Cellulose Digestion and Cellulase Regulation and Distribution in *Fibrobacter succinogenes* subsp. *succinogenes* S85

LI HUANG† AND CECIL W. FORSBERG*

Department of Microbiology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

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*Fibrobacter succinogenes* subsp. *succinogenes* S85 initiated growth on microcrystalline cellulose without a lag whether inoculated from a glucose, cellobiose, or cellulose culture. During growth on cellulose, there was no accumulation of soluble carbohydrate. When the growth medium contained either glucose or cellobiose in combination with microcrystalline cellulose, there was a lag in cellulose digestion until all of the soluble sugar had been utilized, suggesting an end product feedback mechanism that affects cellulose digestion. CI-stimulated cellobiosidase and periplasmic cellodextrinase were produced under all growth conditions tested, indicating constitutive synthesis. Both cellobiosidases were cell associated until the stationary phase of growth, whereas proteins antigenically related to the CI-stimulated cellobiosidase and a proportion of the endoglucanase were released into the extracellular culture fluid during growth, irrespective of the substrate. Immunoelectron microscopy of cells with a polyclonal antibody to CI-stimulated cellobiosidase as the primary antibody and 10-nm-diameter gold particles conjugated to goat anti-rabbit antibodies as the second antibody revealed protrusions of the outer surface which were selectively labeled with gold, suggesting that CI-stimulated cellobiosidase was located on the protrusions. These data support the contention that the protrusions have a role in cellulose hydrolysis; however, this interpretation is complicated by reactivity of the antibodies with a large number of other proteins that possess related antigenic epitopes.

*Fibrobacter succinogenes* subsp. *succinogenes* S85, formerly *Bacteroides succinogenes* (27), is a major cellulolytic bacterium within the rumen (3, 33). One of the reasons for its predominance is its ability to readily degrade various forms of crystalline cellulose (9, 10). However, the physiological mechanism by which the bacterium degrades cellulose is incompletely understood. Elucidation of the mechanism has been tackled by purification and characterization of the enzymes involved in cellulose digestion. The enzymes purified include two cellobiosidases, a CI-stimulated cellobiosidase (14), a periplasmic cellodextrinase (13), and three endoglucanases designated EG1, EG2, and EG3 (23, 25). Although we have detailed knowledge of the properties of these enzymes and their presence in *Fibrobacter succinogenes* subsp. *succinogenes* S85 (15), there is limited information on the regulation of cellulose digestion and of the enzymes involved in the process (9). Therefore, in a recent study by McGavin et al. (24), the regulation and distribution of the endoglucanases was examined. The major objective of the present study was to determine the effect of the carbon source for growth on the rate of cellulose digestion and to correlate this with the production and distribution of cellobiosidase enzymes.

**MATERIALS AND METHODS**

Organism and growth conditions. *F. succinogenes* subsp. *succinogenes* S85 was grown in a 500-ml round-bottom flask with gyration shaking at 150 rpm at 37°C in the medium of Scott and Dehority (31) with the following modifications. Ammonium sulfate was the sole source of nitrogen. Glucose (0.5%, wt/vol), cellobiose (0.5%, wt/vol), Avicel microcrystalline cellulose PH105 (0.3%, wt/vol; FMC Corp., Marcus Hook, Pa.), and amorphous cellulose (acid-swollen cellulose; 0.2%, wt/vol; 30) were used individually or in combination as carbon sources. Samples were removed for processing at the times indicated.

Localization of cellulase components. The cellular distribution of cellulase components of *F. succinogenes* subsp. *succinogenes* S85 grown under various conditions was examined as described previously (12).

Enzyme assays. All enzyme assays were conducted as previously described (12).

Analytical methods. Soluble sugars, i.e., glucose and cellobiose, in culture fluids were measured by using the phenolsulfuric acid method (4). Cellobiose in samples was first washed repeatedly by sedimentation and suspension in water to remove soluble carbohydrates. Sedimented cellulose was then solubilized in 67% (wt/vol) sulfuric acid as described by Updegraff (34) and quantified by using the phenolsulfuric acid method for soluble carbohydrates (4). Glucose was used as the standard. Protein was determined by the method of Bradford (2), with bovine serum albumin as the standard.

Electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described by Laemmli (18), with several modifications (13), by using a 10% acrylamide separating gel.

