Role of Endo-1,4-β-glucanases from Neisseria sicca SB in Synergistic Degradation of Cellulose Acetate

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An enzyme hydrolyzing β-1,4 bonds in cellulose acetate was purified 10.5-fold to electrophoretic homogeneity from a culture supernatant of Neisseria sicca SB, which assimilate cellulose acetate as the sole carbon and energy source. The enzyme was an endo-1,4-β-glucanase, to judge from the substrate specificity and hydrolysis products of cellooligosaccharides, we named it endo-1,4-β-glucanase I (EG I). Its molecular mass was 50 kDa, 9 kDa larger than EG II from this strain, and its isolectric point was 5.0. Results of N-terminal and inner-peptid sequences of both enzymes, and a similarity search, suggested that EG I contained a carbohydrate-binding module at the N-terminus and that EG II lacked this module. The pH and temperature optima of EG I were 5.0–6.0 and 45°C. It hydrolyzed water-soluble cellulose acetate (degree of substitution, 0.88) and carboxymethyl cellulose. The Km and Vmax for these compounds were 0.296% and 1.29 μmol min⁻¹ mg⁻¹, and 0.448% and 13.6 μmol min⁻¹ mg⁻¹, respectively. Both glucanases and cellulose acetate esterase from this strain degraded water-insoluble cellulose acetate synergistically.

Key words: cellulose acetate; biodegradation; Neisseria sicca; endo-1,4-β-glucanase

Cellulose acetate (CA) is the most important synthetic organic ester because of its broad applications, such as in fibers, plastics, films, and membranes, and because it is made from cellulose, which is the most abundant biopolymer on earth. The degree of substitution (DS) is defined as the mean number of acetyl esters per glucose residue in the CA molecule. CA is biodegradable by some unidentified bacteria, Pseudomonas paucimobilis, and Bacillus sp. strain S2055, but the mechanism of biodegradation of CA on the enzymatic level has not been elucidated. Research on the mechanisms of enzymatic degradation of the biodegradable polymers, including CA is needed to understand the assimilatability and safety of degradation products in the environment. The complete degradation of plant cell-wall polymers calls for a set of enzymes with different activities acting in concert. Highly branched polysaccharides like heteroxylans contain many side-chain substituents. In xylan hydrolysis, enzymes act synergistically on the 1,4-β-D-xylan backbone (endo-1,4-β-xylanases) and side-chain-cleaving enzymes (α-L-arabinofuranosidase, acetyl xylan esterase, and α-glucuronidase). The synergistic action between endo-1,4-β-xylanases and acetyl xylan esterase results in the efficient degradation of acetylated xylan. The deacetylation reaction by acetylxylan esterase increases the accessibility of the polysaccharide backbone to attack by endo-1,4-β-xylanase. The endo-1,4-β-xylanase creates shorter acetylated polymers, which are the preferred substrates for esterase.

A bacterium capable of assimilating CA, Neisseria sicca SB, was isolated from soil. We purified and characterized a CA esterase from N. sicca SB that catalyzes the deacetylation of acetylated saccharides and also an endo-1,4-β-glucanase II (EG II) that hydrolyzes the β-1,4 linkages in CA. We have shown synergistic degradation of CA particles by CA esterase and EG II. When we purified the glucanase on a DEAE-Sepharose FF column, the enzyme activities separated into two fractions. The active fractions that passed through the column were named EG I, and the adsorbed fractions were named endo-1,4-β-glucanase I (EG I). This paper describes the purification and characterization of EG I from N. sicca SB, which enzyme hydrolyzed the β-1,4 linkages in the CA molecule.

Materials and Methods

Materials. CA0.88 (DS, 0.88; water soluble), CA1.77 (DS, 1.77; water insoluble), and CA2.45 (DS, 2.45; water insoluble) were obtained from Daicel Chemical Industries, Ltd. CA1.77 and CA2.45 samples were washed extensively with distilled water and ethanol and CA0.88 was washed with methanol to remove low molecular weight substances such as acetic acid, and all were dried at room temperature.

