Comparison of the Flagellins from Different Flagellar Morphotypes of *Escherichia coli*

By A. M. LAWN*

Lister Institute of Preventive Medicine, London SW1W 8RH

(Received 9 February 1977)

The molecular weights of the flagellins of 13 strains of *Escherichia coli*, each with a different H antigen, were estimated using polyacrylamide gel electrophoresis. In each case only one major polypeptide was demonstrated, although some strains possessed apparently sheathed flagella. Considerable differences in the molecular weight of flagellin accompanied the previously described structural differences between flagella from strains with different H antigens. The relationship between flagellar diameter and the molecular weight of the corresponding flagellins was similar for both unsheathed and apparently sheathed flagella. Cross-polymerization occurred between seed consisting of fragments of unsheathed flagella and flagellin solution from apparently sheathed flagella and vice versa. Co-polymerization of flagellin from unsheathed flagella and flagellin from apparently sheathed flagella was also demonstrated. These polymerization experiments indicate that the assembly pattern of flagellin molecules is probably the same in all *E. coli* flagella. The above and other evidence suggests that there is no true sheath, but that the differences in flagellar surface structure between different *E. coli* flagella are the result of differences in the superficial parts of the flagellin molecules.

INTRODUCTION

Considerable variation of surface structure exists between flagella from different H serotypes of *E. coli*, which can be grouped into different morphotypes (Lawn, Ørskov & Ørskov, 1977). The flagellar filaments of some morphotypes have a surface structure which, in other genera of enterobacteria, has been attributed to the presence of a separate sheath (Lowy & Hanson, 1965; Schmitt, Raska & Mayer, 1974). This account describes investigations designed to detect the presence of a separate sheath protein or differences in the pattern of assembly of the protein units to form the filaments of different flagellar morphotypes. The use of sodium dodecyl sulphate-polyacrylamide gel electrophoresis was expected to reveal the presence of an additional sheath protein, and major differences in the pattern of assembly of flagellin, the protein subunit of flagella, were expected to preclude cross-polymerization (block co-polymerization; Asakura, Eguchi & Iino, 1966).

METHODS

Bacterial strains. The strains of *E. coli* were all from the collection of standard H reference strains at the Collaborative Centre for Reference and Research on *Escherichia coli* (WHO), Copenhagen. Before they were used for preparing purified flagella they were passaged through Craigie tubes containing motility medium with a gelatine and agar basis. Such highly motile variants could be stored in liquid nitrogen without loss of motility.

* Present address: Houghton Poultry Research Station, Houghton, Huntingdon, Cambridgeshire PE17 2DA.

Preparation of purified flagella. A 5 l flask containing 1 l Oxoid nutrient broth no. 2 was inoculated with a culture of a highly motile variant of the required strain and grown with shaking at 37 °C for 8 to 16 h. The bacteria with attached flagella were harvested by low-speed centrifugation, resuspended in a small amount of phosphate-buffered saline (PBS, Dulbecco's solution A, pH 7.4) and treated in a Waring-type blender to release the flagella. The bacteria were removed by centrifugation and washed with a small volume of PBS. The washings and the original supernatant were combined, and the flagella were recovered by high-speed centrifugation and resuspended in distilled water. The preparations were purified by alternate high and low speed centrifugation in distilled water until the pellet of flagella appeared almost homogeneous. The flagella from 1 l of culture suspended in 1 ml distilled water formed the stock suspensions for use in all the experiments; their concentration varied considerably between batches. Because of the small scale of the preparations, protein estimation and adjustment of protein concentration was not attempted.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The discontinuous method described by Maizel (1971) was followed. The samples were heated in a boiling water bath for 90 s in the presence of sodium dodecyl sulphate (SDS) and mercaptoethanol and cooled before layering under the electrode buffer covering the upper stacking gel of tubes containing approximately 1 cm of stacking gel [2 % (w/v) acrylamide] and approximately 12 cm of separating gel [8 or 10 % (w/v) acrylamide]. The electrode, sample and stacking gel buffers (pH 9), the separating gel buffer (pH 7) and the marker dye were as described by Maizel (1971). The tubes were run at 3 mA per tube until the sharp marker dye band was a few millimetres from the lower end of the tube. Protein bands were stained with Coomassie brilliant blue.

