Characterization of CenC, an Enzyme from *Cellulomonas fimi* with Both Endo- and Exoglucanase Activities

PETER TOMME, * EMILY KWAN, NEIL R. GILKES, DOUGLAS G. KILBURN, AND R. ANTONY J. WARREN

Department of Microbiology and Immunology and Protein Engineering Network of Centres of Excellence, University of British Columbia, #300-6174 University Blvd., Vancouver, British Columbia, Canada V6T 1Z3

Received 31 January 1996/Accepted 3 May 1996

The cenC gene, encoding β-1,4-glucanase C (CenC) from *Cellulomonas fimi*, was overexpressed in *Escherichia coli* with a tac-based expression vector. The resulting polypeptide, with an apparent molecular mass of 130 kDa, was purified from the cell extracts by affinity chromatography on cellulose followed by anion-exchange chromatography. N-terminal sequence analysis showed the enzyme to be properly processed. Mature CenC was optimally active at pH 5.0 and 45°C. The enzyme was extremely active on soluble, fluorophoric, and chromophoric glycosides (4-methylumbelliferyl β-glycosides, 2'-chloro-4'-nitrophenyl-β-D-cellobioside, and 2'-chloro-4'-nitrophenyl-lactoside) and efficiently hydrolyzed carboxymethyl cellulose, barley β-glucan, lichenan, and, to a lesser extent, glucomannan. CenC also hydrolyzed acid-swollen cellulose, Avicel, and bacterial microcrystalline cellulose. However, degradation of the latter was slow compared with its degradation by CenB, another *C. fimi* cellulase belonging to the same enzyme family. CenC acted with inversion of configuration at the anomic carbon, in accordance with its classification as a family 9 member. The enzyme released mainly cellobiose from soluble celloextrins and insoluble cellulose. Attack appeared to be from the reducing chain ends. Analysis of carboxymethyl cellulose hydrolysis suggests that CenC is a semiprocessive enzyme with both endo- and exoglucanase activities.

Cellulose is an abundant natural polymer with a repeating unit of β-1,4-linked cellobiosyl residues. Plants and other organisms synthesize cellulose in the form of microfibrils consisting of both crystalline and noncrystalline regions (9). Cellulolytic organisms typically produce endo-β-1,4-glucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21) that interact synergistically to degrade cellulose microfibrils. It is thought that endoglucanases randomly attack bonds in noncrystalline regions on the microfibril surface to produce new chain ends for attack by exo-acting cellobiohydrolases. In turn, the erosion of the microfibril surface by cellobiohydrolases reveals further noncrystalline regions for endoglucanase attack. β-Glucosidases prevent the accumulation of cellobiose to inhibitory levels (50).

Although this general model is widely accepted, several aspects of cellulose digestion remain unexplained. For example, some studies indicate a substantial difference in the ability of individual endoglucanases to interact synergistically with cellobiohydrolases. This finding is difficult to explain if all endoglucanases attack noncrystalline regions in the same random fashion. There are other aspects of cellulose digestion that are not explained by the general model, and it is evident that a more complete understanding of the process requires further information about the action of individual enzymes and their interactions in whole systems (50).

Previous studies have demonstrated that the cellulase system of *Cellulomonas fimi*, a gram-positive saprophytic bacterium, includes four enzymes classified as endoglucanases (CenA, -B, -C and -D), two cellobiohydrolases (CbhA and CbhB), and one enzyme (Cex) with both β-1,4-glucanase and β-1,4-xylanase activity. All of these enzymes contain a distinct catalytic domain and at least one cellulose-binding domain (CBD); in some cases, additional domains are present (31, 32, 40). The catalytic domains belong to one of five families of β-1,4-glucanases and β-1,4-xylanases in the established classification of glycosyl hydrolases based on amino acid sequence similarity (22). Most of their CBDs belong to a large group of related domains (family II) in a similar sequence-based classification (50, 51). While most of the *C. fimi* β-1,4-glucanases have recognizable counterparts in other cellulase systems from bacteria and fungi, one of the enzymes, CenC, has structural and functional features that distinguish it from related enzymes. These include two CBDs (N1 and N2) in tandem in the N terminus with distinct structures and specificities and the occurrence of two additional tandem domains (C1 and C2) of unknown functions at the C terminus (13, 14, 50). These features suggest the possibility that CenC has unusual enzymatic properties. Previous studies on CenC have been limited by the rather poor expression of its recombinant gene in *Escherichia coli*; as a result, very little is known about its activity. These considerations prompted us to develop an improved expression system for CenC and to examine its hydrolytic properties in greater detail. The results suggest that the attack of cellulose substrates by CenC is significantly different from that by other *C. fimi* β-1,4-glucanases.