Abbreviations: CA, cellulose acetate; DS, degree of substitution; EG I, endo-1,4-β-glucanase I; EG II, endo-1,4-β-glucanase II
CM-cellulose (DS, 0.6–0.7), p-nitrophenyl-β-D-glucopyranoside, soluble starch, and cellbiose were purchased from Nacalai Tesque, Inc. Bovine serum albumin and birchwood xylan were from Sigma Chemical Co. Avicel, a microcrystalline cellulose powder, was from Merck. Phosphoric-acid-swollen cellulose was prepared as described by Wood.\textsuperscript{15)} DEAE-Sepharose FF, Q-Sepharose FF, Mono Q, and calibration kits for measurement of molecular mass and isoelectric point were from Amersham Biotech. Phenyl-Toyopearl 650M was from Tosoh Corp. p-Nitrophenyl-β-D-cellobioside, cellotriose, cellotetraose, cellopentaose, and cellohexaose were obtained from Seikagaku Corp. Surfactant Plysurf A210G was obtained from Dai-ichi Kogyo Seiyaku Co., Ltd. CA esterase and EG II from \textit{N. sicca} SB were purified as described previously.\textsuperscript{11,12)} All other chemicals were of the highest purity commercially available.

\textbf{Microorganism and culture conditions.} The organism used was \textit{N. sicca} SB, isolated from soil.\textsuperscript{19)} The bacterium was grown in 15 liters of CA medium, which contained 0.1% KH$_2$PO$_4$, 0.1% K$_2$HPO$_4$, 0.2% NH$_4$NO$_3$, 0.05% NaCl, 0.05% MgSO$_4$·7H$_2$O, 0.005% Plysurf A210G, 0.1% peptone, 0.05% yeast extract, and 0.5% CA-177 powder as the carbon source, pH 6.6, at 30°C, stirrer speed with a 180 rpm for 7 days.

\textbf{Enzyme assays.} The enzyme activity for CA0.88 was assayed in a standard reaction mixture containing 50 mM potassium phosphate buffer, pH 6.0, with 1.0% CA0.88 as the substrate.\textsuperscript{12)} The reaction mixture was incubated at 30°C for 60 min. The release of reducing sugars was measured by the dinitrosalicylic acid method,\textsuperscript{10)} and standard curves were prepared under assay conditions for glucose. One unit of the enzyme activity was defined as the amount of enzyme that produces 1.0 μmol of reducing sugar (as glucose) per minute. The effects of pH and temperature on the enzyme activity were examined using CA0.88 as the substrate. The buffers used were KCl-HCl, potassium phosphate, Tris-HCl, and borate. After incubation of the enzyme in various 50 mM buffers for 24 h at 20°C, the remaining activity was measured under the standard assay conditions. After incubation at various temperatures for 60 min in 50 mM potassium phosphate buffer, pH 7.0, the remaining activity was measured under the standard assay conditions. The effects of metal ions for the enzyme activity were examined by addition of metal ions (to the final concentration of 1.0 mM) to reaction mixtures. The effects of various reagents (protein modification reagents and enzyme inhibitors) on the enzyme activity were tested. After incubation of the enzyme at 20°C for 30 min with various reagents, the residual activities toward CA0.88 were measured. Reactions for substrate specificity were done in 50 mM potassium phosphate buffer, pH 6.0, at 30°C except for CM-cellulose in 50 mM sodium acetate buffer, pH 5.0. The following substrates were used: CA1.77, CM-cellulose, acid-swollen cellulose, Avicel, birchwood xylan, and soluble starch. The concentration of each substrate was 1.0% and the incubation time was up to 6 h.

