J. Biochem. 120, 1123-1129 (1996)

Fine Substrate Specificities of Four Exo-Type Cellulases Produced by *Aspergillus niger*, *Trichoderma reesei*, and *Irpex lacteus* on (1→3),(1→4)-β-D-Glucans and Xyloglucan[1]

Yoshihiko Amano,*[,2] Masahiro Shiroishi,* Kazutosi Nisizawa,† Eiichi Hoshino,‡ and Takahisa Kanda*

**Department of Chemistry and Material Engineering, Faculty of Engineering, Shinshu University, Wakasato 500, Nagano 380; †College of Bioresource Sciences, Nihon University, Setagaya-ku, Tokyo 154; and ‡Tochigi Research Laboratories, Kao Corporation, 2606 Akabane, Ichikai, Haga, Tochigi 321-34*

Received for publication, July 5, 1996

To investigate the fine substrate specificities of four highly purified exo-type cellulases (Exo-A from *Aspergillus niger*, CBHI and CBHII from *Trichoderma reesei*, and Ex-1 from *Irpex lacteus*), water-soluble substrates such as barley glucan, xyloglucan from tamarind (*Tamarindus indica* L.), and their oligosaccharides were employed. Four exo-type cellulases immediately hydrolyzed 3-*O*-β-D-cellotriosylglucose to produce cellobiose and laminaribiose. In contrast, CBHII showed no hydrolytic activity towards 3²-*O*-β-D-cellobiosylcellobiose, which was hydrolyzed to cellobiose by the other exo-type cellulases. These cellulases hydrolyzed the internal linkages of barley glucan and lichenan in an endo-type fashion to produce cellobiose and mix-linked oligosaccharides as main products. The $\overline{DP}$-lowering activities of the four exo-type cellulases on barley glucan were in the order of Ex-1, CBHII, Exo-A, and CBHI. Based on gel permeation chromatography analysis of the hydrolysates, Ex-1 seemed to attack the internal cellobiosyl unit adjacent to β-1,3-glucosidic linkages in barley glucan molecule more frequently than did the other cellulases. Xyloglucan was hydrolyzed only by CBHI and CBHII, and produced hepta-, octa-, and nona-saccharides. In addition, a xyloglucan tetradecasaccharide (XG14) was split only to heptasaccharide (XG7) by CBHI and CBHII.

Key words: barley glucan, exo-cellulase, fungi, substrate specificity, xyloglucan.

Native cellulose is highly resistant to enzymatic hydrolysis as compared with its soluble modified derivatives, because of its insoluble crystalline ultrastructure with strong hydrogen bonds. Exo-type cellulases (1,4-β-D-glucan cellobiohydrolase) [EC 3.2.1.91] have been reported to degrade crystalline celluloses more effectively than endo-type cellulases (1,4-β-D-glucan 4-glucanohydrolase) [EC 3.2.1.4], and mainly produced cellobiose (*1, 2*). Thus, the exo-type cellulases seem to play a major role in enzymatic cellulolysis by fungi (*1, 2*). Recently, the cellulolytic exo-type enzymes have been mainly classified in two groups (cellulase families B and C) based on the amino acid sequence of the cellulase components (*3*), and the anomeric configuration of the hydrolysis products (*4*).

In the previous paper (*5*), we reported that four kinds of exo-type cellulases from different fungi may be grouped into two types in terms of their mode of action on chromogenic substrates and cellooligosaccharide degradations. The mode of their action on low-molecular-weight soluble substrates, however, may or may not coincide with that on high-molecular-weight insoluble celluloses.

Although many workers have investigated the hydrolysis of some mix-linked β-glucans such as barley glucan, lichenan, and oat glucan by endo-type cellulases (*6-11*), only a few investigations have been made on the hydrolysis of mix-linked β-glucans by exo-type cellulases (*12, 13*). Henriksson *et al.* reported that CBHI purified from *Trichoderma reesei* degraded barley glucan in a typical endo fashion (*14*). However exo-type cellulases have been believed so far to split off cellobiosyl residues from the non-reducing end of cellulosic substrates. An exo-type cellulase (Ex-1) from *Irpex lacteus* showed exowise splitting of internal glycosidic bonds of the β-glucan from barley and lichenan, but this cellulase strictly requires the cellobiosyl residue to be adjacent to a (1→3)-β-linked glycosyl residue (*15*).

