Synergistic Effects of Cellobiose Dehydrogenase, Cellulases, and 
$\beta$-Glucosidase from *Irpex lacteus* in the Degradation of 
Various Types of Cellulose

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Synergistic effects in the degradation of bacterial cellulose (BC), Avicel and H$_2$PO$_4$-swollen 
Avicel (HA) by cellobiose dehydrogenase (CDH) and $\beta$-1,4-glucanases from *Irpex lacteus* including 
exo-cellulase (Ex-1), endo-cellulase (En-1) and $\beta$-glucosidase were investigated. CDH effecti
vely promoted both the degradation of celluloses by Ex-1 and synergism of Ex-1 and En-1 but 
moderately the degradation by En-1. Kinetic studies indicated that CDH enhances cellulose degra
dation by Ex-1 more effectively than $\beta$-glucosidase does by preventing product inhibition. The en
hancement in the cellulose-degrading capability of cellulases by synergistically acting with CDH 
was significant and independent of the crystallinity of substrate while the synergistic effect of Ex-1 
and En-1 by themselves was limited when substrates were at low crystallinity. Despite the large 
amount of cellobiose consumed by CDH in the presence of electron acceptors, the cellulolytic en
zyme system from *I. lacteus* effectively degraded different cellulose sources and continued produc
ing sufficient glucose for the cell.

Cellulose occurs in various states of purity in plant cell walls, ranging from 90% or more in the 
seed hairs of cotton to 40–60% in wood cell walls.$^{11}$ For the degradation of cellulose, fungi uti
lize the following hydrolytic enzymes, i.e., exo-cell
ulases (EC 3.2.1.91), endo-cellulases (EC 3.2.1.4) 
and $\beta$-glucosidase (EC 3.2.1.21). However, some 
cellulolytic fungi produce extracellular cellobiose-
oxidizing enzymes besides cellulases.$^{2–9}$ The wood-
rotting fungus *Irpex lacteus* is capable of degrad
ing both cellulose and lignin in wood. In addition 
to the hydrolytic enzymes acting on cellulose or 
cellobextrins, *I. lacteus* also produces two cellobiose-
oxidizing enzymes, which have different 
electron acceptor specificity.$^{10}$ One major compo
nent is cellobiose dehydrogenase (CDH, EC 
1.1.99.18) and the other, a minor one, was pre
dicted to be cellobiose: quinone oxidoreductase 
(CBQ). CDH is a hemoflavoprotein and catalyses 
the oxidation of cellobiose and higher cellobextrins 
to their corresponding lactones in the presence of 
electron acceptor such as cytochrome c, quinones 
and Fe (III) complex.$^{10}$ Oxygen also acts as an 
electron acceptor for CDH although it is a poor 
one.$^{11,12}$ Besides its catalytic function, this enzyme 
binds to cellulose as most cellulases do.$^{13–16}$ Our 
 studies suggested that CDH adsorbs a very small 
extent to the surface of the highly crystalline mi
crofibrils, and that the amorphous regions are the 
preferential sites for CDH adsorption.$^{10}$ Moreover, 
Igarashi et al.$^{17}$ have reported that the surface area 
for CDH adsorption increases during cellulase deg
radation by *Phanerochaete chrysosporium*. It is 
moreover reported that CDH could be an essential 
component of the delignification system in white-
rot fungi because of the fact that it can provide Fe 
(II), which together with H$_2$O$_2$, Fenton's reagent 
will be formed to produce hydroxyl radicals that 
can attack both lignin and cellulose.$^{15,18–21}$ Addi
tionally, Bao and Renganathan reported that CDH 
from *P. chrysosporium* enhanced the degradation

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of microcrystalline cellulose at low concentrations in combination with *Trichoderma reesei* cellulases. Recently, Igarashi et al. reported that CDH from *P. chrysosporium* enhanced cellulolytic activity of cellulases in the presence of complete CDH redox system due to the prevention of cellulbiose inhibition of reactions by oxidation of cellulbiose to cellobionolactone. In that study, however, *p*-nitrophenyl-β-D-celllobioside, which is an artificial substance and does not exist in nature, was used as the substrate. In fact, during the growing process of fungi, the cellulose hydrolysis is often done in a heterogeneous milieu, and consequent experiments should preferably employ native cellulose or at least a similar material as the substrate.