\textbf{Enzyme puriﬁcation.} The enzyme was purified as follows. All purification steps were done at 4°C. The culture supernatant was obtained by centrifugation (10,000 × g, 25 min) of the culture. The supernatant was salted out by the addition of ammonium sulfate to 80% saturation. The resulting precipitate was collected by centrifugation (10,000 × g, 25 min) and dialyzed in 10 mM Tris-HCl buffer, pH 8.0. The dialyzed solution was put on a DEAE-Sepharose FF column (2.6 × 40 cm) equilibrated with the same buffer. The enzyme was eluted with a linear gradient of 0–0.5 M NaCl in the buffer. The active fractions were pooled. The pooled solution was put on a Q-Sepharose FF column (2.6 × 40 cm) equilibrated with 10 mM Tris-HCl buffer, pH 8.0. The enzyme was eluted with a linear gradient of 0–0.5 M NaCl in the buffer. The active fractions were pooled and dialyzed against 20 mM potassium phosphate buffer, pH 7.0, containing 1.0 M (NH$_4$)$_2$SO$_4$. The dialyzed solution was put on a phenyl-Toyopearl 650M column (1.5 × 21 cm) equilibrated with the same buffer. The enzyme was eluted by linearly decreasing concentrations of (NH$_4$)$_2$SO$_4$, from 1.0 M to 0 M. The active fractions were pooled and dialyzed against 10 mM Tris-HCl buffer, pH 7.0. The remaining activity was measured under the standard assay conditions. The enzyme was eluted with a linear gradient of 0–0.5 M NaCl in the buffer.
buffer, pH 7.7. The dialyzed solution was put on a Mono Q column (0.5 × 5 cm) equilibrated with the same buffer. The enzyme was eluted with a linear gradient of 0–0.3 M NaCl in the buffer. The active fractions were pooled and dialyzed against 10 mM Tris-HCl buffer, pH 7.3, and used as the purified preparation.

Electrophoretic analysis. Native PAGE was done at pH 8.9 with a 7.5% polyacrylamide gel as described by Gabriel. Endo-1,4-β-glucanase activity in the gel was detected by a modification of the method described by Coughlan with Congo red for staining of polysaccharides. SDS-PAGE was done with a 12.5% polyacrylamide gel as described by Laemmli. Protein was stained with Coomassie brilliant blue R-250. Isoelectric focusing was done with a model 111 mini IEF cell system (Bio-Rad Laboratories) in the pH range of 3–10.

Amino acid sequence. The purified enzyme was blotted onto a polyvinylidene difluoride membrane. The N-terminal amino acid sequence of the enzyme was analyzed by automated Edman degradation with an Applied Biosystems model 471A sequencer. For the preparation of peptides from the enzyme, the enzyme was digested with Staphylococcus aureus V8 protease at 37°C for 2 h. The peptides in the digests were separated on SDS-PAGE and electroblotted onto a polyvinylidene difluoride membrane, and the amino acid sequence of the peptides was analyzed with the protein sequencer. Similarity searches were done of the protein sequence databases of GenBank, PIR, and SwissProt.

Adsorption assay. Substrate binding assays were done for EGs I and II with CA1.77, acid-swollen cellulose, and Avicel. These insoluble β-1,4-glucans were washed several times with 50 mM Tris-HCl buffer, pH 7.3. EG I or II was incubated with a β-1,4-glucan (5 mg) in 0.12 ml of 50 mM Tris-HCl buffer, pH 7.3, at 4°C for 2 h with occasional stirring. After incubation, the suspension was sedimented by centrifugation and the supernatant was analyzed for remaining activity for CA0.88. The results were expressed as the percentage of the total activity.

### Results and Discussion

#### Purification of EG I

EG I, which hydrolyzed the main chain of CA0.88, was purified from the culture supernatant of N. sicca SB. The activity for CA0.88 was separated into fractions by DEAE-Sepharose FF column chromatography (Fig. 1). EG I separated from EG II in this step. One of the adsorbed fractions containing glucanase activity, EG I, was further purified. The enzyme was purified 10.5-fold and had a specific activity of 1.05 U/mg for CA0.88 (Table 1). The enzyme gave a single band on SDS-PAGE (Fig. 2A) and native PAGE, indicating homogeneity of the protein.