Determination of molecular weight. Four reference proteins were used: phosphorylase from rabbit muscle (mol. wt 93 000), bovine serum albumin (65 000), glyceraldehyde phosphate dehydrogenase (36 000) and carbonate dehydrogenase (31 000). In each run, one or two tubes were loaded with a mixture of 2 µg of each of the standard proteins while other tubes were loaded with both the flagellar protein and two or more of the standards. Migration distances were corrected using the distance travelled by the marker dye in individual tubes and the average distance for each standard was plotted against the logarithm of the molecular weight. The resulting points were joined by a hand-fitted, smooth curve. From this line, which was not exactly linear, the molecular weight corresponding to the average migration distance of the flagellar polypeptide was determined. In preliminary runs large amounts of flagellar protein were loaded (up to 0.1 ml) in order to estimate the number of distinguishable polypeptides present in each preparation and their concentration. The reproducibility of the method was checked for H12 flagellin using the results for nine tubes from three runs and for H46 using eleven tubes from five runs. The standard deviations were ± 2450 (mol. wt 61 920) and ± 2020 (mol. wt 56 350) respectively.

Preparation of flagellar protein. The stock suspensions of flagella, or sonicated stock suspensions, were solubilized by treatment with acid or heat. For the former, 0.1 M-HCl was added to the stock suspension, or more usually to a 1:10 dilution of this in distilled water, until the pH was approximately 3.0. Insoluble material was removed by high-speed centrifugation. The supernatant was then dialysed against distilled water (for gel electrophoresis) or PBS (for polymerization). For heat dissociation, the stock suspension, or sonicated suspension, was heated in a water bath at 65 °C for the minimum time required to depolymerize the majority of the flagella, as determined by electron microscopy, and then rapidly cooled. The times used were between 5 and 15 min. The resulting solution was cleared of flagellar fragments by high-speed centrifugation.

Polymerization of flagella. The methods for preparing seed and for repolymerizing flagella on to seed followed those used by Asakura & Eguchi (1964), except that the experiments were carried out with PBS as the vehicle, and a micromethod was used. The seed was obtained by sonication of the stock suspensions of flagella using a miniature ultrasonic bath (Millipore) for three periods of 5 min each at 5 min intervals, the tube containing the suspension of flagella being immersed in ice-cold water throughout. The fragments were recovered by high-speed centrifugation and resuspended in PBS to the initial volume. One-tenth of this suspension was diluted 1:10 with PBS to form the seed suspension and the remainder was used to make soluble flagellar protein (flagellin solution) as described above. Equal quantities of seed suspension and flagellin solution (25 µl of each) were mixed in plastic tubes and left at room temperature for 24 h when an equal quantity (50 µl) of 2.5 % (w/v) glutaraldehyde in PBS was added. After a further 24 h fixation the preparations were prepared for electron microscopy. A copper grid, covered with a carbon-coated formvar membrane and subjected to ionic bombardment to render the membrane hydrophilic, was floated face down on 5 µl of the seed/monomer mixture for several minutes. The grid was then washed in distilled water, uranyl acetate [approximately 0.5 % (w/v)] was added and the surplus was immediately and rapidly withdrawn.

Fig. 1. SDS-polyacrylamide gel electrophoresis of flagellins from flagella of serotypes H12, H21 and H4. The marked difference in mobility between flagellins from the three types of flagella is demonstrated. The gel labelled M was loaded with a mixture of the three proteins, and that labelled S was loaded with four standard proteins: phosphorylase from rabbit muscle (mol. wt 93000), bovine serum albumin (65000), glyceraldehyde phosphate dehydrogenase from rabbit muscle (36000) and carbonate dehydrogenase from bovine erythrocytes (31000).

Table 1. *Some properties of the flagella and of the corresponding flagellins of different H serotypes of Escherichia coli*

The flagellar morphotypes are described by Lawn *et al.* (1977). The diameters include the full extent of any regular projections from the flagellar filament and were determined by negative contrast with uranyl acetate of aldehyde-fixed flagella; each value is the mean of 10 measurements. Molecular weights were calculated, as described in the Methods, from SDS-polyacrylamide gel electrophoresis of flagellins and standard reference proteins; each value is the mean of a number of estimations (given in parentheses) from individual gels.