Therefore, in this study we investigate the degradation of various cellulosic substrates with different crystallinities by cellulases (exo- and endo-cellulases) from *I. lacteus* in the presence of CDH/ferricyanide redox system. The purification of new β-glucosidase from the fungus has facilitated investigations on the synergistic effects of the cellulolytic enzyme system in a manner closer to the natural system which are reported herein.

**MATERIALS AND METHODS**

**Materials.**

**Cellulose substrates.** Avicel, a microcrystalline cellulose powder (Art. 2331), was purchased from E. Merck Darmstadt (Germany). Cotton and cotton linter from Daiwa Shizai Co. (Tokyo) were extracted with chloroform for 9 h in a Soxhlet extractor. Phosphoric acid-swollen cotton, cotton linter and Avicel were prepared as described by TM. Wood. The relative crystallinity index (CrI) of each cellulose preparation was estimated by the method of Wakelin et al. The values obtained were as follows: cotton, 1; cotton linter, 0.910; Avicel, 0.568; H₃PO₄-swollen cotton (HC), 0.140; H₃PO₄-swollen Avicel (HA), 0.037; H₃PO₄-swollen cotton linter (HCL), 0. Bacterial cellulose (BC) was kindly provided by Dr. M. Takai (Hokkaido University, Japan).

**Enzyme source.** Driselase, a commercial enzyme preparation from *Irpex lacteus*, manufactured by Kyowa Hakko Co., was used as the starting material.

**Chemicals.** All chemicals used were of analytical grade.

**Assay of β-glucosidase activity.** β-Glucosidase activity was determined by measuring the amount of *p*-nitrophenol released from *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG) under hydrolyzation of the enzyme. The standard reaction mixture containing 0.1 mL of 5 mM *p*NPG, 0.2 mL of 50 mM sodium acetate buffer pH 5.0, and 0.1 mL enzyme sample was incubated at 30°C for 5 min. The enzyme reaction was stopped by adding 1 mL of 1% Na₂CO₃ and 2 mL distilled water. The amount of *p*-nitrophenol released was measured at 420 nm. One unit of β-glucosidase was defined as the amount of enzyme producing 1 μmol products per min.

**Purification of enzymes.**

**Purification of β-glucosidase.** β-glucosidase was purified as follows. Twenty grams of Driselase powder was dissolved in 150 mL of 20 mM ammonium acetate buffer pH 5.0 (buffer A) with slow magnetic stirring for 5 h at 4°C. The supernatant obtained by centrifugation at 15,000 × g for 15 min at 4°C was applied to the Bio-gel P-100 column (5 × 35 cm) pre-equilibrated with buffer A. Fractions containing β-glucosidase activity were pooled, concentrated, and passed continuously through a DEAE-Sephadex A-50 column (5 × 45 cm) and a CM-Sephadex C-50 column (3.2 × 18.5 cm) which pre-equilibrated with buffer A. β-Glucosidase, which unbound to these columns was collected and concentrated to 5 mL. The sample was then loaded onto a Sephadex G-150 column (2.5 × 113 cm) and eluted by buffer A. Active fractions were pooled, concentrated, and ammonium sulfate was added to the final concentration of 1 M. The sample was then inoculated onto a TSK gel Butyl Toyopearl 650M column (1.1 × 7.5 cm) pre-equilibrated with buffer A containing 1 M ammonium sulfate. A gradient from 1 M to 0 M of ammonium sulfate in buffer A was performed and active fractions were collected. β-Glucosidase was finally purified to homogeneity by the second Bio-gel P-100 column (2.2 × 100 cm). Purified β-glucosidase was estimated to be a 90 kDa protein by SDS-PAGE with a specific activity of 19.0 U/mg.
Synergistic Effects of Cellulolytic Enzymes and CDH

for pNPG. β-Glucosidase had optimal temperature at 50°C and was stable below 50°C for 24 h. It was also stable within a pH range of 3.0 to 7.0 at 30°C, showing the highest activity at a pH around 5.0. Km and Vmax for pNPG was 0.18 mM and 22.7 μmol·min⁻¹·mg⁻¹, respectively.