#### Molecular mass, isoelectric point, and amino acid sequence

The molecular mass of the enzyme was estimated to be 50 kDa by SDS-PAGE, it was larger than EG II, at 41 kDa. The isoelectric point was 5.0. Partial amino acid sequences of EGs I and II were analyzed (Fig. 3). The N-terminal amino acid sequence of EG I was not similar to that of EG II. The peptides from EGs I and II, generated by digestion with Staphylococcus aureus V8 protease, were separated on SDS-PAGE (Fig. 2B). The PAGE pattern of pep-
tides from EG I was the same as that from EG II, with three major bands, and had two amino acid alignments (Fig. 3, fragments 1 and 2). The sequences of the fragments from EG I were almost the same as those from EG II. The inner sequences were similar to the catalytic region of CenA, an endo-1,4-β-glucanase from the cellulolytic bacterium Cellulomonas fimi, and CelA1, endoglucanase 1 from Streptomyces halstedii. On the basis of amino acid sequence similarity of catalytic domains, glycosyl hydrolases have been grouped into families, and CenA and CelA belong to glycoside hydrolase families 6 and 12, respectively. The presence of Mg²⁺, Ca²⁺, Mn²⁺, Fe²⁺, Co³⁺, Ni²⁺, Cu²⁺, and Zn²⁺ had little if any effect. The enzyme was completely inhibited by 1.0 mM sodium fluoride, or metal-chelating reagents (EDTA or o-phenanthroline). These results were the same as those from EG II except for inhibition by Fe³⁺.

Properties of purified enzyme

The pH optimum of the enzyme was 5.0–6.0 in 50 mM buffers. When CM-cellulose was the substrate, the optimum pH was 5.0. The enzyme retained more than 90% activity between pHs 2.5 and 12. The optimum temperature at pH 6.0 was 45°C. The enzyme retained 100% activity when incubated at temperatures up to 40°C, but lost activity at 60°C. The addition of Fe³⁺ or Hg²⁺ inhibited the enzyme activity to 80.1 and 72.2% of the native enzyme activity, respectively. The presence of Mg²⁺, Ca²⁺, Mn²⁺, Fe²⁺, Co³⁺, Ni²⁺, Cu²⁺, and Zn²⁺ had little if any effect. The enzyme was a bifunctional enzyme comprising an N-terminal carbohydrate-binding module and a C-terminal catalytic module. The enzyme was completely inhibited by 1.0 mM N-bromosuccinimide, which modifies tryptophan residues, and 0.1 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide with glycine ethyl ester, which modifies carboxy groups. These results suggested that a tryptophan residue(s) and aspartic or glutamic acid residues were involved in catalytic activity. Glycosyl hydrolase family 6 has highly conserved tryptophan residues near the catalytic residues. The additivity of the native enzyme activity, respectively. The presence of Mg²⁺, Ca²⁺, Mn²⁺, Fe²⁺, Co³⁺, Ni²⁺, Cu²⁺, and Zn²⁺ had little if any effect. The enzyme was completely inhibited by 1.0 mM N-bromosuccinimide, which modifies tryptophan residues, and 0.1 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide with glycine ethyl ester, which modifies carboxy groups. These results suggested that a tryptophan residue(s) and aspartic or glutamic acid residues were involved in catalytic activity. Glycosyl hydrolase family 6 has highly conserved tryptophan residues near the catalytic residues.
hydrate-binding module of EG I from cell walls and to increasing the affinity.27) The carbohydrate-catalytic domain to crystalline cellulose in the plant of reducing sugars increased, but cellooligosaccharides were not found by TLC at the start of the reaction. With the passage of time, cellooligosaccharides were detected as end products in the reaction mixture by TLC. The enzyme hydrolyzed CA0.88 randomly to produce cellooligosaccharides. The hydrolyzates by EG I gave the same pattern on TLC as those by EG II.

EG I bound to acid-swollen cellulose and Avicel, but not to CA1.77, and EG II bound to none of them (Table 3). These results suggest that the carbohydrate-binding module of EG I had high affinity for amorphous cellulose and crystalline cellulose, but not for CA1.77.

