

Macromolecular Organization of the Cellulolytic Enzyme Complex of *Clostridium thermocellum* as Revealed by Electron Microscopy

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Clostridium thermocellum JW20 and YM4 both synthesize cellulolytic enzyme complexes, cellulosomes, when grown on medium containing cellulose. Electron microscopic studies showed that, in the early stages of growth of strain JW20, clusters of tightly packed cellulosomes, i.e., polycellulosomes, were located on the cell surface and were bound to cellulose. The polycellulosome was estimated to have a particle mass of 50×10^6 to 80×10^6 daltons (Da), while that of the cellulosome was estimated to be 2×10^6 to 2.5×10^6 Da and to contain about 35 polypeptides ranging from 20 to 200 kDa. The cellulosome produced by strain YM4 was found to be somewhat larger, with the estimated particle mass being 3.5×10^6 Da, and the number of polypeptides was counted to be 45 to 50, ranging from 20 to 200 kDa. In the early stages of cultivation, the cellulosomes from both species exist as tightly packed complexes (tight cellulosomes). These subsequently decompose to loosely packed complexes (loose cellulosomes) and ultimately to free polypeptides. Examination of the loose cellulosomal particles showed that they contain rows of equidistantly spaced, similarly sized polypeptide subunits, with an apparently identical orientation arranged parallel to the major axis of the cellulosome. It is postulated that on binding of a cellulose chain alongside such a row of subunits a simultaneous multicutting event occurs that leads to the release of celooligosaccharides of four cellobiose units in length (C₄). Rows of smaller-sized subunits with lower center-to-center distances, which are also present in the cellulosome, subsequently cleave the C₄ fragments (or cellulose) to C₂ (celotetraose) or C₁ (cellobiose). In this way the cellulosome can catalyze the complete hydrolysis of cellulose.

Clostridium thermocellum (29), which was first obtained in stable pure culture by McBee (24) over 30 years ago, has the ability to hydrolyze cellulose and ferment the hydrolytic products cellobiose and glucose to ethanol, acetate, and lactate (8, 13). In the years since then, various enzymes associated with cellulose hydrolysis and metabolism have been studied. Among these are the intracellular enzymes cellobiose phosphorylase (2) and cellodextrin phosphorylase (28) and endoglucanases from culture filtrates of different strains of *C. thermocellum* (1, 26, 27). Each of the endoglucanases catalyzes the hydrolysis of carboxymethyl cellulose but is inactive against the crystalline material found in nature.

Demain and co-workers (16, 17) provided a breakthrough in this context by finding that the degradation of crystalline cellulose by the clostridial enzyme system is dependent on the presence of Ca²⁺ and thiols in reaction mixtures. Moreover, it was shown by various groups of investigators (14, 21; J. H. D. Wu and A. L. Demain, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, O79, p. 278) that the ability to hydrolyze crystalline cellulose is the preserve of high-molecular-weight multicomponent complexes; the free endoglucanases are inactive in this regard, whether or not calcium and thiols are present. The molecular weight of the cellulolytic complex produced by strain YS was reported to be 2.1×10^6 and to consist of at least 14 different types of polypeptides ranging in size from 45,000 to 210,000 (16), while the *M_r* of that from strain ATCC 27409 was determined to be 6.5×10^6 (Wu and Demain, Annu. Meet. Am. Soc. Microbiol. 1985). Lamed and colleagues (3, 4, 19, 20), who coined the term

cellulosome to describe these cellulolytic complexes, showed that they are located on the cell surface and that they mediate adherence of the bacterium to the substrate.

During the early stages of growth of *C. thermocellum* JW20, most of the cellulolytically active components produced by the bacterium are bound to cellulose (and, presumably, also to the cell surface). As fermentation proceeds the amount of bound enzyme decreases, while that free in the culture fluid increases (14, 23; H. Hon-Nami, M. P. Coughlan, K. Hon-Nami, and L. G. Ljungdahl, Proc. R. Irish Acad., in press). The bound enzyme, which may be removed from the cellulose by extraction with water (23), consists of two types of complexes (14). The larger of these complexes, termed the originally bound large (OBL) complex, was estimated to have a particle mass of 100×10^6 daltons (Da); while the smaller, termed originally bound small (OBS) complex, was estimated to have a particle mass of 4.5×10^6 Da (12). The enzyme free in the culture fluid of this strain was also resolved into two fractions. One, a multicomponent complex (ca. 4.5×10^6 Da), binds to cellulose in the presence of buffer or salt solutions, i.e., at high ionic strengths. It is termed the free bindable (FB) complex and it is similar, if not identical, to the OBS complex described above. The other free enzyme fraction does not bind to cellulose and is termed the free nonbindable fraction. All four of the fractions described above exhibit endoglucanase (EC 3.2.1.4) activity but only the OBL, OBS and FB complexes hydrolyze crystalline cellulose, with cellobiose being the major (>90%) product (15, 17). The complexes are quantitatively similar in that they each contain about 20 types of polypeptide with *M_r* values ranging from 45,000 to 200,000, although the relative amounts of some types differ (15). Indeed, the available

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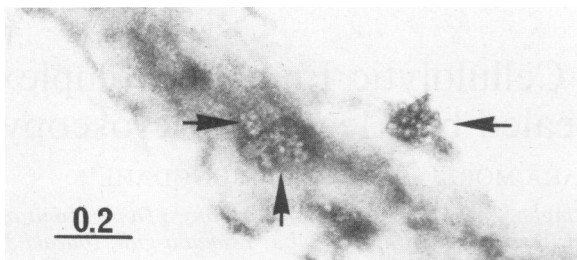


FIG. 1. *C. thermocellum* JW20 exhibiting clusters of spherical particles (cellulosomes; arrows) attached to the cell surface. Bar, 0.2 μm .

evidence suggests that the OBL complex is a polymer of OBS or FB complexes (12, 15). As will be discussed later in this report, we consider the OBS (or FB) complexes to be equivalent to cellulosomes (see above), with OBL being polycellulosomes.