Strain	Flagellar morpho-type	H serotype	Surface structure of flagella	Approx. diam. of flagella (nm)	Mol. wt of flagellin
U9-41	A	4	Thin, long pitch helix pattern	19	37000 (2)
P12b	A	17	Thin, long pitch helix pattern	20	39000 (2)
U4-41	B	5	Thin, subunit pattern	20	46000 (3)
N234	B	25	Thin, subunit pattern	20	50000 (3)
Su4321-41	D	11	Polar subunit pattern	21	54000 (1)
U11a-44	D	21	Polar subunit pattern	22	56000 (3)
Su1242	E	1	Short pitch loop pattern	24	60000 (6)
U5-41	E	7	Short pitch loop pattern	23	61000 (6)
Bi316-42	E	12	Short pitch loop pattern	22	62000 (9)
781-55	F	44	Long pitch loop pattern	25	59000 (3)
5306-56	F	46	Long pitch loop pattern	24	56000 (11)
Bi7575-41	—	9	Thick, long pitch loop pattern	27	69000 (3)
5017-53	—	36	Thick, rope-like pattern	25	61000 (6)

Fig. 2. The relationship between the diameter of the flagellar filament and the molecular weight of the corresponding flagellin for different H serotypes of *Escherichia coli*. There is no consistent difference in distribution between the ringed ●, which represent flagella whose surface structure suggests the presence of a sheath, and the remaining ●, which represent conventional flagella. The straight line is the regression line of d^2 on molecular weight.

RESULTS

Number of polypeptides present in solubilized protein from E. coli flagella of different morphotypes

When acid-soluble and heat-soluble protein from *E. coli* flagella of 13 different serotypes was examined by discontinuous SDS-polyacrylamide gel electrophoresis (Fig. 1), in every case only one major band was consistently observed. The flagella were prepared from the standard reference strains of H serotypes 1, 4, 5, 7, 9, 11, 12, 17, 21, 25, 36, 44 and 46, and included examples of five of the six principal morphotypes described by Lawn *et al.* (1977) (Table 1). In a few preparations a flagellin band was split, but this could be attributed to technical inadequacies and did not occur consistently for any one serotype. Faint additional bands were sometimes observed. The single major band was taken to be the polypeptide, flagellin, from which the flagellar filament is assembled (see Smith & Koffler, 1971).

Molecular weight of flagellins of various serotypes of E. coli

The molecular weights of the flagellins of 13 serotypes (Table 1) were determined by SDS-polyacrylamide gel electrophoresis using standard reference proteins. A considerable range of molecular weights was observed, from 37000 for H₄ flagellin to 69000 for H₉ flagellin.

Relationship between the molecular weight of flagellin and the diameter of the corresponding flagellum

When the molecular weights of flagellins from different *E. coli* flagella were plotted against the square of the diameter (d) of the corresponding flagellum there was apparently no consistent difference in the ratio of d^2 /mol. wt between flagellin from morphotypes which have sheath-like surfaces and those having conventional surfaces (Fig. 2). This result is compatible with a model in which variation in diameter of *E. coli* flagella between different serotypes is due simply to variations in the size of the polypeptide chain of flagellin, without variation in the pattern of assembly of the polypeptides in the flagellar filament.

Table 2. *Cross-polymerization of flagella seed fragments and flagellin solutions from different serotypes of Escherichia coli*

The rows represent seed (fragments of flagella produced by sonication) of different types. The flagellar morphotypes are listed in Table 1. The columns represent solutions of flagellin obtained by heat (H) or acid (A) treatment of suspensions of flagella from different serotypes (see Methods).