The rates of the EG I reaction at 30°C with various concentrations of CA0.88 and CM-cellulose at optimum pH are compared with those with EG II in Table 4. The apparent $K_m$ of EG I was lower for CA0.88 than for CM-cellulose, and the $V_{max}$ was higher for CM-cellulose than CA0.88. The $V_{max}/K_m$ ratios were 4.36 for CA0.88 and 30.4 for CM-cellulose. The kinetic results indicated that EG I was more specific toward CM-cellulose than CA0.88, but that EG II was more specific toward CA0.88 than CM-cellulose. The $K_m$ of EG I for CM-cellulose was lower than that of EG II, but the values for CA0.88 were almost the same. These results indicate that the carbohydrate-binding module in the EG I molecule was important in binding CM-cellulose but not CA0.88, or that the removal of the carbohydrate-binding module from EG I was followed by a change of structure of catalytic domain leading to a decrease of affinity for CM-cellulose.

**Table 2. Substrate Specificity of Endo-1,4-β-glucanases I and II from N. sicca SB**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Specific activity (U/mg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>EG I</td>
</tr>
<tr>
<td>CA0.88</td>
<td>1.05</td>
</tr>
<tr>
<td>CA1.77</td>
<td>0</td>
</tr>
<tr>
<td>CM-cellulose</td>
<td>10.3</td>
</tr>
<tr>
<td>Acid-swollen cellulose</td>
<td>0.236</td>
</tr>
<tr>
<td>Avicel</td>
<td>0.017</td>
</tr>
<tr>
<td>Xylan</td>
<td>0.142</td>
</tr>
<tr>
<td>Starch</td>
<td>0</td>
</tr>
<tr>
<td>p-Nitrophenyl glucoside</td>
<td>0</td>
</tr>
<tr>
<td>p-Nitrophenyl cellobioside</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 3. Adsorption Assay of Endo-1,4-β-glucanases I and II to Various β-1,4-Glucans**

<table>
<thead>
<tr>
<th>β-1,4-Glucan</th>
<th>Activity (mU/ml)</th>
<th>Ratio (%)</th>
<th>Activity (mU/ml)</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>180</td>
<td>100</td>
<td>163</td>
<td>100</td>
</tr>
<tr>
<td>Acid-swollen cellulose</td>
<td>24.5</td>
<td>13.6</td>
<td>144</td>
<td>88.3</td>
</tr>
<tr>
<td>Avicel</td>
<td>20.3</td>
<td>11.3</td>
<td>149</td>
<td>91.4</td>
</tr>
<tr>
<td>CA1.77</td>
<td>133</td>
<td>73.9</td>
<td>146</td>
<td>89.6</td>
</tr>
</tbody>
</table>

**Substrate specificity, adsorption assay, and kinetic properties**

The substrate specificity of the enzyme is shown in Table 2. The purified enzyme had hydrolytic activity for CA0.88 and CM-cellulose, and had low activity for acid-swollen cellulose (amorphous cellulose), Avicel (microcrystalline cellulose), and xylan. No amylase, β-glucosidase, or cellobiosidase activity was detected. These results suggest that the enzyme was an endo-1,4-β-glucanase. The hydrolytic activities of EG I for amorphous cellulose and microcrystalline cellulose were higher than those of EG II. These results suggest that the presence of the carbohydrate-binding module in the EG I molecule facilitates hydrolysis of insoluble cellulose. These results are similar to those of another study in which proteases were used to elucidate the protein structure ofcellulases from *Fibrobacter (Bacteroides) succinogenes* S85 CelF (endoglucanase 2).26) CelF from *F. succinogenes* has a family 11 carbohydrate-binding module and a family 51 catalytic domain of glycoside hydrolase connected with a linker. Trypsin cleaves the enzyme at the linker region. A stable core peptide corresponding to the catalytic domain that retained activity for CM-cellulose was generated by the proteolytic cleavage. The purified catalytic peptide had hydrolytic activity toward CM-cellulose identical to that of the intact enzyme, but activity toward both amorphous and crystalline celluloses was reduced about half, implying a critical role for the carbohydrate-binding module in the hydrolysis of insoluble cellulose. The carbohydrate-binding module of CenA from *C. fimi* contributes to the binding of the catalytic domain to crystalline cellulose in the plant cell walls and to increasing the affinity.27) The carbohydrate-binding module of EG I from *N. sicca* SB may be needed for binding to cellulose and promoting hydrolysis.