Lectin binding. Fluorescence-labeled lectin GS-1 from *Griffonia simplicifolia* (Vector Laboratories, Inc., Burlingame, Calif.) was tested for binding to *F. succinogenes* subsp. *succinogenes* S85 by fluorescence microscopy (20).

Antiserum preparation and immunoblotting. Antisera to cellodextrinase and CI-stimulated cellobiosidase were prepared as described elsewhere (15). Immunoblotting of the samples prepared from various cultures was conducted as described previously (15).

Immunogold labeling of cells. For pre-fixation labeling, a sample (0.5 ml) of an exponentially growing glucose or cellobiose culture (9 h) or a rapidly grown cellulose culture...
(40 h) which contained a substantial amount of residual cellulose was harvested by centrifugation. With cellulose-grown cells, a low centrifugal force was maintained (2,000 × g) to reduce sedimentation of unbound cells. The cell pellet was suspended in 100 μl of 50-fold-diluted rabbit antibody to C1-stimulated cellobiosidase in 20 mM HEPES (N-2-hydroxyethylpiperazine-Ν'-2-ethanesulfonic acid) buffer (pH 6.7)–0.8% (wt/vol) NaCl (HBS). After incubation at room temperature for 30 min, the sample was centrifuged and washed twice in HBS. The washed cell pellet was suspended in 50 μl of 10-fold-diluted goat anti-rabbit immunoglobulin G (IgG)-gold conjugate (10-nm-diameter gold particles; Sigma Chemical Co., St. Louis, Mo.) in HBS supplemented with 0.2% (wt/vol) bovine serum albumin (HBS-BSA). Following incubation at room temperature for 30 min, the sample was centrifuged and washed twice in HBS-BSA and once in 20 mM HEPES buffer (pH 6.7). The cells were then fixed in 0.5% (vol/vol) glutaraldehyde in 20 mM HEPES buffer (pH 6.7). For postfixation labeling, a glutaraldehyde stock solution (1%, wt/vol) in 20 mM HEPES buffer (pH 6.7) was added to the culture to give a final concentration of 0.2% (vol/vol). After incubation at 4°C for 30 min, the sample was centrifuged and washed twice in HBS. The cells were then suspended in HBS containing 0.2 M NH₄Cl and incubated at room temperature for 30 min to inactivate residual reactive aldehyde groups. The samples were then centrifuged, washed twice in HBS, and processed in the same way as for prefixation labeling. The control was a sample of cells treated as described above, except that the first antibody was replaced by preimmune serum or buffer.

Transmission electron microscopy. Transmission electron microscopy was performed in a way similar to that described by Lam et al. (19). Equal volumes of immunogold-labeled cells and 1% (wt/vol) ammonium molybdate (pH 7) were mixed, and a carbon-Formvar-coated 200-mesh copper grid was briefly floated on the surface. Excess solution was removed with filter paper. The samples were examined by using a Philips 300 transmission electron microscope operating at an accelerating voltage of 60 kV.

RESULTS

Growth and cellulase activity of cultures in medium containing a single carbon source. F. succinogenes subsp. succinogenes S85 exhibited a generation time of 3.5 h when grown in chemically defined medium with either glucose or cellulose as the carbon source (Fig. 1A and B). Growth on crystalline cellulose was slower, with a generation time of 10
TABLE 1. Specific activities of cellulytic enzymes of *F. succinogenes* subsp. *succinogenes* S85 grown on various carbon sourcesa

<table>
<thead>
<tr>
<th>Carbon sources</th>
<th>Sp act (nmol min⁻¹ mg of protein⁻¹)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Cellobiosidasea</td>
</tr>
<tr>
<td>Glucose</td>
<td>219 (1.10 ± 0.03)</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>134 (1.08 ± 0.02)</td>
</tr>
<tr>
<td>Amorphous cellulose</td>
<td>154 (ND)</td>
</tr>
<tr>
<td>Avicel</td>
<td>90 (1.11 ± 0.02)</td>
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</table>

*a* Cells grown in synthetic medium (31) with glucose (0.5%, wt/vol), cellobiose (0.5%, wt/vol), or amorphous cellulose (approximately 0.2%, wt/vol) as the carbon source were harvested in the late exponential phase (10 h), while cells grown with Avicel microcrystalline cellulose PH105 (0.3%, wt/vol) as the sole carbon source were harvested after complete digestion of cellulose (50 h). The cells were sonicated for 20-s bursts with 1-min intervals on ice. The cellobiosidase activities were measured in the presence of chloride.