Serotype	Flagellin					
	H5(H)	H11(A)	H11(H)	H21(H)	H7(H)	H46(A)
Seed	H4	NT	NT	+	NT	+
	H17	—	+	NT	NT	+
	H5	+	—	NT	NT	+
	H11	—	NT	NT	+	NT
	H21	—	NT	+	NT	—
	H1	—	—	+	NT	NT
	H44	—	—	+	NT	NT
	H46	+	+	+	NT	—
	H36	+	—	+	NT	(+)
					(+)	(+)

+, Polymerization of flagellar filaments from the flagellin defined by the corresponding column occurred on the seed defined by the intersecting row; (+), possible but doubtful polymerization; —, absence of detectable polymerization using the method described in text; NT, not tested.

Cross-polymerization (block co-polymerization) of flagellin from one flagellar morphotype on the seed of another

Seed was prepared from the flagella of nine different serotypes and solubilized flagellin from eight different serotypes. Only flagellin preparations which gave polymerization with one or more seed preparations are included in Table 2. The flagellin was prepared either by heating or by acid treatment of the sonicated fragments of flagella used as seed, as described in Methods.

In the first experiment seed of H11 and H21 were used with the opposite monomers. As these serotypes both have flagella of morphotype D (polar subunit pattern, Lawn *et al.*, 1977), the seed and newly polymerized filament needed to be distinguished by using specific antiserum (Asakura *et al.*, 1966) derived by cross-absorbing antiserum to H11 flagella with H21 flagella. Only short lengths of H21 polymer were polymerized on H11 seed but H11 flagellin gave up to several wavelengths of new polymer on H21 seed.

Seed of one morphotype was then mixed with flagellin from a different morphotype in a wide variety of combinations (Table 2). The variation in efficiency of polymerization noted with H11/H21 cross-polymerization was even more marked. Some flagellins, for example heat-dissociated H9, would not polymerize on any seed, although examination of this solution by SDS-polyacrylamide gel electrophoresis indicated that it contained as much polypeptide as others that polymerized efficiently. Other preparations, for example H46 and H7, gave only short segments of new polymer whereas heat-dissociated preparations of H5 and H11 flagella gave particularly good polymerization.

Heating appears to have a more drastic effect on flagella of some serotypes than on others. A preparation of H17 flagella precipitated after heating at 65° C for 15 min but the supernatant after high-speed centrifugation contained no flagellin detectable by gel electrophoresis. Heating at the same temperature for 1 min considerably reduced the number of flagella and gave no immediate precipitate but again there was no detectable protein in the supernatant after high-speed centrifugation. The clear supernatant obtained after acid dissociation of H17 flagella gave a precipitate when dialysed against PBS at pH 7.4. Examination of this precipitate in the electron microscope revealed no flagella. None of these 'flagellin' preparations gave polymerization of H17 filaments with a number of different seed preparations. That the H9 flagellin solution contained defective flagellin is suggested

Fig. 3. Cross-polymerization of seed fragments of H36 flagella and flagellin solution from H5 flagella. (a) Low-power micrograph showing that the change from seed to new polymer (arrowed) does not interrupt the overall sinusoidal course of the flagellum. Bar marker represents $0.5 \mu\text{m}$. (b) The junction between seed and new polymer at higher magnification. Bar marker represents 50 nm .

Fig. 4. Cross-polymerization of seed fragments of one flagellar serotype and flagellin solution from another serotype of *Escherichia coli*. Various combinations are shown, in some of which the seed and the new polymer have entirely different surface structures and diameters, yet join smoothly. The serotypes used were: (a) H36 seed and H11 flagellin; (b) H46 seed and H11 flagellin; (c) H17 seed and H46 flagellin; (d) H5 seed and H7 flagellin. Bar marker represents 50 nm .

Fig. 5. Co-polymerization between flagellins from flagella of two serotypes on to seed fragments of a third type. (a), (c) Pure flagellin solutions from flagella of H7 and H5 respectively have polymerized on to seed of H4 flagella to give a new filament with a surface pattern characteristic of the parent (right side) and different from that of the seed (left side). (b) A mixture of equal parts of flagellin solutions from H5 and H7 flagella was added to the same seed. A flagellar filament has polymerized which is not characteristic of either H5 or H7 flagella, but is a co-polymer of the two flagellins. Bar marker represents 50 nm.

by results reported in the following section. In view of the results described above no significance should be attached to the negative findings in Table 2. No attempt was made to optimize the conditions for depolymerization or repolymerization of the flagella, neither were repeated attempts made to obtain polymerization, and the concentrations of the stock suspensions of flagella were not equal.