MATERIALS AND METHODS

Materials. Microcrystalline cellulose (Avicel PH101) was from FMC International, Little Island, County Cork, Ireland. Bacterial microcrystalline cellulose (BMCC) was prepared from cultures of *Acetobacter xylinum* (ATCC 23769) as described previously (18). Acid-swollen cellulose (PASC) was obtained by phosphoric acid treatment of Avicel PH101, as reported previously (13). Carboxymethyl cellulose (CMC; Na salt, low-viscosity grade, nominal degree of polymerization of 400, nominal degree of substitution of 0.7), lichenan, p-nitrophenyl β-glycosides, and 4-methylumbelliferyl β-glycosides were from Sigma Chemical Company, St. Louis, Mo. Barley β-glucan (viscosity, 20 to 30 centistokes) was from Megazyme Ltd., North Rocks, New South Wales, Australia. Cellulodig-
saccharides (>99% pure) were from Seikagaku America Inc., Rockville, Md. 2-Chloro-4'-nitrrophenyl-β-D-cellobioside (CNPGlc2) and 2-Chloro-4'-nitrrophenyl-Lactoside (CNPLac) were synthesized as described previously (12).

**Bacterial strains, plasmids, and growth conditions.** E. coli JM101 [supE thi-1 Δ(lac-proB) F' [traD36 proB lacZΔM15] (54) and TBI [F' Δ(lac-proB) thi-1 hsdR30(Str−) galU galK lacI(lacZ∆M15)167] were used as host strains for maintenance of the plasmids and for production of recombinant protein. Cultures were grown at 30°C in liquid tryptone-yeast-extract-phosphate medium (TYP) (37) or on Luria broth (LB) agar, supplemented with kanamycin (100 μg/ml). Cellulase activity was detected on 0.5% (wt/vol) CMC plates by Congo red (1%, wt/vol) staining (47).

**General DNA procedures.** Plasmid DNA preparation and electrophoresis of DNA fragments were performed by routine procedures (2, 37). Enzymatic treatments of DNA molecules were carried out as recommended by the manufacturer. DNA fragments were recovered after electrophoresis with the GeneClean kit (BIO/ CAN Scientific Inc., Mississauga, Ontario, Canada). Bacteria were transformed by the CaCl2 method (21) or by electroporation (2).

**PCRs.** Oligonucleotide primers were synthesized with an Applied Biosystems 380A automated DNA synthesizer and purified by polyacrylamide gel electrophoresis (PAGE) and reversed-phase chromatography on Sep-Pak columns (Millipore) (1). Each PCR mixture (total volume, 50 μl) contained 10 to 100 ng of template DNA, 25 to 50 pmol (300 ng) of primers, 2 mM MgCl2, 6% dimethyl sulfoxide, 0.2 mM 2'-deoxynucleotide 5'-triphosphates, and 1 U of Taq DNA polymerase in 50 mM Tris-HCl buffer (pH 8.3). Twenty-eight successive cycles were performed as follows: denaturation at 94°C for 15 s, annealing at 57°C for 1.5 min, and primer extension at 72°C for 1.5 min.

**Construction of the expression vector for CenC.** The original vector pTZ-JC2 (13) did not result in useful restriction sites for cloning the cenC gene in pTugEO7K3. Appropriate restriction sites were introduced by PCR (see Fig. 1). A NheI site coinciding with the N-terminal end (Ala-Ser) of the mature protein was introduced as a silent mutation at the 5' end of cenC, with the oligonucleotide 5'TTACCTCATATATTTATTGCCGTGACGATCG-3', where NheI (NheI site underlined) was introduced. An ATG start codon (boldfaced in sequence above) was introduced at the 3' end of cenC with the oligonucleotide 5'AGAAATTCAACGGTGTGGGAGCCGCTGCAC-3' as primer. The resulting 3.24-kb PCR fragment was digested with NdeI and EcoRI and subcloned into the NdeI site of pTSU-100. NheI restriction sites (underlined in Fig. 1) were introduced as primer (NheI site coinciding with the N-terminal end (Ala-Ser) of the mature protein was introduced) in pTugEO7K3. DNA fragments were recovered after electrophoresis with the GeneClean kit (BIO/CAN Scientific Inc., Mississauga, Ontario, Canada). The truncated and modified version, pTugEO7G3, was introduced into E. coli DH5α. The resulting clone was sequenced directly by automated Edman degradation analysis in an Applied Biosystems 470 gas-phase sequencer.

