Abbreviations used are:
P5'N = pyrimidine 5'-nucleotidase.
^{1}H-NMR = proton unclear magnetic resonance.
^{31}P-NMR = phosphorous nuclear magnetic resonance.
CDP = cytidine diphosphate.
UDPG = uridine diphosphate glucose.

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Contact and cellulolysis in *Clostridium thermocellum* via extensile surface organelles

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Summary. The ultrastructural distribution of the cellulosome (a cellulose-binding, multi-cellulase protein complex) of the thermophilic anaerobe, *Clostridium thermocellum*, was investigated. The cellulosome is compacted into protuberant cell surface structures, which, upon interaction with cellulose, form extended contact corridors wherein cellulolysis apparently occurs.
Key words. Cellulase; cellulosome; *Clostridium thermocellum;* exocellular organelle.

Considerable interest has recently been focused on the cellulase system in *Clostridium thermocellum*[1–3]. The cellulolytic apparatus in this bacterium comprises a multiple enzyme system consisting of both exo- and endo-β-glucanases which act synergistically in order to hydrolyze the complicated paracrystalline structure of cellulose. We have recently shown that the major enzymes responsible for cellulose degradation in this organism are arranged into a distinct multisubunit complex which we have called the 'cellulosome'[4–7]. The cellulosome appears both in an extracellular form and in a cell-associated form[4,8]. The latter is considered to comprise a discrete cell surface organelle, which is also responsible for the adherence of the bacterium to its insoluble substrate.

By using histochemical and immunocytochemical means, we demonstrate in the present report the ultrastructural localization of the cellulosome on the cell surface of *C. thermocellum*. In addition, we were able to trace its fate upon binding of the bacterial cell to cellulose.

Materials and methods. Cells of *C. thermocellum* YS were grown anaerobically in serum bottles containing salts, yeast extract and supplemented with either cellobiose (0,8 % w/v) or cellulose (0.8% w/v) as described previously[4]. Cells were harvested during the early stages of growth (usually between 8 and 15 h). Pelleted samples were resuspended gently in 0.7 ml of 0.9 % NaCl (saline) and 0.3 ml Karnovsky's fixative was added[9]. After a period of 20 min, the fixed cells were washed three times with saline, resuspended in 0.7 ml saline and treated either with cationized ferritin (CF; BioYeda, Israel) or by the immunochemical procedure outlined below. CF-treatment entailed the addition to the desired cell suspension of a saline solution (0.3 ml) containing 0.5 mg CF. The cells were incubated for 1 h at room temperature, centrifuged, washed twice (gently) with saline, and fixed with Karnovsky's fixative. Immunocytochemical labeling, using biotinylated anti-cellulosome (S1-specific) antibodies[4], was accomplished according to our previously described method[10,11]. The biotinylated antibody [30 µg in 200 µl 1% (w/v)

bovine serum albumin (BSA)] was added to the desired cell suspension at room temperature for 1 h, after which the samples were washed with BSA three times by centrifugation. A solution of ferritin-avidin conjugates [1 mg protein in 250 µl BSA; prepared by reductive alkylation (Sigma)] was layered over the pellet and the interaction was allowed to proceed for 1 h at 25 °C. The pellet was then washed (very gently) three times with BSA and fixed in Karnovsky's fixative. Labeled cell samples were processed for electron microscopic analysis as described earlier[11].

Results and discussion. When wild type cells were grown on cellobiose (the soluble disaccharide and repeating unit of the cellulose polymer) and labeled with cationized ferritin (a general anion-specific marker), a multitude of novel protuberant surface structures became visible (fig. a). These appeared hemiellipsoidal or hemispherical in shape and ranged between 130 and 200 nm in length and 60 to 100 nm in height. When the same cell was labeled with antibodies specific for one of the 14 cellulosome subunits (the 210 K S1 subunit), the label was also arranged upon these protuberances (fig. b). It is interesting to note that cationized ferritin penetrated these structures completely, whereas the immunochemical stain was mainly restricted to their outer surface. This indicated that additional anionic components comprise the interior portion of the protuberances. Considering the reported size of the isolated cellulosome (about 18 nm)[5], several hundred may be accomodated in one of these structures, thereby suggesting their polycellulosomal nature.