The values in parentheses represent the ratios of the cellobiosidase activity of the disrupted cell suspensions assayed in 0.2 M NaCl to that assayed without NaCl. Each value is expressed as the mean and standard deviation of six measurements. ND, Not determined.

h (Fig. 1C). An unexpected finding was that cells grew on amorphous cellulose (acid swollen) with a generation time of 3.5 h, which was identical to that for cells grown on glucose (Fig. 1D).

When cells were grown on glucose, cellobiose, or crystalline cellulose, the patterns of cellobiosidase synthesis were similar to those of cell biomass increase (Fig. 1A to C). However, the cellobiose-grown culture exhibited low cellobiosidase and cellobiase activities (Table 1). The glucose-grown culture exhibited the highest specific activities of cellobiosidase and endoglucanase, whereas the cellobiose-grown culture expressed the highest cellobiase activity.

Cellulose digestion and cellobiosidase activity of cultures grown in medium containing two carbon sources. Both glucose- and cellobiose-adapted inocula initiated growth and cellulose digestion with no obvious lag when transferred to medium containing cellulose as the sole carbon source (Fig. 2). Furthermore, during growth on cellulose, there was no increase in soluble carbohydrate in the medium.

In a separate set of experiments, *F. succinogenes* was grown in medium containing either glucose plus cellulose or cellobiose plus cellulose. Each of these media was inoculated with cells grown through three serial subcultures on either glucose or cellobiose. In cultures grown with either glucose or cellobiose in conjunction with cellulose, the soluble carbohydrate was consumed in preference to the cellulose (Fig. 3 and 4). Immediately after inoculation, some cellulose was solubilized but solubilization proceeded at a decreasing rate and came to a virtual stop. After the soluble carbohydrate had been consumed, hydrolysis of cellulose resumed at a high rate with no definite lag. Despite the presence of the two distinct phases of cellulose digestion in these cases, the cellobiosidase activity increased in parallel with increasing cell biomass, thereby giving a constant cellular specific activity (Fig. 5).

Cellular distribution of cellulases in cultures grown on various carbon sources. Most cellobiosidase activity (>90%) remained associated with cells which were allowed to grow to the late exponential phase on glucose, cellobiose, and amorphous cellulose or to consume all of the cellulose (Table 2, data shown only for cellulose-grown cells). In contrast to cellobiosidase, considerably larger proportions of endoglucanase activity were released from the cells. Further fractionation of cells grown on cellulose indicated that nearly half of the cell-associated cellobiosidase activity could be released by osmotic shock, suggesting its periplasmic location (Table 2). Small amounts were present in the extracellular culture fluid, the sucrase wash, and the residual membrane fraction. A particularly interesting feature was that all fractions except the periplasmic one exhibited a higher activity when assayed in 0.2 M NaCl, suggesting the presence of CI-stimulated cellobiosidase in all fractions except the periplasm. This result was similar to that previously.
observed for slowly growing chemostat cultures (Table 1 of reference 12), except that there was little indication of cell lysis products in batch culture.

**Cellodextrinase and Cl-stimulated cellobiosidase in cells grown on various carbon sources.** The production of the two cellulases known to be responsible for most of the cellobiosidase activity, i.e., cellobextrinase and Cl-stimulated cellobiosidase, of *F. succinogenes* subsp. *succinogenes* S85 grown on various carbon sources was individually analyzed by immunoblotting. Cellodextrinase was present at similar levels in cells grown to the late exponential phase in medium containing glucose, cellobiose, or Avicel cellulose (Fig. 6). No cellobextrinase was detected by immunoblotting in the culture fluid of glucose- and cellobiose-grown cells, and only a small amount was found in the culture fluid of cellulose-grown cells. Immunoblotting of the same samples with an affinity-purified polyclonal antibody to Cl-stimulated cellobiosidase revealed numerous bands of cross-reactive antigens present both in the cells and in cell-free culture fluid of cells grown in each medium (Fig. 7A). A distinct immunoreactive band migrated just ahead of the Cl-stimulated cellobiosidase standard. The supernatant and cell-associated proteins exhibited some differences in banding patterns. In a separate experiment, when monoclonal antibody H4A was used to detect the specific antigens, they were also found in both the cell extracts and culture fluid. A protein that comigrated with Cl-stimulated cellobiosidase was found in each of the samples (Fig. 7B). Most of the proteins recognized by the monoclonal antibody were smaller than Cl-stimulated cellobiosidase. The banding pattern of the proteins recognized by either the affinity-purified polyclonal antibody or the monoclonal antibody was not affected by the age of the *F. succinogenes* subsp. *succinogenes* S85 culture. The reason for the differences in the labeling of proteins by polyclonal and monoclonal antibodies is unknown, although it is known that the intensity of labeling is different because of the variation in access to antigenic sites on proteins and the quantitative difference in epitopes on each protein.