**RESULTS**

**Production and purification of CenC.** CenC is extremely protease sensitive, and expression of CenC under the control of the tac promoter in the original pTug-JC2 vector resulted in low yields of protein (14). Therefore, the cenC gene was recloned into the high expression vector pTugEO7K3, a derivative of pTugA (20), under the tight control of the tac promoter (Fig. 1). The original CenC leader peptide was replaced with the one

Downloaded from http://j.asm.org/ on June 20, 2017 by guest
from *C. fimi* Cex. This peptide efficiently directs translocation of several proteins to the periplasm of *E. coli*, and upon extended incubation, the proteins leak into the culture medium (34). Furthermore, the processing site located between the two alanines in the Ala-Ala-Ser sequence at the boundary of the leader peptide and the CBD of Cex was correctly recognized by the *E. coli* leader peptidase (34).

After 4 h of induction, 87% of the carboxymethyl cellulase activity was present in the cytoplasm of *E. coli*, with the remainder in the culture supernatant. Further incubation resulted in considerable cell lysis and release of CenC into the culture medium. After 18 h of induction, 55% of the activity was found in the culture medium, 40% was found in the cytoplasm, and only 5% of the total activity was detected in the periplasm. However, longer incubation periods also resulted in substantially more degradation of CenC.

Purification of CenC on Avicel was unsuccessful because the enzyme could not be desorbed under mild conditions even though the polypeptide corresponding to the CBDs N1 and N2 is readily desorbed with distilled water (13). Affinity chromatography of the cell extracts on CF1 cellulose, on the other hand, gave a partially purified fraction (Table 1) containing two major polypeptides with apparent molecular masses of 130 and 40 kDa. A previously observed degradation product with a molecular mass of 120 kDa (14) was absent. The 130-kDa (Fig. 2, peak 1) and 40-kDa (Fig. 2, peak 2) polypeptides were separated by anion-exchange chromatography on MacroQ. The 130-kDa polypeptide was identified as CenC by its activity...
on CNPGlc$_2$ (at 405 nm). CenC was purified approximately 62-fold with a final yield of 14% (Table 1). Purified CenC had a very high specific activity (37°C, pH 7.0) of ~7,600 U/mg on CNPGlc$_2$.

The sequence Ala-Ser-Pro-Ile-Gly-Glu-Gly-Thr-Phe-Asp obtained by Edman degradation analysis of the 130-kDa polypeptide corresponded with that of native CenC and confirmed that the protein was properly processed. However, little activity was detected in the periplasm, suggesting that the leader peptidase functional but that the enzyme was trapped in the inner membrane. Edman degradation analysis and binding to cellulose identified the 40-kDa fragment as N1N2.

Properties of CenC. The apparent molecular mass of CenC determined by SDS-PAGE was 130 kDa (Fig. 2), slightly larger than the molecular mass of 112.2 kDa deduced from the amino acid sequence. This difference may be the result of a slightly aberrant behavior of the highly acidic CenC (calculated pI, 4.2) during electrophoresis. The optimum pH and temperature for hydrolysis of CNPGlc$_2$ or CNPLac were 5.0 and 45°C, respectively.

Activities on soluble and insoluble polymeric substrates. CenC hydrolyzed a variety of β-1,4-glucans (Table 2), and the activity decreased roughly with increasing crystallinity of the substrate (CMC > PASC > BMCC ≥ Avicel). CenA and CenB showed a similar trend (Table 2). The activities of CenC on these substrates resembled most closely those of CenA, with a slightly lower activity on Avicel but a significantly higher activity on BMCC. However, the specific activities of CenC (and CenA) on these crystalline substrates, especially BMCC, were significantly lower than those of CenB and CenD (Table 2). CenB and CenD were eight times more active than CenC and 50 times more active than CenA on BMCC.

On the other hand, with specific activities of ~1,000 mol of glucose residues released per mol of enzyme per min, CenC and CenB were the most active C. fimi carboxymethyl cellulases (Table 2); CenC was originally classified as an endoglucanase on this basis (14). Endoglucanase D (CenD) had a surprisingly low activity (47 μmol · min$^{-1}$ · mg$^{-1}$) on CMC. Although viscometric analysis of CMC hydrolysis suggests that CenD is a true endoglucanase (Fig. 3), its activity on this substrate was about 20-fold lower than the activity of the other C. fimi endoglucanases (Table 2).