On the other hand, xyloglucan and (1→3),(1→4)-β-D-glucans of plant cell wall origin have been reported to be hydrolyzed by endo-type cellulases of plant (*16*). Since xyloglucan in the primary wall contains a (1→4)-β-glucan backbone with xylosyl side chains along the backbone, it seems to be a better substrate to examine the substrate specificities of exo-type cellulases. In addition, there has been no report about xyloglucan degradation by exo-type

[1] This work was supported in part by a Grant-in-Aid (No. 07306005) for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

[2] To whom correspondence should be addressed. E-mail: yoamano @gipwc.shinshu-u.ac.jp

Abbreviations: G_1, glucose; G_2, cellobiose; G3G, laminaribiose; G3G4G, 3²-*O*-β-D-glucosylcellobiose; G4G3G, 3-*O*-β-D-cellobiosylglucose; G4G4G3G, 3-*O*-β-D-cellotriosylglucose; G4G3G4G, 3²-*O*-β-D-cellobiosylcellobiose; XG7, xyloglucan heptasaccharide (glucose/xylose, 4/3); XG14, xyloglucan tetradecasaccharide (glucose/xylose, 8/6); $\overline{DP}$, degree of polymerization.

cellulases, although its degradation by endo-type cellulases has been reported (*17, 18*).

In the present study, therefore, we attempted to elucidate the inherent fine structural differences of exo-type cellulases from *I. lacteus*, *T. reesei*, and *A. niger* in terms of the modes of action on soluble substrates such as mix-linked β-D-glucans, xyloglucan, and their oligosaccharides.

MATERIALS AND METHODS

Enzymes—Driselase, a commercial enzyme product from *I. lacteus* manufactured by Kyowa Hakko Kogyo, and Sumizyme C and Sumizyme AC from *T. reesei* and *A. niger*, respectively, manufactured by Shin Nihon Chemical Co., were used as starting materials. Ex-1 was purified from Driselase according to the methods of Kanda *et al.* (*19*). CBHI and CBHII were purified from Sumizyme C by the modified method reported previously (*20*). CBHI was fractionated by column chromatography using Bio Gel P-100 (Bio-Rad), DEAE-Sephadex A-50 (Pharmacia), and Butyl-Toyopearl 650M (Tosoh). CBHII was obtained by column chromatography using Bio Gel P-100, DEAE-Sephadex A-50, DEAE-Sepharose CL-6B (Pharmacia), Butyl-Toyopearl 650M, and Toyopearl HW-50S (Tosoh). Exo-A was obtained from Sumizyme AC by column chromatography using DEAE-Toyopearl 650M, Butyl-Toyopearl 650M, and Bio Gel P-100. The purified exo-type cellulases each showed a single band on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and the properties of these enzymes are described in another paper (*5*).

Substrates—Barley glucan was obtained from Novo Nordisk Bioindustry. Crude lichenan from Icelandic moss (*Cetraria icelandica*) was purchased from Sigma Chemical and purified according to the procedures described by Perlin and Suzuki (*21*). Avicel, a microcrystalline cellulose powder (Art. 2331) was purchased from E. Merck (Darmstadt, Germany). Crude oligosaccharides such as 3^2-*O*-β-D-glucosylcellobiose (G3G4G), 3-*O*-β-D-cellobiosylglucose (G4G3G), 3-*O*-β-D-cellotriosylglucose (G4G4G3G), and 3^2-*O*-β-D-cellobiosylcellobiose (G4G3G4G) were obtained from lichenan by treating it with endo-β-1,3-glucanase or endo-β-1,4-glucanase. They were purified by preparative thin-layer chromatography following the methods of Kanda *et al.* (*15*). Xyloglucan from the seeds of tamarind (*Tamarindus indica* L.) was provided by Dainippon Pharmaceutical. Xyloglucan tetradecasaccharide (glucose/xylose; 8 : 6, XG14) and heptasaccharide (glucose/xylose; 4 : 3, XG7), obtained from the hydrolysis of xyloglucan, were donated by Dr. Mitsuishi (National Institute of Bioscience and Human Technology).