**Immunolabeling of cells.** To determine the relationship between proteins that reacted with the antibody to Cl-stimulated cellobiosidase with respect to their locations on the cell surface of *F. succinogenes* subsp. *succinogenes* S85, glucose-grown cells were incubated with diluted antibodies to the enzyme and then treated with a goat anti-rabbit IgG

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**FIG. 5.** Specific cellobiosidase activities of *F. succinogenes* subsp. *succinogenes* S85 during growth in batch cultures under various conditions. The growth medium contained glucose (0.3%) plus Avicel cellulose (0.2%) (■ and ○), cellobiose (0.3%) plus Avicel cellulose (0.2%) (▲ and △), or Avicel cellulose (0.2%) only (■ and □). The inoculum was a glucose-grown culture (closed symbols) or a cellobiose-grown culture (open symbols).

**FIG. 6.** Detection of periplasmic cellobextrinase in cultures grown on different carbon sources. Lanes: 1, 14 μg of extracellular proteins from an Avicel-grown culture; 2, 14 μg of extracellular proteins from a cellobiose-grown culture; 3, 14 μg of extracellular proteins from a glucose-grown culture; 4, 0.4 μg of periplasmic cellobextrinase; 5, 27 μg of cellular proteins from an Avicel-grown culture; 6, 27 μg of cellular proteins from a cellobiose-grown culture; 7, 27 μg of cellular proteins from a glucose-grown culture. The first antibody was a polyclonal antibody to periplasmic cellobextrinase, and the second antibody was a 3,000-fold-diluted BioRad goat anti-rabbit IgG antibody conjugated to alkaline phosphatase.

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**TABLE 2.** Location of the cellulase components in *F. succinogenes* subsp. *succinogenes* S85 grown in a batch culture.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>% of sum of activities in each fractiona</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Carboxymethylcellulase</td>
</tr>
<tr>
<td>Cell-free culture fluid</td>
<td>6 (2) [1.30]</td>
</tr>
<tr>
<td>Buffer washes</td>
<td>6 (2) [1.54]</td>
</tr>
<tr>
<td>Sucrose wash</td>
<td>6 (2) [1.12]</td>
</tr>
<tr>
<td>Osmotic shock fluid</td>
<td>44 (14) [1.02]</td>
</tr>
<tr>
<td>Supernatant of sonicated sample</td>
<td>38 (12) [1.15]</td>
</tr>
<tr>
<td>Membrane pellet of sonicated sample</td>
<td>6 (2) [1.11]</td>
</tr>
<tr>
<td>% Recoveryb</td>
<td>110</td>
</tr>
</tbody>
</table>

* Cells grown in a batch culture with 0.3% (wt/vol) cellulose as the carbon source were harvested after virtually complete digestion of cellulose (60 h).

* The values in parentheses are units of enzyme activity (nanomoles per minute per milliliter) or protein concentrations (milligrams per milliliter) in the fractions. The values in brackets represent the ratios of cellobiosidase activities of disrupted cell suspensions assayed in NaCl (0.2 M) to those assayed without NaCl. ND, Not detectable.

* Recovery was calculated on the basis of the total activity or protein content of a sonicated culture.
Antibody conjugated to gold particles. Upon centrifugation, samples incubated with antiserum produced pink cell pellets, while cells first reacted with preimmune serum had unstained pellets, indicating the specific interaction between the antibodies to CI-stimulated cellobiosidase and cell surface antigens. Similar results were achieved when affinity-purified antibodies were used. When glucose-grown cells were labeled with gold before being fixed with 1% (wt/vol) glutaraldehyde, the cell surface structure was not well preserved because of repeated washing of the cells. However, clusters of gold particles were found, indicating that the antigens were not evenly distributed on the cell surface (Fig. 8). No nonspecific background staining by the gold marker was discernible on cells treated with preimmune serum in place of the antiserum. Glucose-grown cells prepared by postfixation labeling retained a well-defined shape, and surface structures were apparent (Fig. 9A). Membranous protuberant structures were observed, and a patchy distribution of the gold particles was observed, except that they bound to the protuberances. When a sample of the sedimented cellulose with attached cells was processed by using the postfixation labeling method, patches of gold particles were also found to be associated with cell surface protuberances (Fig. 9B).

