limiting factor is excluded. It is also difficult to visualize the existence of a core determining the length of the hook, if the observed cross-hatched pattern of the hook (Fig. 3) really represents the superimposed image of the upper and lower surfaces of the hook.

Hooks are supposed to serve as the initiation points for polymerization of flagellin into the filaments. We have obtained provisional evidence that the hooks of *Hr* mutants are involved in the initiation of polymerization of external flagellin into filaments.

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