Hydrolysis of (1→3),(1→4)-β-Linked Oligosaccharides—The reaction mixture consisted of 45 μl of 0.5 wt% mix-linked oligosaccharides, 45 μl of 16.9 μM exo-type enzyme, and 90 μl of 0.05 M buffer at the individual optimum pH. After incubation at 30°C for an appropriate period, 40 μl aliquots of the reaction mixtures were spotted on analytical TLC plates (Silicagel 60, 0.25 mm, Merck) and the reaction products were separated with the solvent system of chloroform-methanol-water (90 : 65 : 15).

Measurement of Saccharification Activities on (1→3),(1→4)-β-D-Glucan—The reaction mixture consisted of 0.1 ml of 0.5 wt% substrate, 0.1 ml of 1.69 μM exo-type enzyme, and 0.2 ml of 0.05 M buffer at the individual optimum pH. After incubation at 30°C for an appropriate period, reducing power produced was measured by the methods of Somogyi and Nelson (*22, 23*).

Hydrolysis Products from Water-Soluble β-D-Glucan—The enzyme reaction was performed under the same conditions as enzymatic hydrolysis of (1→3),(1→4)-β-D-glucan except for the enzyme concentration, which was 16.9 μM. Barley glucan, lichenan, xyloglucan at 0.5 wt% each, and xyloglucan tetradecasaccharides at 0.2 wt% were used as substrates. After incubation at 30°C for an appropriate period, 40 μl aliquots of the reaction mixture were spotted on analytical TLC plates using the solvent system of chloroform-methanol-water (90 : 65 : 15) for barley glucan and lichenan, or that of 1-butanol-ethanol-water (5 : 5 : 4) for xyloglucan.

High-Performance Liquid Chromatography—A multi-solvent delivery system (Waters M600) was coupled to a TSKgel G2500 PW column (21.5 mm I.D. × 30 cm, Tosoh) whose temperature was controlled at 50°C. Refractive index was monitored by a differential refractometer (Waters M410). The solvent system was water, pumped at 3.0 ml/min. Pullulan (molecular weight, 5,800 and 12,200 Da; Showa Denko), 1,3;1,4-mix-linked oligosaccharides (trimer and tetramer), cellobiose (G_2), and glucose (G_1) were used as authentic standards in the gel permeation chromatography.

RESULTS

Degradation of Mix-Linked Oligosaccharides—To investigate the modes of action of the four exo-type cellulases towards (1→3),(1→4)-β-D-glucan in detail, four kinds of mix-linked oligosaccharides, G3G4G, G4G3G, G4G3G4G, and G4G4G3G, were first tested for hydrolyzability. Degradation products were identified by TLC after various incubation periods. The results are summarized in Table I. The mix-linked trisaccharide, G3G4G was not hydrolyzed by any of the four exo-type cellulases, whereas G4G3G was slightly hydrolyzed by Ex-1 and Exo-A in the later stage, but at different linkages. Ex-1 split the β-1,4-linkage to produce G_1 and laminaribiose (G3G) from G4G3G while Exo-A cleaved the β-1,3-linkage to produce G_1 and G_2. All four exo-type cellulases split the middle β-1,4-linkage of G4G4G3G at a high rate to produce G_2 and G3G. G4G3G4G was also hydrolyzed by Ex-1, Exo-A, and CBHI among the four to produce only G_2. CBHII was unable to hydrolyze G4G3G4G in the same way as G3G4G. Thus it was found in this experiment that the rates of hydrolysis of these mix-linked oligosaccharides, other than G4G4G3G, by the exo-

TABLE I. **Substrate specificities of four exo-type cellulases on mix-linked glucooligosaccharides.**

Substrate	Ex-1	CBH I	CBH II	Exo-A
3^2-O-β-D-Glucosylcellobiose	G3G4G	G3G4G	G3G4G	G3G4G
3-O-β-D-Cellobiosylglucose	↓ G4G3G	G4G3G	G4G3G	↓ G4G3G
3^2-O-β-D-Cellobiosylcellobiose	↓ G4G3G4G	↓ G4G3G4G	G4G3G4G	↓ G4G3G4G
3-O-β-D-Cellotriosylglucose	↓ G4G4G3G	↓ G4G4G3G	↓ G4G4G3G	↓ G4G4G3G

Hydrolyzability . ↓ , slow , ↓ , medium , ↓ , rapid.

type cellulases were slow compared with those of cellooligosaccharides found previously (*5*).