While at least 4 and perhaps as many as 10 of the different polypeptides in these complexes are endoglucanases (15, 19), the function of each of the other components has yet to be determined. In genetic experiments, Aubert and colleagues (10, 25) have shown that 10 distinct activities related to cellulose hydrolysis were encoded by *C. thermocellum* DNA cloned in *Escherichia coli*. Seven of these were determined to be endoglucanases in that they hydrolyzed carboxymethyl cellulose, and the genes coding for three of them have been identified and characterized (5, 6, 9, 18). The other three were active against methylumbelliferyl- β -D-cellobioside but were inactive against carboxymethyl cellulose. It is possible that they may be exocellobiohydrolase-type (EC 3.2.1.91) enzymes.

To complement the biochemical and genetic studies described above and to extend previous ultrastructural investigations (4, 12), we undertook detailed transmission electron microscopic examination of the cellulose complexes of *C. thermocellum* JW20 and YM4. The results of these investigations are presented in this paper.

(Preliminary reports of this work have been presented previously [F. Mayer, M. P. Coughlan, and L. G. Ljungdahl, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, K112, p. 212; F. Mayer, Y. Mori, M. P. Coughlan, and L. G. Ljungdahl, Hoppe-Seyler Biol. Chem. abstr. 6.02.54 367[Suppl.]:355, 1986; F. Mayer, Y. Mori, M. P. Coughlan, and L. G. Ljungdahl, Proc. R. Microsc. Soc. 21[Suppl.]:14-15, 1986].)

MATERIALS AND METHODS

Organisms and growth conditions. *C. thermocellum* JW20 (ATCC 31449) (22, 31), was grown at 60°C in a nitrogen atmosphere in prereduced medium (30) containing 1% (wt/vol) Avicel (type 105; FMC Corp., Philadelphia, Pa.). *C. thermocellum* YM4, which was isolated from volcanic soil on the Izu Peninsula, Japan (Y. Mori and K. Kiuchi, Abstr. Annu. Meet. Agric. Chem. Soc. Japan, 1984, p. 388), was grown under similar conditions (L. G. Ljungdahl, M. P. Coughlan, F. Mayer, Y. Mori, H. Hon-Nami, and K. Hon-Nami, Methods Enzymol., in press), but under an atmosphere of carbon dioxide. Cultures of strain JW20 were harvested after 4 days of growth, at which time activity against crystalline cellulose reached maximum values, yet considerable amounts of cellulose were still present (14, 15;

Hon-Nami et al., in press). Strain YM4 produced significantly more cellulase activity than did strain JW20, and under the conditions described here, it digested the cellulose completely within 1 day (Ljungdahl et al., in press). Accordingly, some cultures of this strain were harvested within 1 day, i.e., while residual cellulose was still present. Culture samples were also harvested after 2 and 4 days so as to obtain the free enzyme complexes in the culture fluid.

Samples for electron microscopy. Strain JW20 cultures were passed through fine-pore glass fiber filters. The residual cellulose to which enzyme complexes were bound and to which bacteria adhered was collected. Samples of this material were collected for electron microscopy. The residue described above was suspended in 100 mM triethanolamine-maleic acid buffer (pH 6.9) and allowed to settle. The supernatant was decanted. This process of suspension in buffer, settling, and decantation was repeated several times to remove adhering bacteria. Samples of the washed residue, containing only cellulose with bound cellulase, were collected for electron microscopy. The bound enzyme was eluted from the cellulose by washing with water (23) and examined by electron microscopy. Cultures of strain YM4 were filtered as described above. Enzyme complexes washed from the residual cellulose (<1-day-old cultures) with water and those from culture filtrates (2-day- and

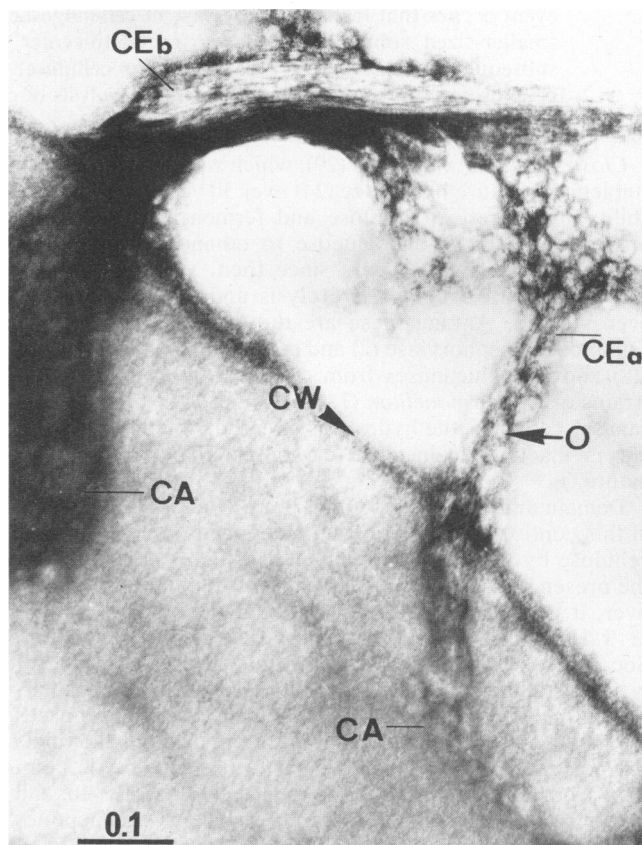


FIG. 2. *C. thermocellum* JW20 with bundles of cellulose fibers (CE_a , CE_b) attached to the cell surface (CW). Attachment appears to be mediated by binding of the cellulose fibers to clusters of cellulosomes (CA) located at the cell surface. Some areas of the cellulose fibers are covered by free cellulosomes (O) of different sizes. Bar, 0.1 μm .