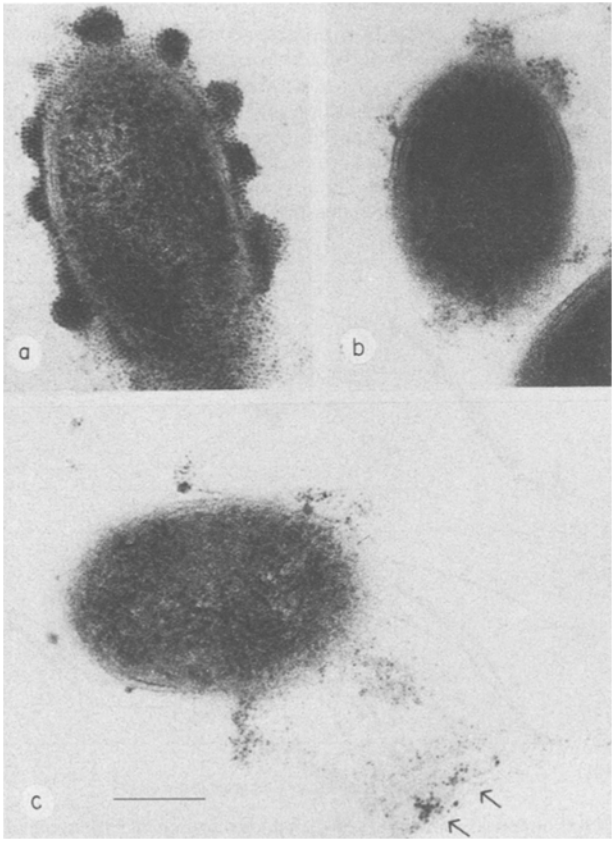
Without the stabilizing and staining effects of either the cationized ferritin or the antibody/ferritin label, only vestiges of these structures could be distinguished on the wild type cell; they were entirely lacking when adherence-defective mutant cells were grown and labeled in an identical manner (data not shown).

Following growth of the wild type cell directly on cellulose, there occurred a dramatic change in the constitution of the exocellular protuberances. When the cell comes into contact with the cellulosic substrate, the structure of some of the polycellulosomal protuberances is transformed yielding an amorphous or fibrous network. A distance of up to 400–500 nm separates the cell itself from the cellulose surface, and the fibrous material appears to connect the two.

Similar fibers on *C. thermocellum* have been reported previously by Wiegel and Dykstra[12] and by ourselves[13] which were then proposed to be involved in the binding of the cell to cellulose. The evidence presented in figure c, however, indicates that the cellulosome represents the major site of attachment to the cellulose, since labeling with anti-cellulosome (S1-specific) antibodies revealed that most of the ferritin marker particles within the fibrous network are arranged in clusters which are intimately associated with the cellulose surface (fig. c, arrows). This is in line with our previously published data[4], all of which connect the cellulosome directly with adherence. The fibrous structures, on the other hand, form a 'contact corridor' which appears to connect the cell to the cellulosome, but is not directly responsible for the binding phenomenon per se.

It is interesting to speculate on the composition and function of the observed fibers. In previous works[12, 13], ruthenium red was used to stain these fibers, indicating perhaps the presence of negatively charged polysaccharides. In the absence of cellulose, the fibers appear to be compacted into the lumen of the cell surface protuberances. Since neither adherence nor cellulolysis is required in cells grown on cellobiose, the latter structures are, in this case, in a dormant state. However, upon primary interaction of the cellulosome complexes with cellulose, the conformation of the lumen material appears to be altered to form the extended fibers. The resultant contact zones may serve to direct cellulose degradation products systematically toward the cell surface.

Indeed, we have previously shown that the cellulosome is responsible for most of the cellulase activity in this organism[7]. It would therefore appear that cellulolysis occurs outside of the cell proper, and that the hydrolysis is mediated by cellulosome clusters at the surface of the insoluble substrate. The transfer of the products toward the cell may also be a highly-ordered efficient process mediated by the fibrous components in the contact zone.



Ultrastructure of *C. thermocellum* and its interaction with cellulose. *a* Cell grown on cellobiose and stained with cationized ferritin. *b* Cell grown as in a, but stained sequentially with biotinylated anti-cellulosome (S1-specific) antibody and ferritin-avidin conjugates. *c* Cell grown on cellulose and stained as in b. Ferritin clusters (arrows) indicate the presence of polycellulosome fragments which are attached to the surface of the cellulosic substrate. The bar marker represents 250 nm.

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