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Ultrastructure of the Basal Organelles of Flagella of *Clostridium chauvoei*

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INTRODUCTION

There has been a growing interest in the structure of the basal organelles of bacterial flagella. The presence of hooks on the basal ends of the flagella was reported by Houwink & van Iterson (1950). Discs have been demonstrated in the basal region of flagella of Gram-negative bacteria (Abram, Koffler & Vatter, 1965; Hoeniger, van Iterson & van Zanten, 1966) and Gram-positive bacteria (Abram, Vatter & Koffler, 1966). Discs in *Clostridium sporogenes* were reported by Betz (1969). De Pamphilis & Adler (1971) proposed a model of bacterial flagellar basal organelles. In this model a hook region was present in the flagella of both Gram-negative and Gram-positive bacteria, the latter having a single pair of discs at the end of a short rod whereas the Gram-negative bacteria had two pairs of flagella discs separated by a rod. The final disc, in all cases, was inserted into the cytoplasmic membrane.

While studying the antigens of *Clostridium chauvoei*, observations were made on the ultrastructure of the basal organelles of the flagella of this organism.

METHODS

A suspension of lysed cells of *C. chauvoei* strain CH3, possessing intact flagella, was prepared by the method of Chandler & Hamilton (1975), the deflagellation step described in that paper being omitted. Cells harvested from liquid culture were lysed by pronase, and the particulate cellular components digested with trypsin and ribonuclease. After sequential washing with physiological saline, phosphate buffer (0·1 M, pH 7·0) and distilled water, the centrifuged deposit from the lysed cell suspension was resuspended in distilled water and a portion examined electron microscopically. Many cell walls were observed, most having cytoplasmic membranes trapped inside and flagella attached. To remove the cell walls, another portion was centrifuged (27000 *g*, 20 min, 4 °C) and resuspended in sodium phosphate buffer (0·1 M, pH 6·9) containing 500 μg lysozyme (Calbiochem, A grade)/ml and thiomersal (0·01 %, w/v). After digestion for 6 h at 37 °C the centrifuged deposit was washed twice with distilled water and finally resuspended in distilled water for electron microscopy. The sample consisted of cytoplasmic membranes and flagella. Many flagella had basal organelles, some of which remained attached to pieces of cytoplasmic membrane.

For electron microscopy, a small drop of sample was placed on to a Formvar-coated copper grid which, after a few seconds, was inverted on to a drop of negative stain. After 30 s, the grid was picked up, the excess strain removed with filter paper and the grid allowed to dry. When dry the grid was examined with a Siemens Elmiskop I electron microscope. Negative stains used in this study were 2% (w/v) sodium phosphotungstate at pH 7·0, 2% (w/v) ammonium molybdate at pH 5·2, and 2% (w/v) uranyl formate at pH 3·8.