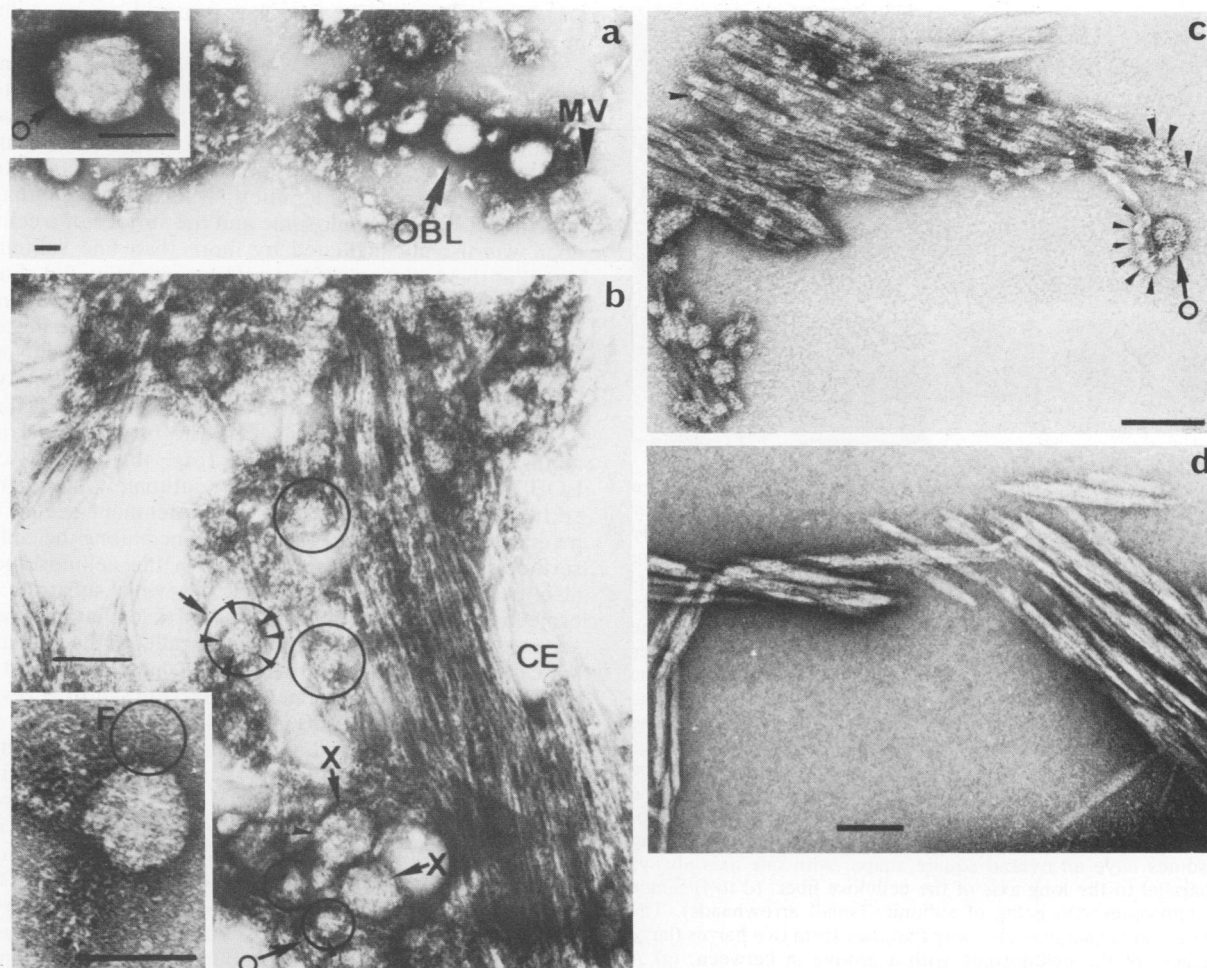


FIG. 3. (a) Sample containing cellulose with enzyme complexes attached. The cells of strain JW20 were removed by washing with buffer before they were mounted for electron microscopy (see text). The cellulase complexes are present as small entities that are not clearly visible (cellulosomes) or as large spherical particles (OBL or polycellulosomes; MV, membrane vesicle). The inset in panel a shows an OBL particle at high magnification. Its surface appears to be covered by a faint "skin" of unknown composition. The OBL particle contains tightly packed cellulosomes (O). (b) A bundle of cellulose (CE) with cellulosomes (circles, arrows, X, and O) of different sizes and clusters thereof attached. The cellulosomes appear to consist of subunits (arrowheads). The inset in panel b shows an OBL particle covered by very fine fibrils (F and circle) which are assumed to be degradation states of cellulose fibers. (c) Bundle of cellulose fibers as shown in panel b but after one washing with water. Cellulosomes (O) and some small particles (arrowheads), which are assumed to be components of formerly intact bound cellulosomes, still adhere to the cellulose. (d) As in panel c, but after intensive washing with water. The cellulase was removed completely. Bars, 50 nm.