CenC was also highly active on mixed β-(1,3-1,4) glucans such as barley β-glucan and lichenan and to a lesser extent on glucomannan (Table 2). This property was shared only by CenB (Table 2). Hydrolysis of glucomannan was probably restricted to the β-1,4-glucosidic linkages since galactomannan and mannnan were not hydrolyzed by either CenC or CenB. The high activity of CenC on barley β-glucan is believed to be a property of endoglucanases, although several celllobiohydrolases, including CBH II from Trichoderma reesei, also slowly hydrolyze this substrate (3). As observed with CMC, CenD had a three- to fivefold-lower activity than CenA, CenB, or CenC on β-glucan.

### Table 1. Purification of CenC from cell extracts of E. coli

<table>
<thead>
<tr>
<th>Step</th>
<th>Vol (ml)</th>
<th>Total protein (mg)</th>
<th>Total activity (U$^*$)</th>
<th>Yield (%)</th>
<th>Sp act (U · mg$^{-1}$)</th>
<th>Purification factor (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell extracts</td>
<td>75.0</td>
<td>1,897.5</td>
<td>233,889</td>
<td>100.0</td>
<td>123.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Streptomycin sulfate step</td>
<td>75.0</td>
<td>1,380.0</td>
<td>188,700</td>
<td>80.7</td>
<td>136.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Cellulose affinity</td>
<td>7.0</td>
<td>13.8</td>
<td>62,160</td>
<td>26.6</td>
<td>4,504.3</td>
<td>36.5</td>
</tr>
<tr>
<td>MacroQ</td>
<td>25.0</td>
<td>4.3</td>
<td>32,200</td>
<td>13.8</td>
<td>7,576.5</td>
<td>61.5</td>
</tr>
</tbody>
</table>

*$^*$ Activities were measured at 37°C and 405 nm with 2'-chloro-4'-nitrophenyl-β-D-cellobioside as the substrate. Units were defined as the amount of enzyme needed to release 1 μmol of 2-chloro-4-nitrophenol per min.

Viscometric analysis of CMC hydrolysis. Hydrolysis of CMC by CenA, CenB, and CenD resulted in a rapid increase of the specific fluidity ($\phi_{sp}$) relative to the amount of reducing sugars released, indicative of a random action of these endoglucanases (Fig. 3). The slopes were 95.9, 72.2, and 88.1 $\phi$ · ml · mmol$^{-1}$ for CenA, CenB, and CenD, respectively. In contrast, the exoglucanase CbhA, acting from the chain ends, resulted in a much lower increase in specific fluidity relative to the sugars released (17.1 $\phi$ · ml · mmol$^{-1}$). A similar value was previously obtained for CbhB, another C. fimi exoglucanase (40). The slope for CenC was 33.4 $\phi$ · ml · mmol$^{-1}$, in between those obtained for the endo- and exoglucanases (Fig. 3).

Activities on small chromophoric and fluorophoric compounds. Several C. fimi cellulases hydrolyzed 4-methylumbelliferyl glycosides with release of the fluorophore (Fig. 4). CenC hydrolyzed both the 4-methylumbelliferyl cellulobioside and the 4-methylumbelliferyl lactoside (Fig. 4, lane 4). In contrast,
Degradation products of cellulose hydrolysis. More than 98% of the total sugar produced from PASC following incubation with 1 nmol of CenC for 24 h was cellobiose, with only trace amounts of glucose (~1.6%) (data not shown). A similar pattern was obtained with BMCC. There was no accumulation of cellobiose or higher-molecular-weight products from either substrate. In contrast, CenB released 20% to 25% glucose and 9 to 12% cellotriose upon incubation with both substrates for 24 h (data not shown).

**DISCUSSION**

Biochemical and kinetic characterizations of CenC reveal several properties which are inconsistent with its classification as a randomly acting endoglucanase. The data obtained in this study also suggest that the classification of β-1,4-gluca- nases in two mutually exclusive groups, endoglucanases and exoglucanases, is inappropriate.

