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Purification and Comparison of the Periplasmic and Extracellular Forms of the Cellodextrinase from *Bacteroides succinogenes*

LI HUANG AND CECIL W. FORSBERG*

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

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Both the periplasmic and the extracellular cellodextrinases from *Bacteroides succinogenes* S85 grown on Avicel microcrystalline cellulose were purified to homogeneity by column chromatography and characterized. Over 70% of the total cellobiosidase activity displayed by cells was accounted for by these enzymes. The periplasmic and extracellular cellodextrinases had identical molecular weights (50,000), as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and identical isoelectric points (4.9). In addition, the two enzymes were similar in catalytic properties, with K_m and V_{max} values of approximately 0.24 mM and 21 μmol/min per mg of protein, respectively. Examination of the two enzymes by using peptide mapping and immunoblotting techniques provided additional evidence indicating their identical nature. Immunoblotting of the extracellular culture fluid with affinity-purified antibody to the periplasmic cellodextrinase revealed one band with a molecular weight corresponding to that of the periplasmic cellodextrinase. The stability of the purified periplasmic cellodextrinase in aqueous solution was markedly enhanced by increased protein content. This enzyme showed a low affinity for crystalline cellulose.

When grown on crystalline cellulose, *Bacteroides succinogenes*, a highly efficient rumen cellulose degrader, has been found to produce two distinctly different cellobiosidase activities, chloride-stimulated and non-chloride-stimulated activities (10; L. Huang, C. W. Forsberg, and D. Y. Thomas, J. Bacteriol., in press). It was found that the chloride-stimulated cellobiosidase was present in the extracellular culture fluid, as well as associated with the cell membranes, while the non-chloride-stimulated enzyme was located in the extracellular fluid and in the periplasm of the cells (10; Huang et al., in press). Recently, the chloride-stimulated cellobiosidase was purified to homogeneity. This enzyme is capable of terminal cleavage of both cellooligosaccharides and acid-swollen cellulose but does not hydrolyze crystalline cellulose to any significant extent (Huang et al., in press). It was also demonstrated earlier that the periplasmic non-chloride-stimulated cellobiosidase could be separated from endoglucanase activity. This cellobiosidase releases cellobiose from cellooligosaccharides but shows little activity with either acid-swollen cellulose or crystalline cellulose and thus is a cellodextrinase (10); however, purification of this enzyme has not been accomplished. Furthermore, the identity and properties of the extracellular non-chloride-stimulated enzyme had not been investigated.

It is now generally accepted that the multicomponent cellulase systems produced by cellulolytic bacteria are different from their fungal counterparts (14; M. P. Coughlan and L. G. Ljungdahl, Abstr. FEMS Symp. Biochem. Genet. Cellulose Degradation, 1987, L-02, p. 12). Cellodextrinases are one class of cellulases, and they are dissimilar in function from any of the three well-characterized fungal cellulases (16, 20). To elucidate the function(s) of the cellodextrinase, it is essential to purify the enzyme to homogeneity and study its properties. In the present study, both the extracellular and the periplasmic cellodextrinases were purified to homogeneity by column chromatography and their structural and biochemical relatedness was examined.

* Corresponding author.

MATERIALS AND METHODS

Organism and growth conditions. The organism used in the present study was *B. succinogenes* S85 (8). This strictly anaerobic bacterium was grown on Avicel microcrystalline cellulose in a Multigen benchtop chemostat (10). The growth medium and its preparation were as described previously (10).

Enzyme assays. Cellobiosidase activity was determined by measuring the amount of *p*-nitrophenol released from *p*-nitrophenyl-β-D-cellobioside by the enzyme (10). Other aryl glycosidase activities were assayed in the same manner, except that *p*-nitrophenyl-β-D-cellobioside was replaced with appropriate *p*-nitrophenyl glycosides. One unit of enzyme activity was defined as the amount of enzyme releasing 1 μmol of *p*-nitrophenol per min.

Endoglucanase, xylanase, laminarinase, and lichenanase activities were assayed as described previously (10). One unit of enzyme activity was defined as the amount of enzyme releasing 1 μmol of reducing sugars (as glucose or xylose) per min.

Protein determination. Protein was measured by using the Coomassie brilliant blue binding reagent (Bio-Rad Laboratories, Richmond, Calif.) with bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Mo.) as a standard (2). The protein content of fractions from column chromatography was estimated by measuring A_{280}.