4-day-old cultures) were purified by affinity chromatography on clean (i.e., washed) yellow affinity substance-cellulose from strain JW20 as described previously (15, 23).

Electron microscopy. Carbon support films (about 4- to 8-nm thick) were prepared by carbon-resistant evaporation onto a clean mica surface. Samples of cellulose with bound enzyme and adhering cells of JW20, cellulose-cellulase (from JW20) aggregates, and isolated enzyme complexes from both strains were negatively stained with an aqueous uranyl acetate solution (3.5% [wt/vol]; pH 4.8) by applying the diffusion technique and using the deep and shallow stain conditions described previously (12). Samples were observed and micrographs taken with a microscope (EM 400ST; Philips) operated at 80 KeV in the conventional transmission mode. Instrument magnification was calibrated as described previously (12). Measurements of particle sizes were made from prints at calibrated magnifications ranging from $\times 168,000$ to $\times 200,000$.

RESULTS

***C. thermocellum* JW20.** Negatively stained cells of strain JW20 exhibited clusters of spherical particles attached to the cell surface (Fig. 1). Occasionally, bundles of cellulose fibers could be seen to be in association with the bacteria (Fig. 2). A close inspection of these associations revealed that the cellulose bundles were attached to clusters of the spherical particles (Fig. 1) and not to the cell surface proper. In addition, it is shown in Fig. 2 that much of the cellulose was covered by spherical particles of various sizes.

After the bacterial cells were removed by centrifugation and the cellulose was washed with 100 mM triethanolamine-maleic acid buffer (pH 6.9), it was found, as expected from earlier observations (12, 23), that spherical particles of different sizes remained attached to the cellulose fibers (Fig. 3a and b). Prominent large-sized particles (ca. 60 nm in diameter; Fig. 3a) were very common. The inset in Fig. 3a

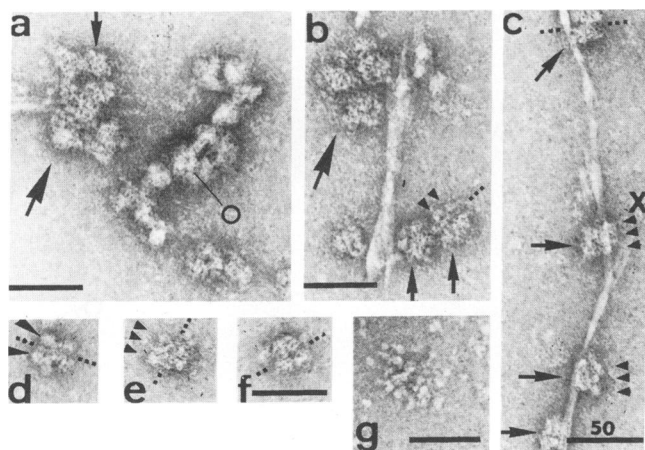


FIG. 4. (a and b) Cellulose fibers covered by clusters (large arrows) of cellulosomes from strain JW20. The cellulosomes occur in two different states. In one state (O) the subunits are tightly packed (TOBS). In the other state (small arrow) the subunits are loosely aggregated (LOBS) and the particle has a spongelike appearance. The arrowheads in panel b point to subunits arranged linearly along a groove (dotted line) running along the midline of one of the loose cellulosomes. (c) Cellulose fiber with four loose cellulosomes (arrows) from strain JW20 bound intimately to the surface of the fiber. This attachment appears to be mediated by the cellulosome subunits, which are visible as white dots aligned along the cellulose surface (arrowheads marked X). Similar subunits of cellulosomes exposed at the surface (arrowheads) can also be seen not to be complexed with cellulose. The dotted line drawn across one of the cellulosomes indicates the presence of a groove within the cellulosome (compare with panel b). The projections of these bound cellulosomes have an overall square shape, with one axis always being parallel to the long axis of the cellulose fiber. (d to f) Single free cellulosomes consisting of subunits (small arrowheads). The subunits are arranged in such a way that they form two halves (large arrowheads) of the cellulosome with a groove in between. (g) A partially decomposed free cellulosome. Bars, 50 nm.

demonstrates further details. The large type of particle appears to be composed of smaller spherical entities with diameters from ca. 16 to 18 nm. Occasionally, a delicate "skin" covering the particle surface could be detected. This skin did not exhibit a particulate substructure. Its chemical composition is as yet unknown. The mass of this large type of spherical particle, termed OBL (12, 15), based on its measured large diameter of 60 nm, was estimated to be ca. 50×10^6 to 80×10^6 Da. The smaller spherical entities, termed OBS (12, 15), would then have a mass of about 2×10^6 to 2.5×10^6 Da, assuming that they are composed of tightly packed subunits. In Fig. 3b is depicted a similar sample at higher magnification. Spherical particles with diameters of 16 to 18 nm can be seen to be bound to cellulose. In addition, a second type of spherical particle, with diameters ranging from 23 to 26 nm, and in rare cases up to 40 nm, could be found to be attached to cellulose. This type of particle had a spongelike appearance with negative-staining salt penetrated into grooves and clefts. This indicates that this type of particle consists of loosely aggregated subunits. We therefore termed it loose OBS (LOBS) to distinguish it from the other type of small spherical particle (diameter, 16 to 18 nm) described above, which we term tight OBS (TOBS). Fine fibrils in close proximity to OBL or OBS particles were frequently observed (Fig. 3b, inset). As the first step in the cellulose degradation process may involve chain separation so as to render the substrate more susceptible to hydrolysis

(11), these fine fibrils may be assumed to be cellulose microfibrils.