The most commonly used criterion to identify endogluca- nases is activity on CMC (47, 53). True exoglucanases would have no activity in the highly sensitive CMC-Congo red plate assay (19, 53). However, at high enzyme concentrations, some cellobiohydrolases do give a positive response in this assay (40). The positive result probably reflects a low intrinsic endogluca- nase activity (40), which can result in erroneous identifi- cation of exocellulases or xylanases as endoglucanases (42). Indeed, it has been suggested previously that there are no strictly exohydrolytic β-1,4-gluca- nases (16, 45). Because detection of exohydrolytic activity requires the use of large amounts of enzyme and long incubation times, slight contamination with endoglucanases, especially for enzymes purified from the culture media of cellulolytic organisms, could account for the apparent endoglucanase activities of the enzymes. However, it appears that CenC has both endoglucanase and exoglucanase activity because it is very active on CMC but it effects a relatively small increase in specific fluidity of CMC per unit of reducing sugar released. This suggests that CenC hydrolyzes CMC by a semiprocessive mechanism; i.e., the enzyme appears

![FIG. 3. Hydrolysis of CMC by C. fimi cellulases. Specific fluidity (φ) is plotted versus the release of reducing sugar upon hydrolysis of CMC by CenA, CenB, CenC, CenD, and ChbA. Reaction mixtures contained 3.6 mM CenA (□), 24 mM CenB (●), 2.5 mM CenC (▲), 20 mM CenD (▲), 500 mM ChbA (□), or 3.2% (wt/vol) CMC in 50 mM sodium citrate (pH 7.0).](http://jb.asm.org/)
to initiate attack on CMC by random attack of internal β-1,4-glucosidic bonds, presumably adjacent to unsubstituted residues, but then proceeds processively from the site of hydrolysis along the β-1,4-glucan chain in an exoglucanolytic manner (Fig. 6B) until one or more substituted glucose residues are encountered. It is not yet clear whether attack of unsubstituted cellulose occurs by a similar mechanism, but such processive activity would explain the almost exclusive release of cellobiose from cellulose. An endo-1,4-β-D-glucanase with a similar semi-processive activity was recently purified from the culture filtrate of Fusarium oxysporum (11). In contrast, the more random action of CenB on cellulose results in the formation of substantial amounts of glucose and cellobiose (Fig. 6C).

The original classification of CenC as a family 9 (formerly family E) cellubiose (14) was based entirely on sequence similarities between its catalytic domain and those of various other β-1,4-glucanases (22). Underlying this classification is the concept that the members of a particular family share a common three-dimensional structure (22) and active-site topology (17). As a result, conservation of the catalytic machinery, dictated by the spatial arrangement of catalytic residues (see below), and of the reaction mechanism, as indicated by the stereochemistry of the product, is expected. The finding that hydrolysis by CenC, like several other family 9 enzymes (17, 30) such as CenB from C. fimi, proceeds with inversion of anomeric configuration (Fig. 6C) is consistent with this concept.

Glycosyl hydrolase family 6 contains both endoglucanases and exocellulbiohydrolases. The endoglucanases have a groove-shaped active site, whereas in the celllobiohydrolases, the active site is enclosed by two large surface loops resulting in a tunnel-shaped structure (36, 44). Regions that contribute to the formation of these loops are evident as insertions when the amino acid sequences of the family 6 endoglucanases and exocellulbiohydrolases are compared (31, 36, 44). The tunnel-shaped active site of the celllobiohydrolases is consistent with a processive mechanism in which the enzymes continually release cellobiose from the ends of cellulose molecules while remaining bound to the substrate (Fig. 6A). Family 9 (subfamily 1) currently contains nine enzymes. In contrast to family 6, sequence comparison of the family 9 enzymes does not reveal the presence of celllobiohydrolases and most enzymes were identified as endoglucanases. CelD from Clostridium thermocellum is the only family 9 subfamily 1 enzyme for which structural information is available (24). Its active site forms an extended cleft composed of six substrate-binding sites, A to F (24).

Comparison of CenC and the other family 9 enzymes does not reveal any obvious insertions that might correspond to extended surface loops enclosing the CenC active site. It therefore seems reasonable to assume that the CenC active site has a cleft-like structure similar to that of CelD. The active site of CenC appears to be readily accessible to the cellulose chains, and such a structure is consistent with the ability of CenC to attack CMC randomly. Presumably, binding is sufficiently tight to prevent rapid dissociation of the substrate after the initial attack, allowing the enzyme to continue processively (Fig. 6B).