**Purification of CDH, Ex-1 and En-1.** CDH, Ex-1 and En-1 were purified by the same procedures as reported previously.¹⁰,²⁷,²⁸

**Kinetic studies.** The Michaelis-Menten constants (Km), the maximal velocities (Vmax) and the equilibrium constant for inhibitor binding (K) were determined by using Lineweaver-Burk and Dixon plots. The assay of CDH activity using potassium ferricyanide as the electron acceptor was determined by the decreasing of absorbance at 420 nm with ε = 1.04 mM⁻¹·cm⁻¹. β-Glucosidase activity toward cellobiose was measured by the glucostat method using the standard curve of glucose.²⁹

**Synergistic effect studies of CDH, β-glucosidase, and cellulases in the degradation of celluloses.** Each cellulose and 2.5 nmol of Ex-1, 2.5 nmol of En-1 (alone or in combination) was incubated with and without 0.5 nmol of CDH or/and β-glucosidase in 50 mM sodium acetate buffer (pH 5.0), containing 5 mM potassium ferricyanide in total volume of 2.4 mL at 30°C with mechanical shaking (100 rpm). At each point of time after incubation (1, 3, 6 and 9 h for CDH or 6 h for β-glucosidase), an aliquot was withdrawn from the reaction mixture and the residual cellulose was removed by centrifugation (4000 X g, 3 min). The amount of total sugar was determined by the anthrone-sulfuric acid method using cellobiose or glucose as standards.³⁰ The oxidized cellooligosaccharides were determined by the Ferricyanide method with some modifications.³¹ Briefly, the supernatant (0.4 mL) obtained after incubation was terminated by adding 0.4 mL of the SDS-phosphoric acid reagent (0.3 g SDS, 9.5 mL phosphoric acid and water to 100 mL). It was then carefully mixed with 0.1 mL of 2.5% ferric sulfate solution following by 2.5 mL of water and left at 30°C for 30 min to allow for full color development. The absorbance was measured at 660 nm. The amount of oxidized cellooligosaccharide was calculated using the standard curve.

Concentration of celluloses (cotton, cotton linter, Avicel, BC, HA, HC and HCL) used in these experiments was, 2.5, 2.5, 2.5, 1.25, 2, 2 and 2 mg/mL, respectively.

The ratios of cellooligosaccharide formed in reaction mixtures were analyzed by high-performance liquid chromatography (HPLC) on TSK gel G-Oligo-PW column (7.8 mm i.d. by 30 cm; Tosoh Corp., Tokyo) using Jasco 805-GI system (Japan Spectroscopic Co., Tokyo) combined with a Waters 410 differential refractometer (Millipore Corp., Milford, Mass.). The degradation percentages of cellulose were calculated from amount of soluble sugars formed and the total sugar of substrate.

**Other methods.** Cellobiose dehydrogenase (CDH) activity was assayed by monitoring the increase of absorbance at 550 nm of cytochrome c as reported previously.¹⁰ SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as described by Laemmli.³²

**RESULTS**

**Cellulose degradation by cellulases in the presence of CDH/Ferricyanide redox system.**

Due to the partial oxidation of hydrolytic cellulase products in the presence of CDH/ferricyanide redox system in reaction mixture, the use of conventional method (Somogyi and Nelson method) for measurement the reducing sugars was not suitable. In these investigations, a useful method for measuring the amount of reducing sugars was developed as described in MATERIALS AND METHODS. Three different kinds of celluloses (BC, Avicel, and HA) were used as substrates.

Concerning the degradation of BC and HA by Ex-1 and En-1 shown in Fig. 1, the degradation percentages quickly reached a plateau after 3 h or 6 h for Ex-1 or En-1/alone, respectively. In the case of Avicel substrate, the degradation percentages continued to increase slightly after 9 h but generally the pattern was the same as for BC and HA. When Ex-1 and En-1 synergistically hydrolyzed celluloses the degradation percentage of BC and Avicel continued to increase slightly after 9 h
Fig. 1. Cellulose degradation by cellulases from *I. lacteus* in the presence of CDH/ferricyanide redox system.

The experiments were carried out as described in MATERIALS AND METHODS. (A) Bacterial cellulose, (B) Avicel, (C) H₃PO₄-swollen Avicel. □, Ex-1 alone; ■, Ex-1 + CDH; △, En-1 alone; ◇, En-1 + CDH; ○, Ex-1 + En-1; ●, Ex-1 + En-1 + CDH. The values are means of two different experiments performed in duplicate.

Table 1. Effect of the crystallinity of cellulose substrate on the synergism of cellulolytic enzymes and CDH from *I. lacteus*.