Degradation of (1→3),(1→4)-β-D-Glucans—Time courses of reducing sugar productivity by the four exo-type cellulases on barley glucan and lichenan, which consist of (1→3)- and (1→4)-linked β-D-glucosyl residues, are shown in Fig. 1. The four enzymes attacked both substrates with different productivities of reducing sugar. CBHI and Exo-A produced small amounts of reducing sugars during the hydrolysis of lichenan as compared with Ex-1 (Fig. 1A). In the hydrolysis of barley glucan, only small differences in reducing sugar productivity were found among the four

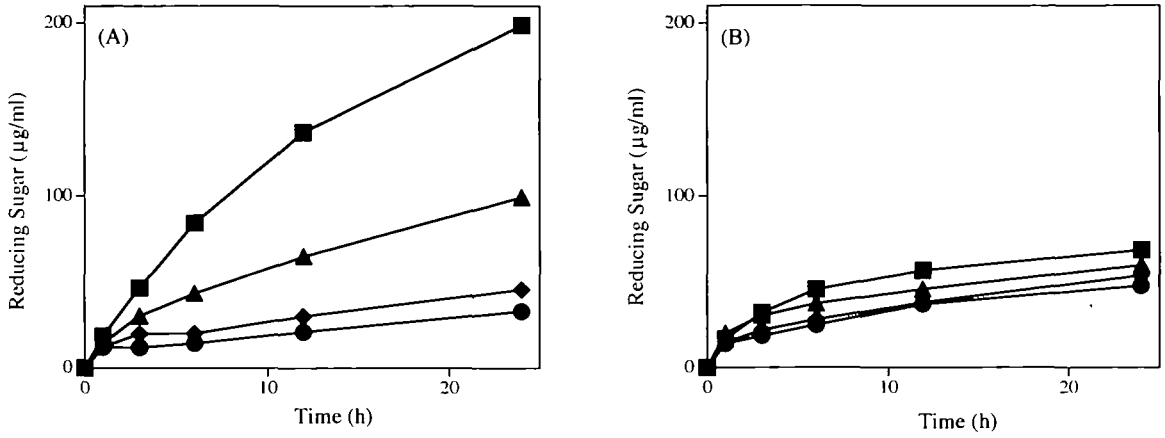

Fig. 1. **Time course of the hydrolysis of lichenan (A) and barley glucan (B) by exo-type cellulases.** ■, Ex-1; ◆, Exo-A; ●, CBHI; ▲, CBHII.