In spite of these limitations cross-polymerization was obtained with a number of different combinations some of which gave new filaments attached to seed of an entirely different morphotype (Figs 3 and 4). When a sufficient length of new filament was formed it continued the sinusoidal curve of the seed perfectly (Fig. 3) and the junction of seed and new filament always occurred without detectable deviation of the axis (Figs 3 and 4).

When H11 flagella were used as seed or monomer their strong polarity (Lawn *et al.*, 1977) established that polymerization of the new filament was always initiated from the fork-shaped end, as described by Asakura *et al.* (1966). With flagellin from flagella of morphotypes E and F, which apparently have sheaths, the sheath-like appearance was present throughout the length of the new filament.

The observation of J. Pye (quoted by Asakura *et al.*, 1966) that polymerization was not initiated by flagella attached to bacteria, was confirmed.

Co-polymerization of flagellins from flagella of two different morphotypes

The results reported in the previous section, i.e. that flagellin from flagella of one morphotype would polymerize on to seed of fragments of flagella of an entirely different morphotype, suggested that the assembly pattern of the subunits of all these flagellar filaments was similar. It was decided to use a more stringent test for this hypothesis by attempting to assemble flagellin from flagella of two different morphotypes into one flagellar filament (Asakura *et al.*, 1966). The polymerization experiments were performed as before except that instead of using a pure flagellin from one H serotype, the same volume of a mixture of two different flagellins was used. In different tubes the ratios of the volume of one flagellin solution to that of the other were 125:1, 25:1, 5:1, 1:1, 1:5, 1:25, and 1:125. Three types of polymerization mixture were used, in each case with seed of H17 and with heat-dissociated flagellin from H5 as two of the components. The third component was heat-depoly-

merized H7, H9 or H46 flagellin. As the amount of flagellin in each preparation varied and was not known, the ratio of the volumes of the flagellin solutions did not necessarily define the ratio of the numbers of molecules of each flagellin present per unit volume of the final polymerization mixture.

When the ratio of the volume of H5 flagellin to the volume of H7, H9 or H46 flagellin was 5:1 or more, long lengths of H5 filament were polymerized on to the H17 seed fragments and the presence of the other types of flagellin in the polymer could not be convincingly demonstrated, but isolated molecules might well have been undetectable. When the ratio was 1:125, short segments of H7 or H46 filament were found on some of the seed fragments but there was no detectable polymerization of H5 filaments. No polymerization of any kind was detected at this ratio when the second flagellin was from H9 flagella. With 1:1 mixtures of H5 and either H7 or H46 flagellin solutions, short segments of filaments were polymerized on some seed fragments but their surface structure was not completely like that of either of the parent flagella (Fig. 5). With a 1:1 mixture of H5 and H9 flagellin solutions there was no detectable polymerization. The interpretation of these results must be influenced by the fact that when these same H5, H7, H9 and H46 flagellin solutions were mixed individually with suitable seed, H5 polymerized excellently, H7 and H46 poorly and in only short segments, and H9 showed no convincing evidence of polymerization.

DISCUSSION

Two principal hypotheses can be advanced to explain the differences in surface structure between *E. coli* flagella of different morphotypes. One assumes that some of the differences arise from the presence in some morphotypes of two layers of protein molecules, one forming the core and the other a separate sheath (Lowy & Hanson, 1965; Schmitt *et al.*, 1974), whereas the other supposes that all the flagella are constructed from only one layer of flagellin molecules and that the different surface structure results from differences in the arrangement of the outer parts of these molecules. Three types of evidence in favour of the second hypothesis are provided by results reported here. The first is that only one major polypeptide band was present when acid- or heat-soluble protein from purified flagella of 13 different serotypes was examined by SDS-polyacrylamide gel electrophoresis. Five of these serotypes were from morphotypes E and F which are apparently sheathed (Lawn *et al.*, 1977), or six if H9 is accepted as having a sheath-like surface. Thus, unless in all six cases the molecular weight of the sheath protein is indistinguishable from the accompanying core protein using this technique, there is no separate sheath protein. The sheath protein might be insoluble in acid conditions or after heating, but samples of the same solutions of flagellin that polymerized to form normal filaments, apparently sheathed, gave single bands in SDS-polyacrylamide gel electrophoresis. Schmitt *et al.* (1974) also found a single flagellin band using apparently sheathed flagella from *Pseudomonas rhodos*.