Preparation of samples for column chromatography. Cellulose-grown culture, harvested from the chemostat, was centrifuged at 13,200 × *g* for 20 min at 4°C. The cells were subjected to osmotic shock, and the periplasmic fraction thus released was concentrated by ultrafiltration through a membrane (PM10; Amicon Canada Ltd., Oakville, Ontario). The culture supernatant was concentrated by using a Pellicon filtration system (Millipore Corp., Bedford, Mass.) fitted with membranes with a nominal molecular weight cutoff of 10,000. The concentrated material was ultracentrifuged at 100,000 × *g* for 2 h at 4°C. The nonsedimentable fraction served as the source of extracellular enzyme. The details of these procedures have been reported elsewhere (10; Huang et al., in press).

Column chromatography. Chromatography of the periplasmic cellodextrinase activity on DEAE-Sepharose CL-6B, hydroxylapatite HTP, and Bio-Gel P-150 was conducted in this order as described previously (10). The sample from the Bio-Gel P-150 column was applied to a chromatofocusing column (1.0 by 30 cm) which was packed with Polybuffer exchanger PBE94. The column had been preequilibrated with 12 bed volumes (300 ml) of 0.025 M piperazine hydrochloride (pH 5.5). Following sample application, the proteins were eluted with 10 bed volumes (250 ml) of 10-fold-diluted Polybuffer 74 hydrochloride (pH 4.0). The fractions containing cellobiosidase activity were pooled and loaded onto a Sephadex G-100 column (2.6 by 67 cm) which was equilibrated with 0.05 M sodium phosphate buffer (pH 6.5). The enzyme was eluted with the same buffer.

Purification of the extracellular cellodextrinase was achieved similarly by column chromatography on DEAE-Sepharose CL-6B, hydroxylapatite HTP, Polybuffer exchanger PBE94, Bio-Gel P-150, and Sephadex G-100. All chromatographic columns were prepared according to the instruction of the manufacturer. The experiments involving column chromatography were performed at 4°C.

Gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in slab gels was performed by using the method described by Laemmli (11), with a few modifications (Huang et al., in press). The gels were stained with Coomassie brilliant blue R-250. For glycoprotein determination, the protein samples were separated by SDS-PAGE as described above, except that a 12% separating gel was used. Glycoproteins were stained with the periodic acid-Schiff reagent, as described by Beeley (1). Bovine glycoprotein (Sigma) was used as a positive control. Analytical isoelectric focusing of the purified enzymes was performed in a slab gel covering a pH range of 2.5 to 6.0, as described previously (10).

Peptide mapping. The procedure used in this study was similar to that described by Cleveland et al. (3). The periplasmic and the extracellular cellodextrinases, at concentrations of 33 and 19 μg/ml, respectively, were incubated at 37°C for 1 h in a final volume of 56 μl with *Staphylococcus aureus* V8 protease (36 μg/ml). Following the addition of 5 μl of 20% SDS and 6 μl of mercaptoethanol, proteolysis was stopped by boiling the samples for 2 min. Each sample (40 μl) was analyzed by SDS-PAGE as described above. The separating gel contained 14% acrylamide. The gel was stained with Bio-Rad silver stain.

Production of periplasmic cellodextrinase antiserum and purification of antibodies. The partially purified periplasmic cellodextrinase (greater than 90% pure) was subjected to SDS-PAGE on a 10% preparative gel. The gel was then briefly stained with Coomassie brilliant blue. After destaining to give a clear background, the enzyme band was excised from the gel and lyophilized. The lyophilized gel was crushed and emulsified in Freund complete adjuvant for the primary injection and in incomplete adjuvant for the subsequent injections. Approximately 100 μg of protein was injected into the muscle of the hindquarters of a New Zealand White rabbit. Three subsequent injections of 100, 50, and 50 μg of protein were given at 7, 14, and 29 days, respectively, following the first injection. Antiserum was obtained 1 week after the final administration of the antigen.

Specific antibodies to the periplasmic cellodextrinase were affinity purified from this antiserum by using a modification of published methods (18, 19). The purified periplasmic cellodextrinase was subjected to SDS-PAGE and electrophoretically transferred to a nitrocellulose sheet. A narrow vertical guide strip was cut from the middle of the sheet and stained with amido black. The position of the antigen band was located, and the corresponding horizontal strip was cut from the unstained nitrocellulose.

