Electron Microscopic Demonstration of Connective Fibrils between Outer Sheath and Wall-Membrane Complex in *Treponema phagedenis*

Kuniyoshi Masuda,* and Tomio Kawata

Department of Food Microbiology, Tokushima University School of Medicine, Tokushima, Tokushima 770

(Accepted for publication, December 20, 1985)

Spirochetes possess an outer sheath as an outermost membranous structure and their protoplasmic cylinder is covered by a wall-membrane complex (peptidoglycan-cytoplasmic membrane complex) (4). The wall-membrane complex, not the outer sheath, is considered to be the principal determinant of the helical shape specific for spirochetal cells, because the complex contains peptidoglycan (3-5). Therefore, we have assumed that the outer sheath must be connected with the wall-membrane complex by cellular substances (linkers) in order to form and maintain the helical appearance. This paper describes the presence of fibrils connecting the outer sheath and the wall-membrane complex as revealed by electron microscopy of thin sections of *Treponema phagedenis*.

*T. phagedenis* biotypes Reiter and Kazan were grown anaerobically at 37 C in a modified Kawata's thioglycolate medium as described previously (8). Cells harvested from a 5-day culture were washed once with cold 0.05 M phosphate buffered saline (pH 7.2) by centrifugation, fixed with 1% OsO₄ and treated with 0.5% uranyl acetate according to the method of Kellenberger et al (6). Thin sections were then prepared and stained with lead citrate as described previously (9). Specimens were examined with a Hitachi HU-11E electron microscope operating at 75 kV.

Electron micrographs of thin sections of both biotypes of *T. phagedenis* showed that a triple-layered outer sheath about 10 nm in width is present as an outermost layer of the cells (Fig. 1). A wall-membrane complex immediately surrounds the protoplasmic cylinder. In cross-sectioned cells flagella (axial filaments) could be observed as electron-dense spots about 30-40 nm in diameter in the space between the outer sheath and the wall-membrane complex.

The most striking feature was the presence of fibrils by which the outer sheath was connected with the wall-membrane complex. The connective fibrils were observable as moderately electron-densely stained, fluffy filaments about 8-12 nm in thickness. The helical shape of spirochetal cells seems to be principally determined by the wall-membrane complex since it contains peptidoglycan (3-5). On the other hand, the outer sheath may not participate in forming the helical shape since it consists mainly of protein, phospholipid and carbohydrate and does not
Fig. 1
contain peptidoglycan (1, 9, 12). Therefore, the connective fibrils are considered to play an important role as linkers in the formation and maintenance of the helical appearance of treponemal cells by connecting the outer sheath with the wall-membrane complex. Although fluffy material has been seen infrequently in the space between the outer sheath and the wall-membrane complex, in thin-sectioned cells of *Spirochaeta stenostrepta* (5) and tannic acid-treated *T. phagedenis* biotype Reiter (10), these observations have not been referred by these authors.

Helical cells of *T. phagedenis* biotype Reiter are converted into spherical forms by exposure to hypotonic conditions upon separation of the outer sheath from the protoplasmic cylinder (2). Similar spherical conversion of spirochetal cells is also found during the stationary growth phase of spirochetes (5, 7). The conversion of helical cells into the spherical form may be caused by disintegration of the connective fibrils between the outer sheath and the wall-membrane complex followed by expansion of the outer sheath, because the protoplasmic cylinder still maintains a helical shape within the spherical form (1, 7, 11).

REFERENCES


(Received for publication, August 5, 1985)