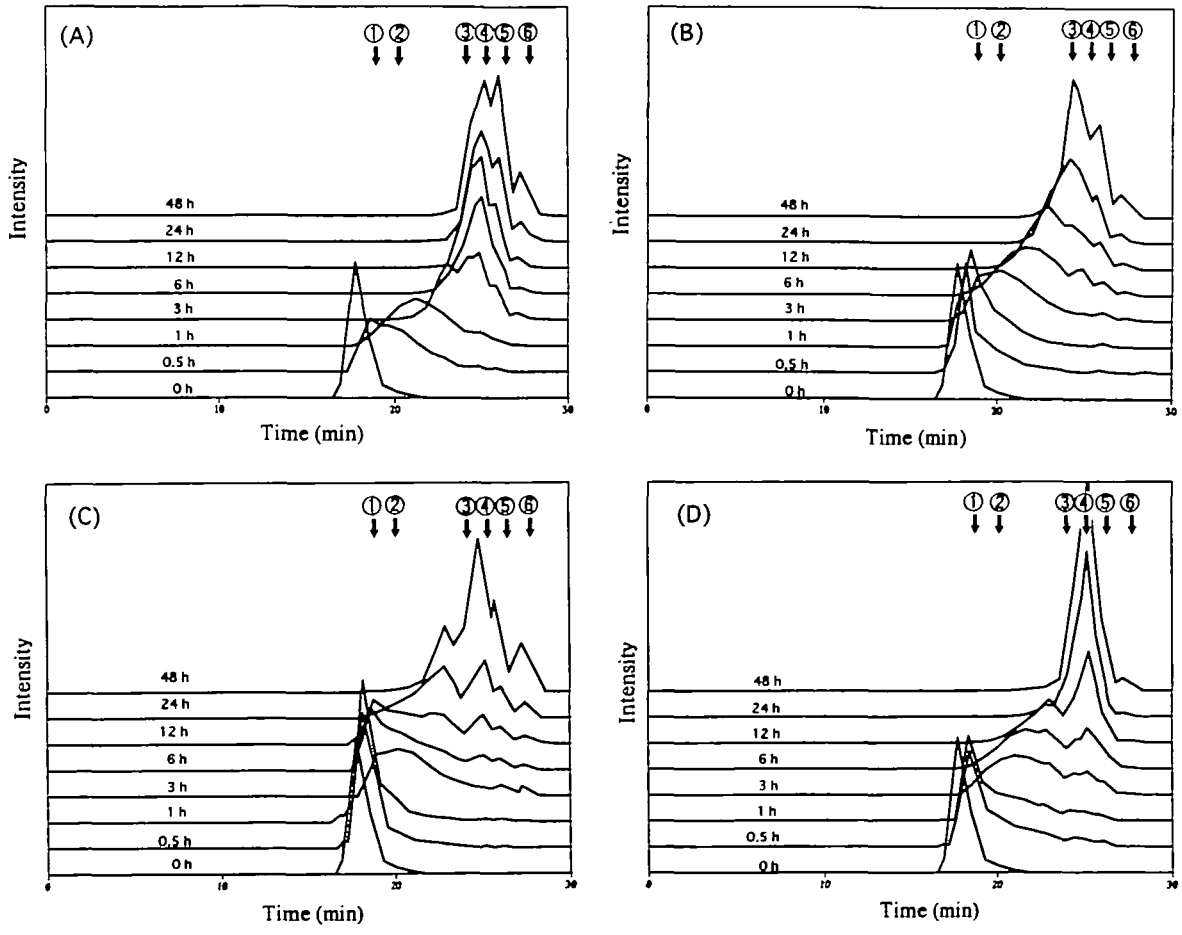

Fig. 2. **HPLC profiles of hydrolysis products from barley glucan by Ex-1 (A), Exo-A (B), CBHI (C), and CBHII (D).** 1, pullulan standard (MW 12,200); 2, pullulan standard (MW 5,800); 3, tetramer; 4, trimer; 5, dimer (cellobiose); 6, glucose.

exo-type cellulases (Fig. 1B).

In contrast, Ex-1 showed a higher activity for lowering the degree of polymerization (DP) of barley glucan than the other enzymes. The peak of native barley glucan disappeared from the elution profile of the degradation products after incubation with Ex-1 for 0.5 h, and peaks of smaller molecular weight components were detected (Fig. 2). Compared with the DP-lowering activity of Ex-1 towards barley glucan, those of the other enzymes were relatively low, and the elution profiles of the reaction mixture during hydrolysis showed no rapid decrease in the molecular weight of the substrate.

The hydrolysis products formed from barley glucan and lichenan by exo-type cellulases were examined by TLC after various incubation periods (Fig. 3). The hydrolysis products generated by CBHII were detected by TLC analysis after 3 h and those by Ex-1, CBHI, and Exo-A were detected after 6 or 12 h. Cellobiose and mix-linked oligosaccharides were detected as main hydrolysis products formed by each cellulase. However G_1 was not detected among the hydrolysis products formed by CBHII from barley glucan even after prolonged incubation, whereas small amounts of G_1 were produced by the other enzymes. From the R_f values of the products on TLC, there are at least three different oligomers among mix-linked oligosaccharides, and they were named M-1, M-2, and M-3 (Fig. 3). CBHII and Exo-A produced M-1 and M-3 from barley glucan as main hydrolysis products while Ex-1 produced M-2 and M-3, and CBHI produced only M-2 as mix-linked oligosaccharides. From the R_f values of authentic sugars (G3G4G, G4G3G, G4G3G4G, and G4G4G3G), M-1 and M-2 may be identified as trisaccharides, and M-3 may be a tetrasaccharide. Based on previous work (*15*), the trisaccharides M-1 and M-2 formed by Ex-1 may be identified as