The strip was quenched in 3% gelatin–20 mM Tris hydrochloride–0.5 M NaCl (pH 7.5) and then incubated with 50-fold-diluted periplasmic cellodextrinase antiserum. The strip was washed three times for 10 min each with 20 mM Tris hydrochloride–0.5 M NaCl–0.5% Tween-20 (pH 7.5). The bound antibodies were eluted from the immunoblot with 0.2 M glycine hydrochloride–0.5 M NaCl (pH 2.7) by incubating at room temperature for 5 min. This step was repeated once. The eluates were immediately neutralized with 1.5 M Tris hydrochloride (pH 8.8).

Immunoblotting. Samples were separated on an SDS-polyacrylamide gel as described above. After electrophoresis, the proteins were electroblotted onto a nitrocellulose sheet in a Trans-Blot apparatus (Bio-Rad) containing 25 mM Tris hydrochloride, 192 mM glycine, and 20% (vol/vol) methanol (pH 8.3). The specific antigens bound to nitrocellulose were detected by using the procedure described in the Bio-Rad Immunoblot assay kit. The first antibody was either diluted antiserum (1:200) or affinity-purified antibody. The second antibody was diluted protein A conjugated to alkaline phosphatase (Sigma).

Analysis of hydrolysis products of cellooligosaccharides and acid-swollen cellulose by high-pressure liquid chromatography. Hydrolysis of cellobiose, cellotriose, cellotetraose, cellopentaose, cellohexaose, and acid-swollen cellulose by the purified periplasmic and extracellular cellodextrinases was examined as described previously (10).

Binding of cellodextrinase to Avicel microcrystalline cellulose. Binding of the purified periplasmic cellodextrinase to Avicel microcrystalline cellulose was examined as described elsewhere (Huang et al., in press).

Chemicals. Carboxymethyl cellulose, methylcellulose, xylan, laminarin, lichenan, and aryl glycosides were obtained from Sigma. Avicel microcrystalline cellulose PH 105 was from FMC Corp., Philadelphia, Pa. Cellotriose, cellotetraose, and cellopentaose were from Pfanstiehl Laboratories Inc., Waukegan, Ill. Cellohexaose was a generous gift from R. B. Hespell. DEAE-Sepharose CL-6B, Polybuffer exchanger PBE94, and Polybuffer 74 were obtained from Pharmacia, Uppsala, Sweden. Hydroxylapatite HTP was purchased from Bio-Rad. Isoelectric focusing carrier ampholyte was from LKB, Bromma, Sweden. All other reagents were at least reagent grade.

RESULTS

Purification of periplasmic and extracellular cellodextrinases. In our previous studies, the presence of a unique periplasmic cellodextrinase was demonstrated, and this enzyme was separated from endoglucanase activity by column chromatography (10). Attempts to completely remove the contaminant proteins from the enzyme, however, encountered considerable difficulties. By taking advantage of the high resolution offered by chromatofocusing, the purity of the enzyme preparation was improved (Fig. 1). After further purification by gel filtration on Sephadex G-100, an electrophoretically homogeneous enzyme preparation was obtained (Fig. 2 and 3). A summary of the purification of the periplasmic cellodextrinase is presented in Table 1. An approximately 500-fold increase in the specific activity of the enzyme preparation was achieved. However, most of the starting activity was lost during purification, especially in the

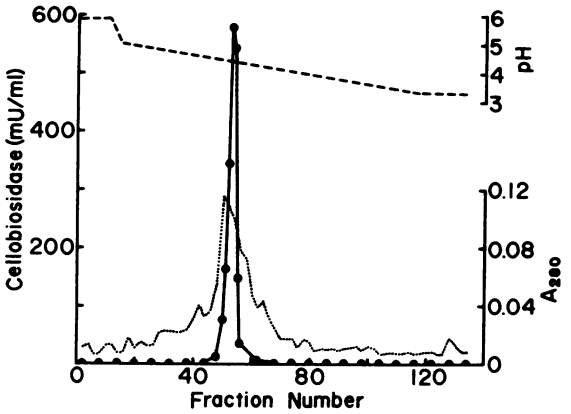

FIG. 1. Chromatofocusing of periplasmic cellobiosidase activity fractions obtained from Bio-Gel P-150 chromatography. The column (1.0 by 30 cm) was packed with Polybuffer exchanger PBE94. Elution conditions: start buffer, 0.025 M piperazine hydrochloride (pH 5.5); elution buffer, 10-fold-diluted Polybuffer 74 hydrochloride (pH 4.0); flow rate, 15 ml/h; fraction volume, 2 ml; temperature, 4°C. Symbols: ●, cellobiosidase; · · · ·, protein; – – – –, pH gradient.