FIG. 7. Detection of CI-stimulated cellobiosidase in cultures grown on different carbon sources. Lanes: 1, 14 µg of extracellular proteins from an Avicel-grown culture; 2, 14 µg of extracellular proteins from a cellulose-grown culture; 3, 14 µg of extracellular proteins from a glucose-grown culture; 4, 0.2 µg of CI-stimulated cellobiosidase; 5, 27 µg of cellular proteins from an Avicel-grown culture; 6, 27 µg of cellular proteins from a cellulose-grown culture; 7, 27 µg of cellular proteins from a glucose-grown culture. The first antibody was a polyclonal antibody to CI-stimulated cellobiosidase (A) or undiluted monoclonal antibody H4A (B), and the second antibody was 3,000-fold-diluted protein A conjugated to alkaline phosphatase (A) or a 5,000-fold-diluted goat anti-mouse F(ab')2 antibody conjugated to alkaline phosphatase (B).

FIG. 8. Immunolabeling of glucose-grown cells. The first antibody was a polyclonal antibody to CI-stimulated cellobiosidase, and the second antibody was a goat anti-rabbit IgG antibody conjugated to gold particles. The cells were fixed with 1% (wt/vol) glutaraldehyde after gold labeling. Bar, 0.5 µm.

Lectin binding. F. succinogenes was tested for lectin binding by using fluorescence-labeled lectin GS-1 from G. simplicifolia, since binding has been correlated with the presence of cellulases in some cellulolytic bacteria (20). However, no binding of the lectin to F. succinogenes subsp. succinogenes S85 cells was detected.

DISCUSSION

In this study, we examined the influence of growth conditions on digestion of cellulose and production and distribution of cellobiosidases in F. succinogenes subsp. succinogenes S85. A fascinating observation on the growth characteristics was that cells grew as rapidly on amorphous cellulose as on glucose or cellulose, whereas growth was threefold slower on crystalline cellulose. Therefore, it appears that the actual solubilization process, involving disruption of the crystalline structure of cellulose and not hydrolysis of the glucan strands of amorphous cellulose, limits cell growth on crystalline cellulose (5). In other words, as we proposed previously, endoglucanase action which catalyzes the breakdown of amorphous cellulose is not a growth-limiting factor (6). This conclusion is further supported by the observation that soluble carbohydrate did not accumulate during growth of F. succinogenes subsp. succinogenes S85 on crystalline cellulose.

When cells were grown in medium containing a combination of either glucose or cellulose with crystalline cellulose, the growth rate decreased after utilization of the soluble carbohydrate, but no distinct lag in growth was observed and hydrolysis of cellulose followed immediately. Furthermore, no lag in initiation of growth on crystalline cellulose was observed when either glucose- or cellulose-grown cells were used as the inoculum. These observations suggest that cells constitutively synthesize the enzymes necessary for
Figure 9. Immunolabeling of cells grown on glucose (A) or Avicel cellulose (B). The first antibody was a polyclonal antibody to Cl-stimulated cellobiosidase, and the second antibody was a goat anti-rabbit IgG antibody conjugated to colloidal gold. The cells were fixed with 0.2% (wt/vol) glutaraldehyde for 30 min before labeling. Bar, 0.5 μm. The arrowheads indicate cell surface protrusions labeled with immunogold.

digestion of crystalline cellulose. This is indeed true of cellobiosidase synthesis. Cellobiosidase production by *F. succinogenes* subsp. *succinogenes* S85 correlated closely with the increase in cell protein under all conditions of growth. Immunoblots of cellular and extracellular proteins of cells grown on glucose, cellobiose, or cellulose demonstrated that periplasmic cellobextrinase and Cl-stimulated cellobiosidase were synthesized in similar proportions by cells grown on each of the three carbon sources. In addition to cellobiosidase activity, endoglucanase and cellobiase activities were also found in cells grown on glucose, cellobiose, or cellulose medium.