The progressive removal of cellulase particles from the cellulose surface by washing with water is documented in Fig. 3c and d. After one wash, the cellulase was not completely removed. Subunits that were formerly situated at the periphery of the cellulosomes could still adhere to the substrate (Fig. 3c). In addition, it can be seen that the contact between a cellulosome and the surface of a cellulose fiber was usually mediated by more than one subunit exposed at the periphery of the cellulosome (Fig. 3c). Further details on the binding of cellulosomes to cellulose and on some fine-structure aspects of LOBS particles are presented in Fig. 4. In Fig. 4a several TOBS particles and clusters of LOBS are depicted. It can be seen that only one or two of the LOBS in a cluster made contact with the cellulose. Subunits in a cellulosome appeared to be linearly arranged along the midline of the complex (Fig. 4b). In Fig. 4c four LOBS particles can be seen to be intimately bound to the surface of a cellulose fiber. This attachment seems to be mediated by cellulosome subunits aligned along the cellulose surface (Fig. 3c). Similar subunits in the cellulosome can also be seen not to be complexed with substrate. The projections of these bound LOBS particles have an overall square shape with one axis always parallel to the long axis of the cellulose fiber. Comparison of the shape of bound LOBS (above) with those of isolated LOBS (Fig. 4d to f) revealed that the structural integrity of the LOBS particle was stabilized by binding to cellulose. The free LOBS particles, although visibly consisting of ordered subunits and divided into halves separated by a groove, lost their regular square shape. A further state of structural decomposition is depicted in Fig. 4g. The number of subunits in such particles was counted to be ca. 35, with masses ranging from 20 to 200 kDa. The structural deformation above might have been caused by the mounting and staining procedures used for electron microscopy. However, our earlier observations (15; Hon-Nami et al., in press) indicate that the cellulolytic complexes synthesized in the early stages of growth undergo an inexorable path of decomposition that leads ultimately to the appearance of the original components as free polypeptides in the culture fluid.

C. thermocellum YM4. OBL particles were not seen on

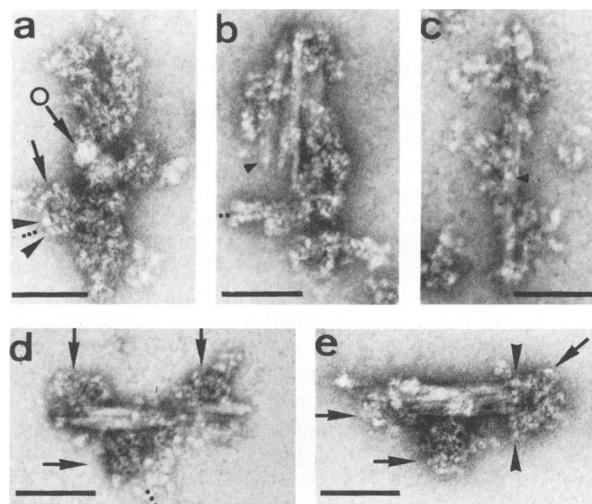


FIG. 5. *C. thermocellum* YM4. See Fig. 4 legend for a description of the panels, abbreviations, and symbols.

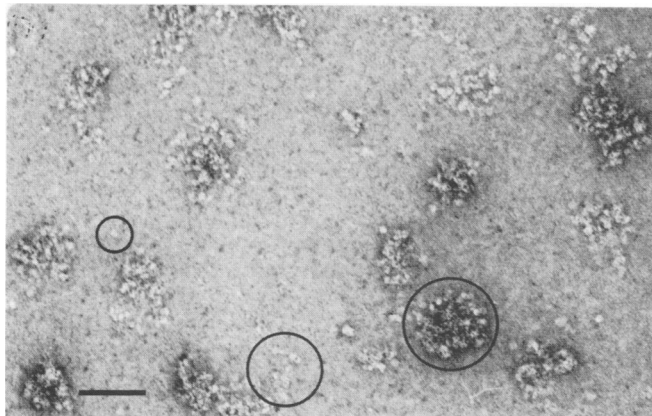


FIG. 6. Isolated cellulosomes from strain YM4. These cellulosomes are larger than those from strain JW20 (cf. Fig. 4). Some of the cellulosomes appear to be structurally preserved (large circle). Many are decomposed into clusters made up of chains of subunits (mid-size circle). Free subunits (smallest circle) can also be seen. Bar, 50 nm.

microscopic inspection of YM4 cultures regardless of the time of harvesting. With one exception, however, all other major structural aspects of its cellulosomes were found to be similar to those of JW20 (Fig. 5 and 6), with the exception being that the diameters (23 to 30 nm) of the LOBS particles of strain YM4 were larger. This indicates that they have a higher mass and a larger number of subunits than those of