two gel filtration steps, presumably because of the poor stability of the enzyme in a dilute protein solution, as shown below. It was found that purification of samples containing more enzyme protein gave higher yields.

In the extracellular fluid of *B. succinogenes* cultures grown on Avicel cellulose in a chemostat, non-chloride-stimulated cellobiosidase was found to constitute nearly half of the total cellobiosidase activity (Huang et al., in press). This enzyme was purified by following a procedure similar to that used for the purification of the periplasmic cellodextrinase. The first step in this procedure, chromatography on DEAE-Sepharose, is illustrated elsewhere (Huang et al., in press). The non-chloride-stimulated cellobiosidase is clearly separated from the chloride-stimulated enzyme. The activity peak from the Sephadex G-100 column contained a single protein species, as revealed by SDS-PAGE (Fig. 3). The purification of the extracellular non-chloride-stimulated cellobiosidase (cellodextrinase) is summarized in Table 2.

Properties of purified periplasmic and extracellular cellodextrinases. The characteristics of the two purified enzymes are shown in Table 3. The molecular weights of the two enzymes were determined under both denaturing and non-

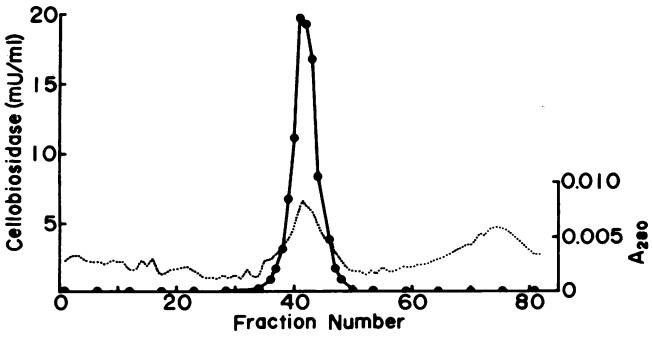

FIG. 2. Gel filtration of periplasmic cellobiosidase activity fractions obtained from chromatofocusing column on Sephadex G-110. Conditions: column, 2.6 by 67 cm; flow rate, 20 ml/h; fraction volume, 4.8 ml; temperature, 4°C. Symbols: ●, cellobiosidase; · · · ·, protein.

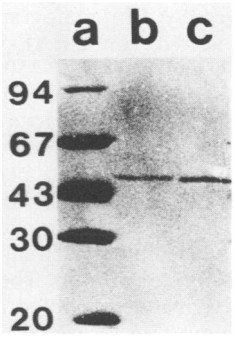

FIG. 3. SDS-PAGE of periplasmic and extracellular cellobiosidases obtained from Sephadex G-100 chromatography. Lanes: a, Pharmacia low-molecular-weight standards; b, 0.4 μg of purified periplasmic cellobiosidase; c, 0.4 μg of purified extracellular cellobiosidase.

denaturing conditions. It is apparent that both enzymes are monomers. In addition, the two enzymes seem to have identical molecular weights, as revealed by SDS-PAGE (Fig. 3). The isoelectric points of the two enzymes were the same. Staining of the purified periplasmic cellodextrinase with the periodic acid-Schiff reagent produced a negative result, suggesting that the enzyme was not glycosylated.

The two purified enzymes were most active in the same temperature range; whereas they differed slightly in optimum pH. A comparison of the kinetic parameters of the two enzymes further indicated their similarity. The activities of the periplasmic and the extracellular cellodextrinases on various carbohydrates and aryl glycosides were also compared (Table 4). Similar to the periplasmic cellodextrinase, the extracellular cellodextrinase exhibited hydrolytic activity on two *p*-nitrophenyl glycosides, *p*-nitrophenyl-β-D-cellobioside and *p*-nitrophenyl-β-D-lactoside. The ratios of the activities of the two enzymes on these two substrates were very close. The activity of the extracellular enzyme on carboxymethyl cellulose was found to be negligible.