The enzyme hydrolyzed cellotetraose to produce cellobiose. The enzyme also hydrolyzed cellobentaose and cellohexaose to yield cellobiose and cellotriose. When CA0.88 was the substrate, the release of reducing sugars increased, but cellooligosaccharides were not found by TLC at the start of the reaction. With the passage of time, cellooligosaccharides were detected as end products in the reaction mixture by TLC. The enzyme hydrolyzed CA0.88 randomly to produce cellooligosaccharides. The hydrolyzates by EG I gave the same pattern on TLC as those by EG II.

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The rates of the EG I reaction at 30°C with various concentrations of CA0.88 and CM-cellulose at optimum pH are compared with those with EG II in Table 4. The apparent $K_m$ of EG I was lower for CA0.88 than for CM-cellulose, and the $V_{max}$ was higher for CM-cellulose than CA0.88. The $V_{max}/K_m$ ratios were 4.36 for CA0.88 and 30.4 for CM-cellulose. The kinetic results indicated that EG I was more specific toward CM-cellulose than CA0.88, but that EG II was more specific toward CA0.88 than CM-cellulose. The $K_m$ of EG I for CM-cellulose was lower than that of EG II, but the values for CA0.88 were almost the same. These results indicate that the carbohydrate-binding module in the EG I molecule was important in binding CM-cellulose but not CA0.88, or that the removal of the carbohydrate-binding module from EG I was followed by a change of structure of catalytic domain leading to a decrease of affinity for CM-cellulose.

**Synergistic effects on degradation of CA by CA esterase and endo-1,4-β-glucanase**

The deacetylation of CA0.88 by EG I and CA esterase is shown in Fig. 4A. The same amounts of acetic acid were released by CA esterase alone as by the mixture of CA esterase and EG I. Deacetylation by CA esterase was not affected by the action of EG I. When CA1.77 was used as the substrate, a small amount of acetic acid was released by CA esterase alone (Fig. 4B). More acetic acid was released by the mixture of CA esterase and EG I. The deacetylation
of insoluble CA by CA esterase was increased by the action of EG I. When CA0.88 was the substrate, a larger amount of reducing sugars was released by the mixture of enzymes than by EG I alone (Fig. 4C). EG I had no activity toward CA1.77 (Fig. 4D). A much greater amount of reducing sugars was produced by EG I with CA esterase. The mixture of the enzymes catalyzed the extensive degradation of CA1.77. These results indicate that hydrolysis of β-1,4 linkages in the CA molecule by EG I was increased by the action of CA esterase, which deacetylates CA, creating a region that EG I can hydrolyze. Degradation of CA involved synergism between CA esterase and EG I, especially for insoluble CA.

The synergistic action between endo-1,4-β-glucanases I and II from \textit{Neisseria sicca} SB resembled heteroxylan degradation in its synergistic action. In particular, the action of CA esterase in degradation of CA was similar to that of acetylxylan esterase in the degradation of acetylxylan. We previously reported the enzymatic degradation of CA particles, and suggested the synergistic degradation of CA particles by CA esterase and EG II. The action of EG I in the degradation of CA was similar to that of EG II, and the efficiency of CA degradation by EG II with CA esterase was slightly greater than that with EG I and CA esterase. These results are compatible with the finding that the \( V_{\text{max}}/K_m \) ratio of EG II for CA0.88 was higher than that of EG I.