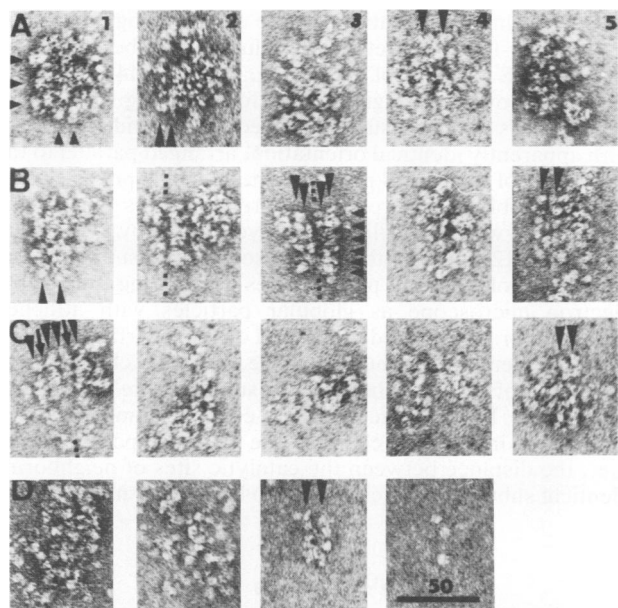


FIG. 7. Gallery of loose cellulosomes (strain YM4) from the sample depicted in Fig. 6. (Row A) Full-size cellulosomes. There are indications (arrowheads) for an ordered arrangement of the subunits. (Rows B and C) Examples of cellulosomes with clearly visible ordered arrangement (arrowheads) of subunits of different sizes with string connections between individual polypeptides (arrows in row C) and indications for subdivision of the cellulosome into halves (dotted lines). The subunits in each half appear to be packed in paracrystalline arrays. (Row D) Cellulosomes in different states of decomposition. The arrowheads show small-sized ordered aggregates. Bar, 50 nm.

the LOBS particles from JW20. A structural feature of the LOBS particles depicted in Fig. 7 is that the majority of the subunits making up these particles are arranged in straight parallel rows, with five to eight equidistant subunits in each row. As before, these rows were (often) arranged in parallel pairs, thereby giving rise to lines of symmetry. Thus, the major mass of a LOBS particle appears to be organized as a paracrystalline array of subunits. The peripheral subunits, which were frequently visible in a nonordered state, may be naturally nonordered. On the other hand, they might have been altered from a formerly defined position in the paracrystal by the mounting and staining procedures used in this study (see above). Ultrathin fibrils connecting polypeptides in an individual row and joining pairs of rows were occasionally seen (Fig. 7). These fibrils are better resolved in Fig. 8 and 9. The LOBS particles depicted here were nearly completely decomposed into chains of subunits held together by ultrathin fibrils of unknown composition. The number of subunits in an average LOBS particle from strain YM4 was counted to be between 45 and 50. Their masses, as estimated from their diameters, may be between 20 and 200 kDa (see introduction). Ultrathin fibrils were especially evident in Fig. 9b to d. Because of intensive flattening and additional artificial deformation, the straight parallel rows of subunits (Fig. 9a) were bent into clusters, each of which contained several subunits that were connected by ultrathin fibrils to a central string of unknown composition. In Fig. 9d the ultrathin fibrils can be seen to be attached to the masses of the subunits proper and to a central mass. The diameter of an ultrathin fibril is at the limit of the electron microscopic resolving power, but it may be about 1 nm.

DISCUSSION

Results of the studies presented above confirm our earlier assumptions (12, 15) that OBL complexes attached to the

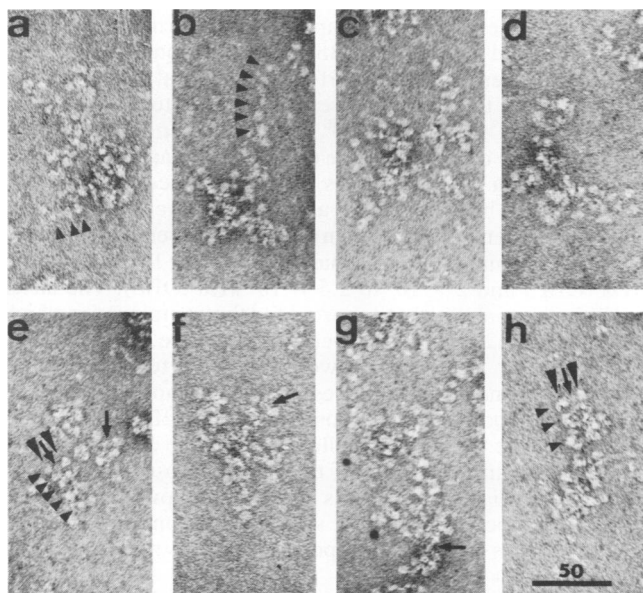


FIG. 8. (a to h) Gallery of loose cellulosomes (strain YM4) from the sample depicted in Fig. 6. These cellulosomes are almost completely decomposed into chains of subunits held together by ultrathin fibrils (arrows) of unknown composition. In some of the cellulosomes the ordered arrangement of the subunits is still visible (arrowheads). Bar, 50 nm.

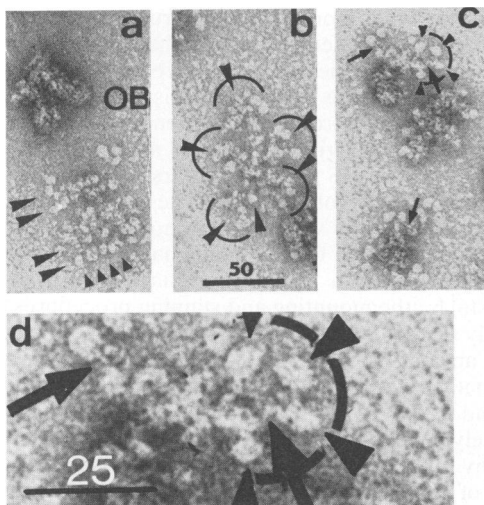


FIG. 9. Gallery of partially decomposed loose cellulosomes (strain YM4) from the sample depicted in Fig. 6. (a) Apart from an intact cellulosome (OB), a flattened artificial cellulosome is shown. Its subunits (small arrowheads) are arranged in parallel rows (large arrowheads) that are held together by structural components (not visible). (b and c) As described for panel a; however, because of intensive flattening and additional artificial deformation by the preparation for electron microscopy, the formerly straight parallel rows have been bent into clusters (semicircles and arrowheads), each of which contains several subunits connected to a central string of unknown material by ultrathin fibrils (arrows). (d) Higher magnification of an area depicted in panel c. The ultrathin fibrils can be seen to be attached to the (white) globular masses of the subunits proper and to a central mass. Bar in panel b; 50 nm; bar in panel d, 25 nm.