The hydrolytic action of the two enzymes on cellulose and its derivatives was studied. Neither enzyme effected a detectable hydrolysis of cellobiose, acid-swollen cellulose, or Avicel cellulose. However, both enzymes degraded the cellooligosaccharides cellotriose, cellotetraose, cellopentaose, and cellohexaose, indicating that they were cellodextrinases.

Peptide mapping of periplasmic and extracellular cellodextrinases. As shown above, the periplasmic and the extracellular cellodextrinases were similar in both physical and catalytic characteristics. To examine the structural relationship between these two enzymes, the Cleveland peptide mapping technique was used (3). The two purified enzymes were partially digested with *S. aureus* V8 protease. The resultant peptides were separated by SDS-PAGE. The two enzymes displayed seemingly identical patterns of peptide banding (Fig. 4). The presence of a couple of minor protein bands (Fig. 4, lane e) appeared to be due to degradation of the purified enzyme during storage, a phenomenon which was observed several times in our studies and did not result in any complication.

Examination of the relatedness of periplasmic and extracellular cellodextrinases with specific antibody. The reactivity of the specific antibodies to the periplasmic cellodextrinase with both enzymes was tested by using the Western blotting (immunoblotting) technique. Both enzymes were recognized

TABLE 1. Purification of periplasmic cellodextrinase

Purification step	Total protein (mg)	Total activity (U)	Sp act (U/mg of protein)	Yield (%)	Purification (fold)
Periplasmic fraction	246.1	24.4	0.10	100	1
DEAE-Sepharose CL-6B	34.8	15.1	0.43	62	4
Hydroxylapatite	13.2	13.9	1.1	57	10
Bio-Gel P-150	0.52	2.16	4.2	9	42
Chromatofocusing	0.17	1.24	7.3	5	73
Sephadex G-100	0.012	0.60	50.0	3	500

by the antibodies (Fig. 5). It was also found that the affinity-purified monospecific antibodies to the periplasmic cellodextrinase detected only one protein in the extracellular culture fluid and that this protein was identical in size to the periplasmic enzyme (Fig. 6). Therefore, it seems that in extracellular culture fluid the cellodextrinase is the only protein species that is antigenetically related to the periplasmic enzyme, and these two enzymes presumably are identical.

Stability of periplasmic cellodextrinase. It was found that the stability of the purified enzyme preparation was significantly affected by the protein content. This effect was investigated by using BSA. When incubated at 39°C in the absence of BSA, the enzyme (2.5 μg/ml) was inactivated by 80% within 4 h. However, no decrease in enzyme activity was detected after 24 h of incubation in the presence of a minimum of 100 μg of BSA per ml. Approximately 63% of the activity remained after incubation for 3 days, and 22% of the activity was left after 1 week. The enzyme stability was also enhanced to various degrees by some other reagents, e.g., ribonuclease (100 μg/ml) and Triton X-100 (0.05%).

Binding of periplasmic cellodextrinase to Avicel microcrystalline cellulose. In the presence of BSA (100 μg/ml), which was used to stabilize the enzyme, 23% of the total enzyme (0.4 μg) was found to bind to cellulose. However, an increase in BSA concentration to 800 μg/ml completely prevented the enzyme from binding, indicating the low affinity of the periplasmic cellodextrinase for Avicel cellulose.

DISCUSSION

B. succinogenes grown on Avicel cellulose in a continuous culture system produces considerable amounts of the enzymes active on *p*-nitrophenyl-β-D-cellobioside, an analog of cellotriose (10; Huang et al., in press). As reported previously, approximately 60% of the total activity was cell bound and primarily in the periplasm of the cells, whereas the rest of the activity was extracellular (10; Huang et al., in press). When the nonsedimentable extracellular enzymes were fractionated on a DEAE-Sepharose CL-6B column, two activity peaks were identified (Huang et al., in press). One was stimulated by chloride and the other was not. Estimation of the activity associated with the two peaks appears to suggest that each accounted for approximately one-half of the total extracellular activity. In other words, over 70% of the total activity produced by cells was not stimulated by chloride.