However, there was a lag in the digestion of cellulose when either glucose or cellobiose was present in conjunction with cellulose. The lag was not immediate, as might have been expected, but this could be attributed to the fact that Avicel microcrystalline cellulose contains as much as 30% amorphous cellulose (17). The physiological basis of the lag in digestion is not known, but it must be due to a type of feedback inhibition of enzyme catalysis or binding or inhibition of cell adherence rather than repression of synthesis. The data also suggest that the inhibition is of an enzyme(s) that acts at an early stage in the disaggregation of crystalline cellulose. The type of enzyme affected may be one with the unique ability to partially degrade insoluble cellulose to lower-molecular-weight insoluble fragments, and thus it may be similar to the endoglucanase isolated from *Ruminococcus albus* (29). Inhibition by cellobiose is readily explained by inhibition of binding by the cellulase-binding domain, as demonstrated by Ghangas and Wilson (7); however, inhibition by glucose is difficult to account for but must affect catalysis.

The observation of the constitutive nature of cellulase action in *F. succinogenes* subsp. *succinogenes* S85 made in this study differs from data presented by Hiltner and Dehority (11). They observed that inocula from either glucose- or cellobiose-grown cultures of *F. succinogenes* subsp. *succinogenes* S85 exhibited a lag before initiating cellulose digestion, and it was suggested that the lag was due to the inducible nature of cellulase action, removal of a metabolic inhibitor, or time required for attachment. Their observed lag in cellulase digestion can perhaps be accounted for by a carryover of either glucose or cellobiose which affects cellulase digestion, since the cellulase enzymes seem to be constitutive and glucose-grown cells bind efficiently to crystalline cellulose (8).

Cellobiose has been reported to cause repression of cellulase production in both *Clostridium thermocellum* (16) and *Bacteroides cellulosolvens* (28). In addition, feedback inhibition of the cellulase system of *B. cellulosolvens* by cellobiose was noted but glucose had no deleterious effect on either cellulase production or activity (28). However, the cellulase of *Acetobacter cellulolyticus* was not subject to end product inhibition by either glucose or cellobiose (21), whereas the cellulase of *Streptomyces flavogriseus* was inhibited by cellobiose but not by glucose (22). In contrast to the various responses described for other bacteria, both glucose and cellobiose have been reported to cause inhibition of cellulase activity of *R. albus* (32). These data indicate that regulation of cellulase digestion by *F. succinogenes* subsp. *succinogenes* S85 resembles that in *R. albus* by being feedback inhibited by both glucose and cellobiose.

The cellobiosidase activity was mostly cell associated, while 45% of the endoglucanase was released from the cells; these data were confirmed by McGavin et al. (24). The cellular location of cellobiosidase activity determined under these conditions is consistent with the initial observations by Huang and Forsberg (12, 13) which showed that the cellobiosidase not stimulated by chlorite was mainly periplasmic, while Cl-stimulated cellobiosidase was present in all cell fractions except the periplasm and the extracellular culture fluid. By using immuno electron microscopy with polyclonal antibodies specific for Cl-stimulated cellobiosidase, the enzyme and/or other related antigens were found located in patches on the cell surface. In cells fixed with glutaraldehyde before labeling, the antibody was found associated with.
membranous protrusions or blebs in both glucose- and cellulose-grown cells. These structures are similar in appearance to the polycellulosomes initially identified in *Clostridium thermocellum* (1) and apparently related structures observed in other cellulosytic bacteria, including *B. cellulolyticus*, *A. cellulolyticus* (20), and more recently, *R. flavefaciens* and *F. succinogenes* (26). The distribution of cellulosidase-common antigen(s) is different from that of EG2, another cell-associated enzyme from *F. succinogenes* subsp. *succinogenes* S85 (24). EG2 was rarely detected at the cell surface by immunoelectron microscopy, and then only where there was disruption of the integrity of the outer membrane. The probable presence of Cl-stimulated cellulosides on the cell surface is complicated by the fact that the enzyme shares epitopes with a large number of other cellular proteins of *F. succinogenes* subsp. *succinogenes* S85 (15). Because of this complication, it cannot be concluded unequivocally that Cl-stimulated cellulosidase is at the cell surface. Obviously, it is of considerable interest to elucidate the nature and functions of those proteins immunologically related to Cl-stimulated cellulosidase.

The experimental data available strongly support the cell surface as the location for crystalline cellulose digestion; however, there are a number of missing pieces in this puzzle. Furthermore, the question remains as to what the precise activators are that bring the various cellulase enzymes into juxtaposition for highly efficient crystalline cellulose hydrolysis.

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**LITERATURE CITED**