The effects of a serine hydrolase inhibitor on CA hydrolysis were tested with a crude preparation of CA-degrading enzyme from \textit{N. sicca} SB (not shown). The culture supernatant from \textit{N. sicca} SB was salted out with ammonium sulfate, dialyzed against 10 mM Tris-HCl buffer, pH 7.3, and used as the crude enzyme preparation. The enzyme preparation was treated with 2.0 mM phenylmethylsulfonyl fluoride, which is an inhibitor of CA esterase\(^{11}\) and does not inhibit EG I and EG II,\(^{12}\) before measurement of hydrolysis activity for CA1.77. The untreated enzyme preparation hydrolyzed CA1.77 with release of reducing sugars and acetic acid. With the treated enzyme, the release of reducing sugars and acetic acid released were decreased to about one-tenth of the base line. These results suggested that the synergism of CA esterase, EG I, and EG II is important for degradation of CA particles; it is unlikely that an enzyme hydrolyzes β-1,4 linkages in CA particles alone.

We suggest the mechanism of the degradation of CA by \textit{N. sicca} SB to be as follows. CA esterase and EG I are produced from \textit{N. sicca} SB during its growth in CA medium. At the early stage of the degradation of CA, deacetylation by CA esterase proceeds to produce CA with lower DS than before.\(^{13}\) During the deacetylation, a portion of EG I is cleaved by a protease(s) from \textit{N. sicca} SB at the linker region and EG II, the catalytic domain of EG I, is generated. EG II is faster than EG I in the cleavage of β-1,4 linkages in the CA molecules. DS and the degree of polymerization of CA molecules decrease because of the synergistic reactions of CA esterase and EG II. EG I hydrolyzes the deacetylated regions in the CA molecules produced by CA esterase and EG II. EG II also hydrolyzes such regions. As these and additional enzyme reactions occur, CA molecules are degraded into small molecules such as cellooligosacchalides, cellobiose, and glucose, which are incorporated into cells. CA esterase is important in facilitating the hydrolysis of the CA backbone by EG I and II, which are important in exposing the in-

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>( K_m ) (%)</th>
<th>( V_{\text{max}} ) (μmol min(^{-1}) mg(^{-1}))</th>
<th>( V_{\text{max}}/K_m )</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG I</td>
<td>CA0.88</td>
<td>0.296</td>
<td>1.29</td>
<td>4.36</td>
</tr>
<tr>
<td></td>
<td>CM-cellulose</td>
<td>0.448</td>
<td>13.6</td>
<td>30.4</td>
</tr>
<tr>
<td>EG II</td>
<td>CA0.88</td>
<td>0.242</td>
<td>2.24</td>
<td>9.26</td>
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<tr>
<td></td>
<td>CM-cellulose</td>
<td>2.28</td>
<td>12.8</td>
<td>5.61</td>
</tr>
</tbody>
</table>

Table 4. Kinetic Parameters of Endo-1,4-β-glucanases I and II from \textit{Neisseria sicca} SB

![Fig. 4. Changes with Time in CA Degradation by CA Esterase and Endo-1,4-β-glucanase I from \textit{N. sicca} SB. CA0.88 (A, C) and CA1.77 (B, D) were the substrates. A substrate was incubated with CA esterase (×), EG I (○), or a mixture of CA esterase and EG I (●) from \textit{N. sicca} SB. The reaction mixture was assayed for the acetic acid released (A, B) and reducing sugars (C, D) at the times indicated. The concentration of EG I was 0.2 U/ml, and the concentration of CA esterase was 0.2 U/ml for CA0.88 and 1.2 U/ml for CA1.77.](image-url)
side of CA particles and decreasing the molecular weight of CA. EG I, which has a carbohydrate-binding module, is superior to EG II in hydrolyzing insoluble cellulose. EG I is secreted as the enzyme that hydrolizes natural cellulose. EG II, derived from EG I, is important in the degradation of CA, an artificial organic ester. The enzyme specificity is changed by removal of the carbohydrate-binding module of EG I. The physiological role of the enzyme is not known.

References


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