In our previous study, the periplasmic cellodextrinase was freed from endoglucanases (10). In the present investigation, this enzyme was purified to homogeneity. The characteristics of the purified cellodextrinase were found to be in good agreement with those of the partially purified enzyme preparation. The molecular weight of the enzyme determined by SDS-PAGE was only slightly greater than the value obtained by gel filtration, indicating the monomeric nature of the enzyme. Compared with the two *p*-nitrophenyl-β-D-cellobioside-hydrolyzing enzymes purified from *Ruminococcus albus* (17) and *Ruminococcus flavefaciens* (6), the *B. succinogenes* enzyme is considerably smaller. However, the affinity of the *B. succinogenes* enzyme for *p*-nitrophenyl-β-D-cellobioside was much higher than that reported for the *R. flavefaciens* enzyme, with the K_m being 0.27 mM for the former and 3.08 mM for the latter. The purified cellodextrinase was not stable at 39°C in a dilute preparation. But the stability of the enzyme was substantially improved by added proteins such as BSA and maximized when the concentration of BSA was raised to 100 μg/ml. This requirement of the enzyme for a relatively high protein content for maintenance of stability can be satisfied in the protein-rich periplasm of the cells, from which the enzyme was isolated. The purified cellodextrinase exhibited a low binding affinity for crystalline cellulose, suggesting that it functions at a late stage of cellulose degradation (i.e., on cellooligosaccharides). This finding also agrees with the hydrolytic properties of the enzyme.

As mentioned above, the extracellular non-chloride-stimulated cellobiosidase (cellodextrinase) constitutes a significant portion of the total cellobiosidase activity. To ascertain the physicochemical and catalytic properties of this enzyme, it was purified by using a purification procedure similar to that for the periplasmic cellodextrinase. Characterization of the purified extracellular cellodextrinase revealed its similarity to the periplasmic enzyme. Notably, both enzymes had

TABLE 2. Purification of extracellular cellodextrinase

Purification step	Total proteins (mg)	Total activity (U)	Sp act (U/mg of protein)	Yield (%)	Purification (fold)
Nonsedimentable cell-free culture fluid	2,093	122.6	0.06	100	1
DEAE-Sepharose CL-6B	234	26.7	0.11	22	2
Hydroxylapatite	59.8	13.6	0.23	11	4
Chromatofocusing 1	5.15	12.5	2.4	10	40
Chromatofocusing 2	1.24	5.8	4.7	5	80
Bio-Gel P-150		2.9			
Sephadex G-100	0.023	0.5	21.7	0.4	360

TABLE 3. Properties of the cellodextrinases

Cellodextrinase	Mol wt by: SDS-PAGE	Mol wt by: Gel filtration	pI	K_m (mM)	V_{max}[a]	Optimum pH	Optimum temp (°C)
Periplasmic	50,000	40,000	4.9	0.27	22	6.1	45–50
Extracellular	50,000	40,000	4.9	0.21	20	6.3–6.7	45–50

[a] Expressed as micromoles per minute per microgram of protein.

identical molecular weights, as judged by SDS-PAGE, and identical isoelectric points. To conclusively demonstrate the identical nature of the two enzymes, peptide mapping and immunoblotting techniques were used. The results clearly showed that these two enzymes had practically the same pattern of peptide banding and were immunologically related.

A question arises concerning the cellular location of the enzyme, that is, the site where the cellodextrinase plays a role in cellulose degradation. Does the enzyme act in the extracellular culture fluid, on the cell surface, in the periplasm of the cells, or perhaps in all of these locations? If a combined action of cellodextrinase and other cellulase components is required for effective hydrolysis of cellulose, all these enzymes have to be exposed to the substrate outside the cells. However, if degradation of cellulose to cellooligosaccharides and further breakdown of cellooligosaccharides are separate processes and the outer membrane of cells is permeable to cellooligosaccharides, it is conceivable that cellodextrinase can function in the periplasm, as well as in the extracellular fluid.

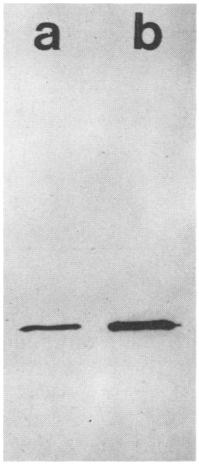

FIG. 5. Analysis of relatedness of periplasmic and extracellular cellodextrinases by electrophoresis and immunoblotting. Purified periplasmic and extracellular enzymes were subjected to SDS-PAGE and then electrophoretically transferred onto a nitrocellulose sheet. Immunoblots were developed with affinity-purified antiserum to the periplasmic cellodextrinase. Lanes: a, purified extracellular cellodextrinase; b, purified periplasmic cellodextrinase.