The second type of evidence arises from a comparison between the molecular weight of a flagellin and the diameter of the corresponding flagella (Fig. 2). If a flagellum has a separate sheath layer, then its diameter will be the sum of the thicknesses of the core and the sheath layer. Variation of the molecular weight of one of these proteins would not be expected to give a simple relationship between diameter and molecular weight. However, Fig. 2 shows that the relationship between d^2 and molecular weight is approximately linear, and that the flagellins of apparently sheathed flagella are not distributed differently from those of un-sheathed flagella.

The third type of evidence arises from the observation that throughout this investigation not one of the so-called sheathed flagella was ever seen as a sheathless core of constant diameter in even part of a filament. This was true of sonicated flagella, of newly polymerized flagella and of flagella in various stages of dissociation as a result of acid conditions.

The results of polyacrylamide gel electrophoresis of flagellins and those summarized in

Fig. 2 do not exclude the possibility that the same protein, assembled in different ways, forms both sheath and core (Schmitt *et al.*, 1974), but an analysis of the results of the polymerization experiments provides further evidence against the presence of a separate sheath. In this case the core of the flagellum would be a complete layer of protein molecules and comparable to the entire filament of a non-sheathed flagellum. Measurements of the diameter of this core show that it has approximately the same diameter as that of the entire filament of the flagella of morphotype A, i.e. those of serotypes H4 and H17. However, the molecular weight of the flagellins from apparently sheathed flagella is in every case considerably greater than the molecular weight of H4 and H17 flagellins. If two molecules of very different molecular weight are to be assembled into a rod of the same diameter then there will either be considerably more fluid-filled space within the rod formed from the smaller molecules (for example, H4 or H17 flagella), for which there is no evidence, or the number of molecules per unit length, and therefore the assembly pattern of the molecules, must differ in the two cases. If it can be shown, on the contrary, that the assembly pattern is the same in both instances, then a consideration of the molecular weight relationships rules out the presence of a separate sheath.

The ability of one type of flagellar filament to polymerize on to seed consisting of fragments of another without detectable deviation of the axial direction suggests that the assembly pattern of the two types is nearly, if not absolutely, identical and it is shown here that thin, unsheathed flagella and thicker flagella that are apparently sheathed can be cross-polymerized in this way. The ability of flagellin from apparently sheathed flagella and flagellin from unsheathed flagella to co-polymerize, forming a rod with the form of a flagellum which continues the axis of the seed but has a surface structure indicating a mixture of the two types of flagellin, is even stronger evidence. It is difficult to believe that molecules with bonding regions adapted to the formation of one helical lattice could be inserted into spaces in another helical lattice without disrupting assembly, unless the two lattices were nearly identical. There is evidence that defective flagellin molecules disrupt assembly (see below).

If the evidence from cross-polymerization and co-polymerization is accepted as proving that the flagellin subunits are assembled into a nearly identical lattice in both apparently sheathed and in unsheathed flagella of *E. coli* then, as discussed above, a separate sheath cannot be present. In this case the linear relationship between d^2 and molecular weight is easily explained for, in an identical lattice, a large molecule must have a greater radial extent than a small molecule. The variation from an exact linear relationship in Fig. 2 can be attributed to the variation in degree of space-filling by flagellin in different types of flagella which is demonstrated by the differing extent of penetration of the negative-contrast agent, and also to inaccuracies of measurement.