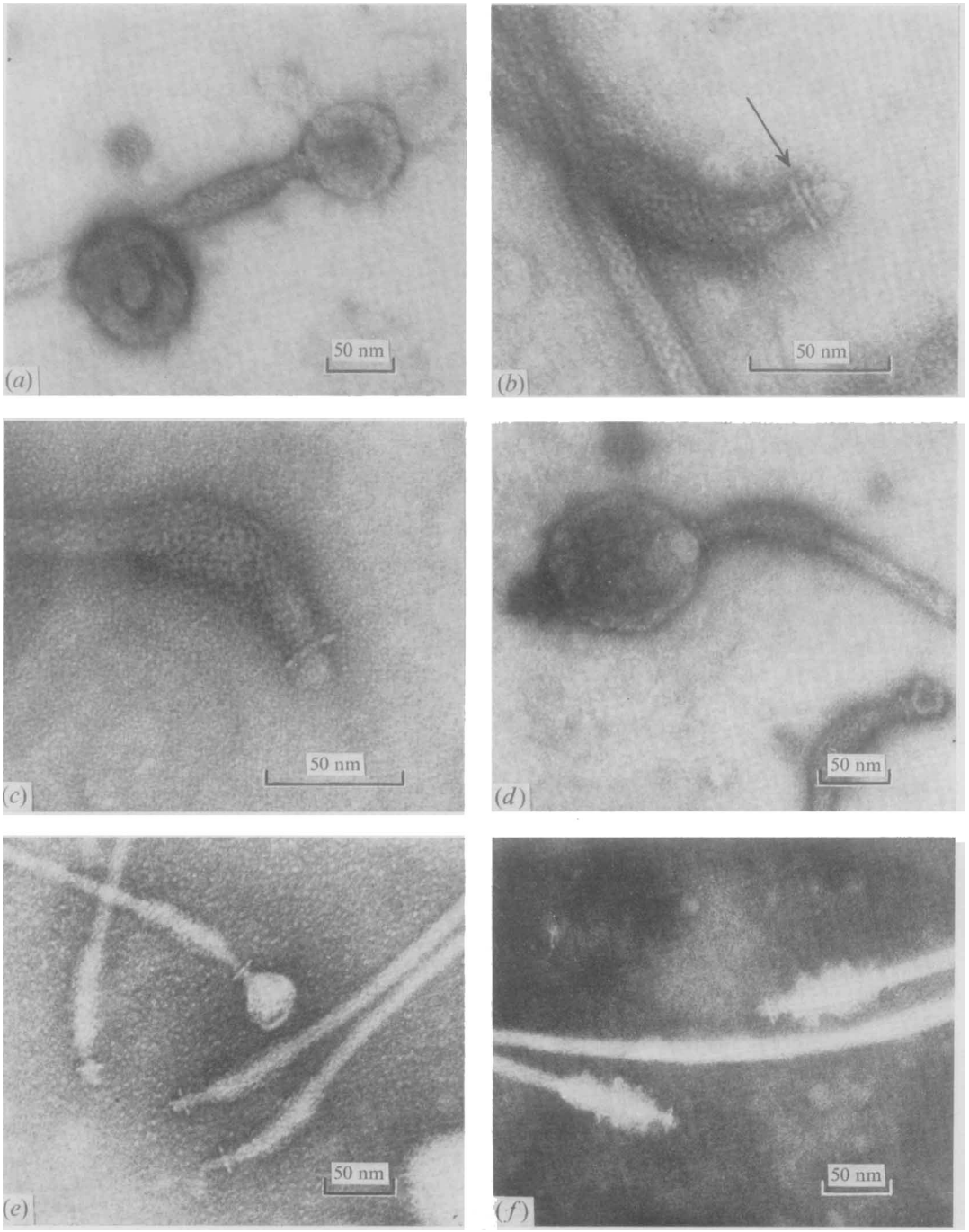

Fig. 1. Basal organelles of flagella of *C. chauvoei*. Note the presence of two discs in (*b*) (arrow). (*a*), (*b*), (*c*) and (*d*) were stained with uranyl formate, (*e*) with ammonium molybdate, and (*f*) with sodium phosphotungstate.

RESULTS

The flagellar basal organelles of *C. chauvoei* consisted of a hook, a rod and a pair of discs; one disc lay immediately outside the cyptoplasmic membrane, while the other appeared to be incorporated in it.

Hooks were observed which were straight (Fig. 1 *a*, *f*), slightly bent (Fig. 1 *d*, *e*), or bent at an angle of almost 90° (Fig. 1 *b*, *c*). When stained with uranyl formate, the hook was 76 nm long and 27 nm in diameter and had a substructure which appeared to be composed of circular subunits (Fig. 1 *a*, *b*, *c*, *d*). However, when stained with sodium phosphotungstate the hook region was fibrous in appearance, although the length of the hook was similar (Fig. 1 *f*). When ammonium molybdate was used the hook had a similar appearance to the uranyl formate image, although little substructure was apparent (Fig. 1 *e*).

The rod was 12 nm long and 11 nm in diameter, whilst the flagellar filament was 13 nm in diameter.

Observation of flagella which were attached to cytoplasmic membranes showed a disc 22 nm in diameter immediately outside the cytoplasmic membrane (Fig. 1 *a*, *d*, *e*). Where the flagellum was free of the cytoplasmic membrane two discs were occasionally observed (Fig. 1 *b*). As the two discs were only seen when the flagella were free of the cytoplasmic membrane, we believe that the second disc was either incorporated into the cytoplasmic membrane or lay immediately inside this membrane. In preparations containing whole lysed cells it was observed that the hook lay outside the cell wall, with one disc between the cell wall and the cytoplasmic membrane. The second disc was not apparent.

DISCUSSION

The basal organelles of the flagella of *C. chauvoei* resembled those of *C. sporogenes* as reported by Betz (1969). The first flagellar disc is more clearly resolved in our micrographs than his, and we have observed a second flagella disc. The hooks of *C. sporogenes* appeared to be more consistently bent than those of *C. chauvoei*. No grommets through the cell wall were observed in the present study.

Our observations on the ultrastructure of the basal organelles of the flagella of *C. chauvoei* fit the general model of basal organelle ultrastructure proposed by De Pamphilis & Adler (1971) for Gram-positive bacteria. There is a hook separated by a rod from a pair of discs, one of the latter lying immediately outside the cytoplasmic membrane while the other appears to be incorporated within it. The rod corresponds to the region of the flagellum which passes through the cell wall.

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REFERENCES

ABRAM, D., KOFFLER, H. & VATTER, A. E. (1965). Basal structure and attachment of flagella in cells of *Proteus vulgaris*. *Journal of Bacteriology* **90**, 1337–1354.

ABRAM, D., VATTER, A. E. & KOFFLER, H. (1966). Attachment and structural features of flagella of certain bacilli. *Journal of Bacteriology* **91**, 2045–2068.

BETZ, J. V. (1969). Structure of the proximal ends of flagella of *Clostridium sporogenes*. *Canadian Journal of Microbiology* **15**, 761–764.

CHANDLER, H. M. & HAMILTON, R. C. (1975). The preparation of immunogenic cell walls from a highly protective strain of *Clostridium chauvoei*. *Journal of General Microbiology* **88**, 27–35.

DE PAMPHILIS, M. L. & ADLER, J. (1971). Fine structure and isolation of the hook–basal body complex of flagella from *Escherichia coli* and *Bacillus subtilis*. *Journal of Bacteriology* **105**, 384–395.

HOENIGER, J. F. M., VAN ITERSON, W. & VAN ZANTEN, E. N. (1966). Basal bodies of bacterial flagella in *Proteus mirabilis*. II. Electron microscopy of negatively stained material. *Journal of Cell Biology* **31**, 603–618.

HOUWINK, A. L. & VAN ITERSON, W. (1950). Electron microscopical observations on bacterial cytology. II. A study of flagellation. *Biochimica et biophysica acta* **5**, 10–44.