<table>
<thead>
<tr>
<th>Enzyme combination</th>
<th>HCL</th>
<th>HA</th>
<th>HC</th>
<th>A</th>
<th>CL</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulases and CDH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex-1 and CDH</td>
<td>1.70</td>
<td>1.65</td>
<td>1.55</td>
<td>1.15</td>
<td>1.10</td>
<td>1.07</td>
</tr>
<tr>
<td>En-1 and CDH</td>
<td>1.21</td>
<td>1.20</td>
<td>1.11</td>
<td>1.05</td>
<td>1.04</td>
<td>1.03</td>
</tr>
<tr>
<td>Ex-1, En-1 and CDH</td>
<td>1.41</td>
<td>1.40</td>
<td>1.33</td>
<td>1.31</td>
<td>1.31</td>
<td>1.30</td>
</tr>
<tr>
<td>Exo- and endo-cellulases</td>
<td>Exo-1 and En-1</td>
<td>1.06</td>
<td>1.09</td>
<td>1.16</td>
<td>1.43</td>
<td>1.49</td>
</tr>
</tbody>
</table>

*Degree of synergism is defined as the ratio of the soluble sugars produced by the combined action of cellulase components or cellulase with CDH to the sum of the amount of sugars produced by their independent actions. *Crl*, relative crystallinity index of cellulose. Cotton (C), cotton linter (CL), Avicel (A), H₃PO₄-swollen cotton (HC), H₃PO₄-swollen Avicel (HA), H₃PO₄-swollen cotton linter (HCL). The values are means of two independent experiments performed in duplicate.

of reaction whereas it reached a plateau in the case of HA. The degradation of each cellulose was enhanced in the presence of the CDH/ferricyanide redox system compared with that in the presence of Ex-1 alone, while no or very little enhancing effect was observed in the case of En-1 (Fig. 1). In addition, cellulose was degraded more rapidly by the combination of Ex-1 and En-1 than by either cellulase alone in the presence of CDH (Fig. 1). The degradation percentages of BC and HA in 9 h were 25 and 45% which were about 7-fold and 13-fold higher than that for microcrystalline Avicel (3.5%), respectively. In the presence of CDH/ferricyanide redox system, these values were increased to 38, 72 and 5% for BC, HA and Avicel, respectively. These results indicated that product inhibition might occur with Ex-1 and there might be a relation between degradation percentage and crystallinity of celluloses. The following investigations were to demonstrate the possible role of CDH in cellulose-degradation.

**Effect of the crystallinity of cellulose on the synergism between cellulases and CDH.**

The synergistic action of cellulases and CDH was investigated using six celluloses with *Crl*
Synergistic Effects of Cellulolytic Enzymes and CDH

Fig. 2. Inhibition effect of cellobiose on p-nitrophenyl-β-D-lactopyranoside (pNPL) hydrolysis by Ex-1 from \textit{L. lacteus}.

The reaction mixtures containing pNPL (0.75–1.5 mM), Ex-1 (1 μM), and cellobiose (0–0.2 mM) in 50 mM sodium acetate buffer (pH 5.0) were incubated at 30°C for planned times. The enzyme activity was measured as described in MATERIALS AND METHODS. (A) Lineweaver-Burk plot. (B) Dixon plot. The values are means of two different experiments performed in duplicate.

ranging from 0 to 1 (Table 1). The synergistic action between Ex-1 and En-1 increased proportionally with the increase of \( Cr_1 \) of cellulose, while it decreased when CDH coreacted with Ex-1 or En-1. However, the synergistic effect of Ex-1, En-1, and CDH was likely independent on \( Cr_1 \) of cellulose (Table 1).

**Kinetic studies.**

Product inhibition phenomenon by cellobiose, the major product of cellulose hydrolysis has been reported with several exo-cellulases. In the present study, \( p \)-nitrophenyl-β-D-lactopyranoside (\( p \)NPL), an artificial soluble substrate for exo-cellulase, was employed for kinetic studies to unveil the inhibitory mechanism. The inhibitory effect of cellobiose on \( p \)NPL hydrolysis by Ex-1 was dependent upon cellobiose concentration and Lineweaver-Burk plots shown in Fig. 2A indicated that the inhibition is typically non-competitive. The equilibrium constant for inhibitor binding (\( k_i \)), obtained from Dixon plots, was 0.42 mM as shown in Fig. 2B.

To clarify the extent of the role of CDH in preventing product inhibition in the presence of both CDH and β-glucosidase, kinetic studies on cellobiose oxidation by CDH and cellobiose degradation by β-glucosidase were carried out and the results obtained are shown in Table 2. The \( K_m \) values for β-glucosidase and CDH were 23.7 and 0.09 mM, respectively. The \( k_{cat}/K_m \) of CDH was 105 mM⁻¹·s⁻¹ which is 14 times higher than that of β-glucosidase (7.4 mM⁻¹·s⁻¹).