TABLE 4. Substrate specificity of the cellodextrinases

Substrate	Sp act (μmol/min per mg of protein): Periplasmic cellodextrinase	Sp act (μmol/min per mg of protein): Extracellular cellodextrinase
p-Nitrophenyl-β-D-cellobioside	30.7	19.2
p-Nitrophenyl-α-D-glucopyranoside	<0.5	<0.5
p-Nitrophenyl-β-D-glucopyranoside	<0.5	<0.5
p-Nitrophenyl-β-D-lactoside	11.8	8.5
p-Nitrophenyl-β-D-xylopyranoside	<0.5	<0.5
Carboxymethyl cellulose	<0.5	1.0
Acid-swollen cellulose	<0.5	<0.5
Avicel cellulose	<0.5	<0.5
Xylan	<0.5	<0.5

Under the growth conditions used in this study, approximately 30% of the cellodextrinase activity produced by the cells was in the extracellular medium, whereas the remaining 70% was located in the periplasm of the cells. The mechanism of the release of this enzyme from the periplasm is not yet clear. In some gram-negative bacteria, e.g., *Escherichia coli* and *Salmonella* spp., the secretion of periplasmic proteins into the culture medium appears to be very low, even in the late stationary phase of growth (12, 15). However, a number of periplasmic-leaky mutants have been isolated

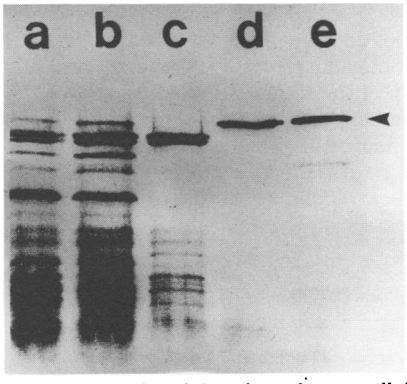

FIG. 4. Peptide maps of periplasmic and extracellular cellodextrinases on SDS–14% polyacrylamide gel. Following digestion with *S. aureus* V8 protease, samples of the two enzymes were separated on a SDS–14% polyacrylamide gel as described in Materials and Methods. Lanes: a, peptides of the periplasmic cellodextrinase; b, peptides of the extracellular cellodextrinase; c, V8 protease (2.76 μg); d, undigested periplasmic cellodextrinase (0.6 μg); e, undigested extracellular cellodextrinase (0.4 μg).

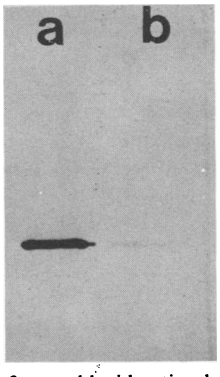

FIG. 6. Detection of non-chloride-stimulated cellodextrinase in supernatant of cellulose-grown culture with affinity-purified antibodies to periplasmic cellodextrinase. Purified periplasmic enzyme and extracellular culture fluid were subjected to SDS-PAGE and then blotted onto a nitrocellulose sheet. Affinity-purified antibody to periplasmic cellodextrinase was used to detect specific antigens. Lanes: a, purified periplasmic cellodextrinase (0.5 μg); b, extracellular culture fluid (35 μg of protein).

(13). It was found that lipoprotein was absent or abnormal in many of these mutants (5, 13). Because of this defect, cells were shown to release membrane vesicles or blebs from the outer membrane (9). Information on the chemistry and structure of the envelope of *B. succinogenes* is not available at the present time, but the examination of the cells by electron microscopy has revealed the absence of a strong adhesion between the outer membrane and the underlying thin peptidoglycan and the plasticity of the cell walls (4, 7). Membrane vesicles were also found to be released from the cells, and their occurrence varied with growth conditions (4, 7). It is therefore speculated that the unstable nature of the *B. succinogenes* cell envelope may help explain the release of the periplasmic cellodextrinase. Obviously other explanations for the release of the cellodextrinase are also tenable. Careful analysis of the cellular location of the enzyme throughout the various growth phases will be particularly valuable.

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