A model of the structural gene for the flagellin of *E. coli* can be constructed to explain these observations. This hypothesis assumes that the gene can be divided into two parts, which may be termed the constant and variable regions. One end of the polypeptide, the code for which occupies the constant part of the gene, folds to form the part of the flagellin molecule which is innermost in the assembled flagellar filament, and which carries the specific bonding regions required to assemble a rigid flagellum possessing a spiral conformation. Mutations in this region of the gene are contra-selected as they abolish motility either because the flagellin cannot be assembled or because the resulting filament is unsatisfactory as a propulsive organ, for example straight or flexible. Martinez *et al.* (1968) have presented evidence that a single amino-acid substitution can produce a straight flagellum; this substitution would correspond to one in the constant part of the flagellin gene of *E. coli*. The part of the polypeptide whose code occupies the variable region of the gene lies on the outside of the flagellum and plays no part in its assembly so that massive insertions or deletions are permissible. This part is responsible for the surface properties of the flagellum including the exposed antigenic determinants, and mutations may frequently be advantageous because they allow the bacterium to escape the effect of the immobilizing antibody produced by the

host organism. Such mutations would be responsible for creating the different H serotypes of *E. coli*.

The surface structures seen in different morphotypes must, then, be the result of different arrangements of the outer portions of the flagellum molecules, but the nature of the associations between neighbouring molecules is not immediately apparent. For example, the surface pattern of H5 flagella is relatively simple, but a calculation of the surface area occupied by a space-filling protein molecule of molecular weight 46000 in a filament 21 nm in diameter gives an area three or four times less than that occupied by each apparent subunit. There is no evidence from the degree of penetration of the contrast agent to suggest that the molecules are widely separated from each other in H5 flagella and so it must be assumed that an apparent subunit is composed of several neighbouring polypeptide chains. Of course the subunit-like pattern might arise from the superimposition of front and back images of a flagellar filament possessing helical surface grooves which are not further subdivided.

An observation that requires comment is that some flagellin preparations only polymerized to form short lengths of filament, whose length was relatively constant even when different types or amounts of seed were used. The most likely explanation is that a proportion of flagellin molecules were denatured, and when by chance one, or a group, of these defective molecules was attached at the growing end of the filament further addition of molecules was prevented. This finding suggests a similar explanation for the observation that an H9 flagellin preparation gave no detectable polymerization but inhibited the polymerization of H5 flagellin; the majority of the H9 flagellin molecules might have been defective. Many other explanations for the phenomenon are, however, possible, and the polymerization of H9 monomer on H9 seed was not investigated.

The author is grateful to the Collaborative Centre for Reference and Research on *Escherichia coli* and Drs F. and I. Ørskov for the generous gift of bacterial strains and to Ivor Osborne for excellent technical assistance.

REFERENCES

- ASAKURA, S. & EGUCHI, G. (1964). Reconstitution of bacterial flagella *in vitro*. *Journal of Molecular Biology* **10**, 42-56.
- ASAKURA, S., EGUCHI, G. & IINO, T. (1966). *Salmonella* flagella: *in vitro* reconstruction and overall shapes of flagellar filaments. *Journal of Molecular Biology* **16**, 302-316.
- LAWN, A. M., ØRSKOV, I. & ØRSKOV, F. (1977). Morphological distinction between different H serotypes of *Escherichia coli*. *Journal of General Microbiology* **101**, 111-119.
- LOWY, J. & HANSON, J. (1965). Electron microscope studies of bacterial flagella. *Journal of Molecular Biology* **11**, 293-313.
- MAIZEL, J. V. (1971). Polyacrylamide gel electrophoresis of viral proteins. In *Methods in Virology*, vol. 5, pp. 179-246. Edited by K. Maramorosch and H. Koprowski. New York: Academic Press.
- MARTINEZ, R. J., ICHIKI, A. T., LUNDH, N. P. & TRONICK, S. R. (1968). A single amino acid substitution responsible for altered flagella morphology. *Journal of Molecular Biology* **34**, 559-564.
- SCHMITT, R., RASKA, I. & MAYER, F. (1974). Plain and complex flagella of *Pseudomonas rhodos*: analysis of fine structure and composition. *Journal of Bacteriology* **117**, 844-857.
- SMITH, R. W. & KOFFLER, H. (1971). Bacterial flagella. In *Advances in Microbial Physiology*, vol. 6, pp. 219-339. Edited by A. H. Rose and J. F. Wilkinson. London: Academic Press.