**Table 2. Determination of kinetic parameters for cellobiose oxidation by CDH and cellobiose hydrolysis by β-glucosidase.**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>( K_m ) (mM)</th>
<th>( k_{cat} ) (s⁻¹)</th>
<th>( k_{cat}/K_m ) (mM⁻¹·s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDH</td>
<td>0.09</td>
<td>9.5</td>
<td>10.5</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>23.7</td>
<td>175</td>
<td>7.4</td>
</tr>
</tbody>
</table>

**The effects of CDH and β-glucosidase on cellulose degradation by cellulases.**

Bacterial cellulose (a highly crystalline cellulose with a large surface area) and \( H_2PO_4 \)-swollen Avicel (an amorphous cellulose substrate that could be used to investigate product inhibition phenomenon during hydrolyzing by Ex-1 and En-1 as shown in Fig. 1) were used for comparing the role of CDH and β-glucosidase for cellulose degradation by cellulases. Concerning the BC degradation, the degradation percentage by synergism of Ex-1 and En-1 was 20.6% and cellobiose was the main product (92.1%) while glucose only constituted 7.9% (Table 3). The degradation percentage increased to 25.7% when β-glucosidase was added to the reaction mixture but 12.7% of cellobiose still remained in the reaction mixture. In the presence of CDH, all cellobiose was oxidized to cellobionolactone and the degradation percentage of BC rose up to 32.2% (Table 3). When CDH, Ex-1, En-
Table 3. The effects of CDH and β-glucosidase (β-glu) on cellulose degradation by cellulases from I. lacteus after 6 h incubation.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Degradation (%)</th>
<th>Relative degradation (%)</th>
<th>Hydrolysis and oxidation products (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>G1</td>
</tr>
<tr>
<td>Bacterial cellulose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulases only</td>
<td>20.6</td>
<td>100</td>
<td>7.9</td>
</tr>
<tr>
<td>Cellulases + β-glu</td>
<td>25.7</td>
<td>125</td>
<td>87.3</td>
</tr>
<tr>
<td>Cellulases + CDH</td>
<td>32.2</td>
<td>156</td>
<td>7.3</td>
</tr>
<tr>
<td>Cellulases + β-glu + CDH</td>
<td>33.9</td>
<td>165</td>
<td>14.3</td>
</tr>
<tr>
<td>H2PO4-swollen cellulose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulases only</td>
<td>39.5</td>
<td>100</td>
<td>8.9</td>
</tr>
<tr>
<td>Cellulases + β-glu</td>
<td>47.6</td>
<td>121</td>
<td>88.8</td>
</tr>
<tr>
<td>Cellulases + CDH</td>
<td>56.8</td>
<td>144</td>
<td>9.1</td>
</tr>
<tr>
<td>Cellulases + β-glu + CDH</td>
<td>59.1</td>
<td>150</td>
<td>22.4</td>
</tr>
</tbody>
</table>

*a The degradation percentages of cellulose were calculated from the ratio between the soluble sugars produced and the amount of sugar equivalents in the substrate. b The hydrolysis and oxidation products are defined as the percentage of each generated product in the total degradation percentages of cellulose; G1, glucose; G2, cellobiose; G3, cellotriose; GnL, cellooligo-lactone. —, not detected. c The combination of exo-cellulase (Ex-1) and endo-cellulase (En-1) was used. The values are means of two independent experiments performed in duplicate.

1 and β-glucosidase synergistically degraded BC, the degradation percentage increased to 33.9%, slightly higher than for the synergism of CDH, Ex-1 and En-1. The main product was cellobionolactone (85.7%) while the rest was glucose (Table 3).

When HA was substrate, there was a change in product of hydrolysis by cellulases. Cellotriose (13.4%) was formed in addition to cellobiose (77.7%) and glucose (8.9%). The degradation percentages also went up to 39.5%, which is nearly twice as high as that of BC. In the presence of β-glucosidase or CDH or the combination of the two in the reaction mixture, the degradation percentages increased to 47.6, 56.8 and 59.1%, respectively (Table 3). However, 4.7% of cellobiose still remained in the reaction mixture even in the presence of CDH, which was different from BC. The amount of cellobiose was even higher (8.9%) in the co-existence of CDH and β-glucosidase compared with β-glucosidase alone (Table 3). Additionally, the generation of glucose was significantly increased in the presence of CDH/ferricyanide redox system (constituting 7.3% in the total 32.2% of BC degradation and 9.1% in the total 56.8% of HA hydrolysis compared to 7.9% in 20.6% of BC degradation and 8.9% in 39.5% of HA hydrolysis using only cellulase, respectively).