cell surface of strain JW20, to cellulose fibers (Fig. 1 to 4), or both consist of a number of OBS complexes (see Fig. 3a). In our preliminary investigations (12), the diameters of these particles in electron micrographs were determined to be 60 nm (OBL) and ca. 20 nm (OBS). On the assumption that the particles observed were perfectly spherical, as they appeared to be, their masses were calculated to be ca. 100×10^6 kDa (OBL) and 4.5×10^6 kDa (OBS). In this study we confirmed the diameters to be 60 nm (OBL) and 16 to 18 nm (OBS). Tilting of the samples in the microscope, however, showed that both particles are rotational ellipsoids, i.e., flattened spheres, rather than perfect spheres. Thus, more accurate estimates of their masses are 50×10^6 to 80×10^6 kDa (OBL) and 2×10^6 to 2.5×10^6 kDa (OBS). The latter value is the same as that reported for the complex, for which Lamed et al. (20) coined the term cellulosome, from cultures of strain YS. Accordingly, we refer hereafter to the OBS complexes (and FB complexes, since they appear to be the same; see above) as cellulosomes. The OBL complexes would then be termed polycellulosomes. The composition of the faint skinlike covering of the polycellulosomes of strain JW20 (see Fig. 3a, inset) is as yet unknown. From the observed appearance of the skin in electron micrographs, however, it is tempting to speculate that it may consist of peptidoglycan remnants.

Polycellulosomes were not observed in electron micrographs of isolated complexes of strain YM4, regardless of the time of harvesting (Fig. 5 to 9), nor were they isolated from cultures of this strain by biochemical procedures (Ljungdahl et al., in press), although we do not preclude the fact that they were present on the cell surface at early stages

of growth. However, the OBS complexes, i.e., cellulosomes, produced by this strain were somewhat larger (diameter, 23 to 30 nm; mass, 3.5×10^6 Da) than those of strain JW20 and were composed of a greater number of polypeptide subunits (cf. Fig. 4g and 8). This would account for the greater total cellulolytic activity but similar specific activity of strain YM4 relative to that of JW20 (Ljungdahl et al., in press). On the basis of gel filtration behavior, the molecular mass of the cellulolytic complex produced by strain ATCC 27409 was calculated to be 6.5×10^6 Da (Wu and Demain, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985). Thus, the cellulosomes from this strain may also be larger than those from JW20.

On the basis of results of previous investigations, we conclude that polycellulosomes (OBS complexes), which are located on the surface of strain JW20 and which make contact with cellulose fibers, decompose and ultimately form free polypeptides in the culture fluid (15; Hon-Nami et al., in press). Results of the electron microscopic studies reported here confirm such conclusions. The probable paths of decomposition of the complexes of strains JW20 and YM4 are illustrated in Fig. 10. One may note that the loss of order evident in the electron microscopic studies appears to parallel the loss of activity against crystalline cellulose as determined by assay (Hon-Nami et al., in press). Because there is no evidence that proteases were present in these cultures of *C. thermocellum* (Ljungdahl et al., in press), various other possible explanations must be considered for the observed decomposition of the enzyme complexes during cultivation. Lysis of the bacterium, hydrolysis of cellulose with the consequent release of polycellulosomes, or both may remove a stabilizing influence. Alternatively, the accumulation of ethanol, acetate, lactate, cellobiose, and glucose during fermentation may be destabilizing influences.

The ultrastructural details of cellulosomes, best resolved in the LOBS particles of JW20 (Fig. 4) and YM4 (Fig. 7 and 9a), are shown in diagrammatic form in Fig. 11. These exhibit rows of equidistantly spaced polypeptide subunits, with apparently identical orientation, arranged parallel to the major axis of the LOBS particle. These details provide clues as to possible structure-function relationships and to the mechanism whereby cellulose is hydrolyzed by this organism (Fig. 12). Thus, the cellulosome is assumed to be composed of sets of polypeptides (appearing under the electron microscope as globular particles with attached ultrathin fibrils) arranged in ordered chainlike arrays and in a defined orientation. Four or more of these chains, each composed of five to eight identical subunits, are assumed to be present. The average center-to-center distance between individuals in a single chain of the largest type of subunit (i.e., the distance between the catalytic sites of neighboring identical subunits) was estimated to be about 4 nm. We note

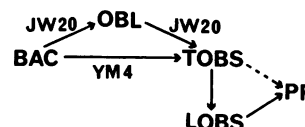


FIG. 10. Time course of decomposition during fermentation of the cellulolytic enzyme complexes of strains JW20 and YM4. Probable pathways are indicated by full arrows, and a possible pathway is indicated by a dotted arrow. Abbreviations: BAC, bacterial cell surface; LOBS, loose cellulosome; PP, polypeptide subunits; TOBS, tight cellulosome; JW20 and YM4, clostridial strains.