Until recently, it was thought that all celllobiohydrolases hydrolyzed cellulose from the nonreducing ends. However, there is now evidence that hydrolysis by CbB from C. fimi (40), CBH I from T. reesei (52), and Avicelase II from Clostridium stercorarium (7) proceeds from the reducing end. Although the determination of the exact bond cleavage frequencies requires the introduction of a radioisotope label, HPLC analysis of the hydrolysis patterns for cleavage of cellobiose and cellodextrins indicates that CenC also liberates cellobiose from the reducing ends. Assuming similar active-site topologies for CenC and CelD, CenC would bind the reducing chain end in subsite F and cleave the glucosidic bond between sub-sites D and E (24), resulting in release of cellobiose from the reducing chain end. This assumption is supported by the finding that most functionally important residues identified in the six substrate-binding sites of CelD are conserved in CenC. In this context, it is interesting to note that both CenC (this study) and CelD (10, 23, 48) are highly active on chromophoric and fluorophoric glycosides derived from β-p-n,4-chellobiose and lactose. The absence of significant activity of CenB on these substrates supports the classification of CenC and CenB in different subfamilies (50).

Hydrolysis by CenC of the agluconic bond of chromophoric glycosides (especially cellobiosides) is not inconsistent with the

![FIG. 4. Analysis of the hydrolysis of 4-methylumbelliferyl glycosides by CenA, CenB, CenC, CenD, and Cex. Hydrolysis of the fluorophoric glycosides was assessed qualitatively by UV illumination after incubation of 0.1 to 0.5 pmol of enzyme with 200 to 500 μM glucoside (MeUmbGlc), cellobioside (MeUmbGlc2), cellotrioside (MeUmbGlc3), lactoside (MeUmbLac), xyloside (MeUmbXyl), or arabinoside (MeUmbAra) for 30 min (A) or 2 h (B) at 37°C.](image-url)
preferential release of cellobiose from the reducing end of natural substrates because the hydrophobicity of the aglucons is known to affect the mode of binding of the glycosides in the active site (5, 29). For example, CBH I from *T. reesei* readily hydrolyzes CNPGlc₂ and CNPLac with release of the phenol (49), even though kinetic analysis using 3H-labeled cellooligosaccharides (52) and structural evidence obtained from co-crystallization of CBH I with substrate analogs (15) indicate that hydrolysis proceeds by removal of cellobiose from the reducing ends.

In summary, the data presented here suggest that the classification of cellulases into endoglucanases and exoglucanases is artificial. It is now apparent that at least some exoglucanases have measurable, albeit low, endoglucanase activity. Similarly, some endoglucanases, such as CenC, appear to act processively at the ends of cellulose molecules following an initial, random attack. Furthermore, the long-held view that all exoglucanases attack cellulose from the nonreducing end is clearly untenable. The topology of the active site probably determines the direction of attack, although a cellulose-binding domain, if present, may also be influential.

FIG. 5. Products of the hydrolysis of cellohexaose by CenC. The products were analyzed by HPLC. (A) Hydrolysis products of cellohexaose after 30 s of incubation with CenC at 25°C; (B) hydrolysis products after incubation at 37°C for 30 min, conditions which allow mutarotation at the anomeric carbon; (C) hydrolysis products of cellohexaose after 30 s of incubation with CenB at 25°C. The β- and α-anomers of cellotriose (peak 3 and 4) and cellotetraose (peak 5 and 6) are resolved; those of glucose (peak 1) and cellobiose (peak 2) are not.

FIG. 6. Attack by processive (A), semiprocessive (B), and nonprocessive (C) cellulases. In each case, the cellulose substrate is represented by three adjacent β-1,4-glucan chains. Each chain consists of 16 cellobiosyl residues with the reducing end denoted by an asterisk. Numbered arrows show the sequence of nine successive hydrolytic events. (A) A processive enzyme (exoglucanase [arrows 1 to 6] or endoglucanase [arrows 7 to 9]) that initiates attack at a reducing end and continues by removing many successive cellobiosyl residues from the same chain before jumping to a new site nearby; (B) a semiprocessive enzyme that initiates attack at an internal β-1,4-glucosidic bond and removes only a few successive cellobiosyl residues from the new reducing end before jumping to another site; (C) a nonprocessive enzyme that jumps to a new site after each attack. The mechanisms shown are similar to those proposed by Robyt and French (35) to describe the attack of starch by α-amylases.

ACKNOWLEDGMENTS

We thank Edgar Ong for the pTugEO7K3 vector and Gary Lesnicki for use of the HPLC.

This work was supported by the Protein Engineering Network of Centres of Excellence.

REFERENCES