**DISCUSSION**

Similarly, our investigations on secretion of cellulolytic enzyme system indicated that I. lacteus produced synergistical enzymes including cellulases and CDH when it was cultivated in cellulolytic medium (data not shown). In addition a CDH-deficient mutant of *Trametes versicolor* is viable and retains its capabilities in delignification. These results suggested that the biological role of CDH in the degradation of cellulose should be considered. In the present study we focused on the synergistic effects of the cellulolytic enzyme system including CDH, β-glucosidase, exo-cellulase, and endo-cellulase from *I. lacteus*. Because ferricyanide does not inhibit activity of cellulases (data not shown) and could serve as electron acceptor for CDH, the CDH/ferricyanide redox system was used in this study, based on a hypothesis that the CDH/electron acceptors naturally exist in the medium.

Our first investigations on cellulose degradation of cellulases and the synergism of cellulases with CDH (Fig. 1) indicated that cellulolytic enzyme system from *I. lacteus* shares properties with enzymes from other fungi.
cellulose degradation capability of exo-cellulase (Ex-1) more effectively than endo-cellulase (En-1). Since only cellulases and CDH were available at the time, reports of the biological role of CDH in the enhancement of the cellulose degradation capability of exo-cellulase by Ander et al.\textsuperscript{35} and Igarashi et al.\textsuperscript{17} did not convince researchers very much. The question was why the cell would use CDH when $\beta$-glucosidase could carry out the function more effectively for the cell than CDH does.\textsuperscript{34,36} This encouraged us to study the synergistic effects of the cellulolytic enzyme system from \textit{I. lacteus} to provide proof of the role of CDH. As expected, kinetic studies indicated that Ex-1 was strongly inhibited by cellobiose with $K_i$ of 0.42 mM (Fig. 2). This result can explain why the degradation percentage of Ex-1 rapidly reached a plateau while in the presence of CDH the effect was reduced (Fig. 1). The obtained kinetic parameter values of CDH and $\beta$-glucosidase (Table 2) provide proof for the role of CDH. The larger $k_{cat}/K_m$ value obtained from CDH indicated CDH prevents cellobiose inhibition much more effectively than $\beta$-glucosidase could do. As shown in Table 3, in the presence of $\beta$-glucosidase the relative degradation percentage of cellulose averaged an increase of 23% and up to 50% for CDH, while in the presence of both CDH and $\beta$-glucosidase the increase was 57% in comparison with synergism of Ex-1 and En-1. Among electron acceptors checked,\textsuperscript{40} ferricyanide was not the best one; therefore the effect of CDH on cellulose degradation might be stronger if better electron acceptors were coupled.

Additionally, in the presence of CDH or both CDH and $\beta$-glucosidase the glucose concentration remained at a significant level for the cell. This data proved that the consumption of cellobiose by CDH does not limit the sugar nutrient in the medium for the cell. Moreover, the data shown in Table 2 suggested that CDH might support the adaptation of cellulases in the degradation of different cellulose sources. In general, with the results obtained and the reduction of the natural electron acceptors was required or cellobionolactone enters into a useful secondary metabolism as well as becoming a signal for the cell, we therefore conclude that the biological role of CDH in oxidation of cellobiose is explainable.

Nevertheless, the whole picture of the biological role of CDH should be laid out when additional studies are done on the natural electron acceptors, the protein-protein interaction in the cellulolytic enzyme system in the absence of electron acceptors, and the role of the cellulose-binding domain, as well as the metabolism in which cellobionolactone is involved.

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Synergistic Effects of Cellulolytic Enzymes and CDH


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Irpex lacteus 由来のエキセルラーゼ（Ex-1），エンドセルラーゼ（En-1），β-グルコンフラーゼおよびセルラーゼは主生成物であるセロビオースを生成する。これらの役割を果たすが，これらの生成物の一部を有するCDH はβ-フララーゼよりもEx-1 のセルラーゼ分解を促進することが示され，CDH を加えて他の効果を確認されなかった。電子受容体が存在するとき，CDH により多くのセロビオースが消化されるが，本菌のCDH はセルラーゼの生成物障害の解消だけでなく，種々のセルラーゼの構造に寄与するものと考えられる。