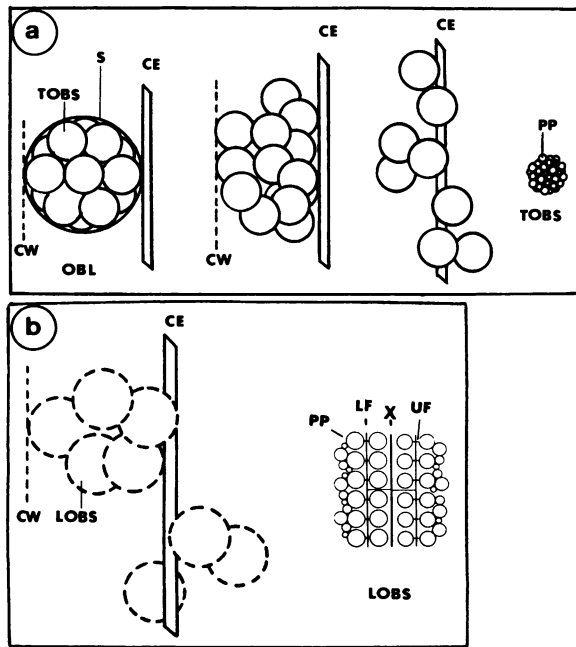


FIG. 11. (a) Diagrammatic view of some of the structural aspects of the cellulolytic enzyme system of strain JW20. Abbreviations: CE, cellulose; CW, cell wall; OBL, a spherical particle of defined diameter (60 nm) consisting of a number of tight cellulosomes; S, skin sometimes seen to be covering OBL particles; PP, polypeptide subunits in the cellulosome; TOBS, tight cellulosome. Clusters of cellulosomes, with or without a skin and attached to the cell wall or not, can be seen to be bound to cellulose fibers. Usually, only a few of the cellulosomes in a cluster were in immediate contact with the cellulose fiber. (b) Diagrammatic view of some of the structural aspects of the cellulolytic system common to strains JW20 and YM4. Abbreviations: CE, cellulose fiber; CW, cell wall; LF, central string of unknown material; LOBS, loose cellulosomes; PP, polypeptide subunits (some ordered, and some at the periphery non-ordered); UF, ultrathin fibrils connecting the subunits to the central string; X, the long axis of the cellulosome referred to as the groove at the midline of cellulosomes in Fig. 4, 5, and 7.

from information reported in the literature (7) that this is the length of a cellooligosaccharide of four cellobiose units long (C_4). If we assume that these subunits represent individual polypeptides with enzymatic activity, these findings could imply that a simultaneous multicutting event takes place along a cellulose fiber (C_n) aligned beside such a row of subunits. Smaller cellooligosaccharides (C_2 , C_1) might then be formed from C_4 units or from cellulose, either by a similar multicutting event mediated by chains of smaller subunits, with smaller center-to-center distances also present in the same cellulosome (not shown in Fig. 12, but see Fig. 9a for electron microscopic evidence), or by single cuts. This could take place within the cellulosome, assuming that the C_4 units diffuse to the cellulosome. Such a series of proposed reactions is consistent with the findings that many polypeptide components of cellulosomes possess enzymatic activity (see above) and with the finding that cellobiose accounts for greater than 90% of the products of cellulose hydrolysis by isolated cellulosomes (15, 17). In the latter context, we must assume that cellooligosaccharide fragments (e.g., C_4 , C_2) do not diffuse from the complex. It is clear from the mechanism proposed above that cellulosomes, in which various types of endoglucanases appropriately juxtaposed with respect to one another and with respect to the substrate, can account for

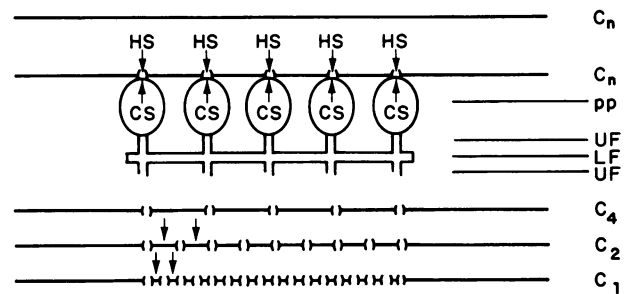


FIG. 12. Diagrammatic view of the main aspect of the structural concept of the *C. thermocellum* cellulosome from strains JW20 and YM4 and of the inherent structure-function relationships. Abbreviations: CS, catalytic site; HS, hydrolysis site, i.e., the site on the cellulose chain at which a glycosidic bond is cleaved; UF, ultrathin fibrils; C_n , C_4 , C_2 , C_1 ; cellooligosaccharides of various lengths (C_n is cellulose and C_1 is cellobiose). See the text for details.

the complete hydrolysis of cellulose without the need for the operation of enzymes of the exocellobiohydrolase type. We do not preclude the possibility that such enzymes may be among the many components of the cellulosomes (cf. reference 27), but their existence has yet to be verified.

In the electron micrographs shown in Fig. 7 to 9 above, ultrathin fibrils were seen to be joining individual subunits in a given row as well as connecting pairs of rows. The measurable diameter of these fibrils is consistent with the fact that they are composed of carbohydrate. In this context we note that many of the individual types of polypeptide in cellulosomes of JW20 are known to be glycoproteins (15) and that the cellulosomes proper of strains JW20 and YM4 contain from 6 to 13% carbohydrate (Ljungdahl et al., in press).

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