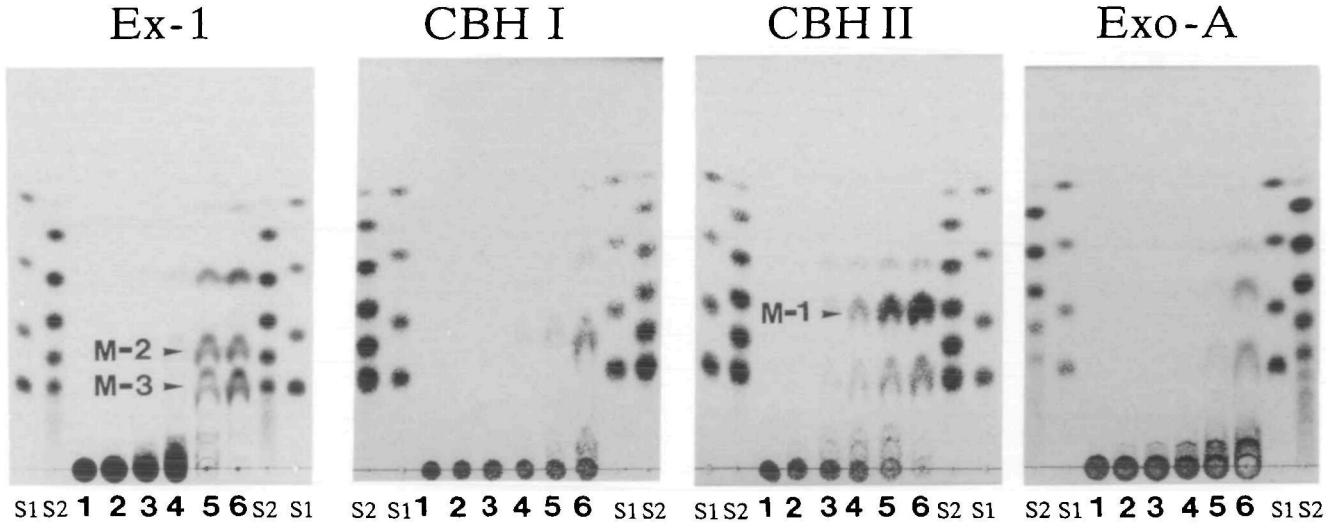

Fig. 3. **Thin layer chromatographic analysis of hydrolysis products from barley glucan by exo-type cellulases.** Incubation times are 0, 1, 3, 6, 12, and 24 h for 1-6, respectively. S1: standard sugars (glucose to cellotetraose). S2: standard sugars (laminaribiose to laminarihexaose). M-1-M-3: mix-linked oligosaccharides produced from barley glucan.

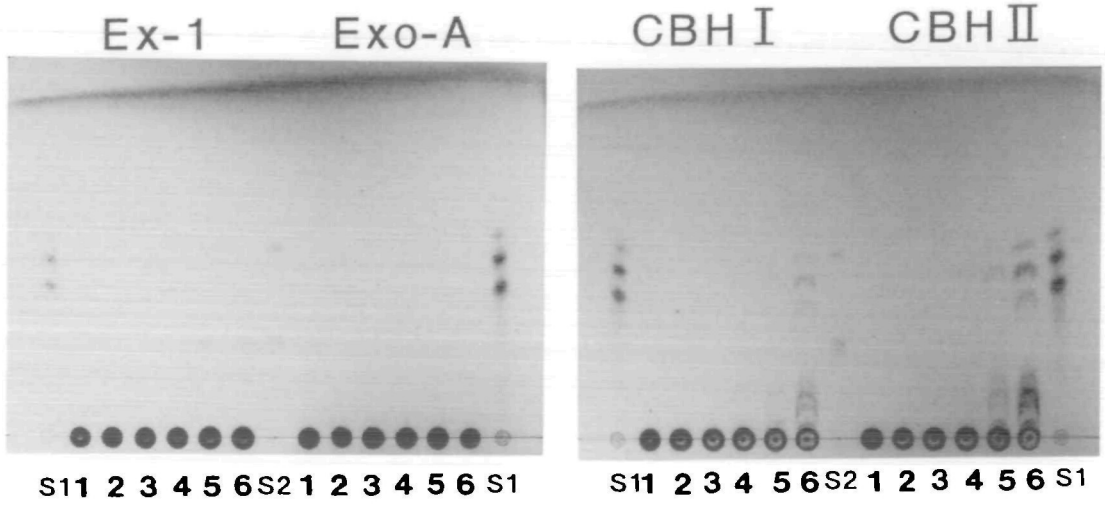

Fig. 4. **Thin layer chromatographic analysis of hydrolysis products from xyloglucan by exo-type cellulases.** Incubation times are 0, 1, 3, 6, 12, and 24 h for 1-6, respectively. S1 and S2: standard sugars of xyloglucan oligosaccharide, heptamer to nonamer, and heptamer and tetradecamer, respectively.

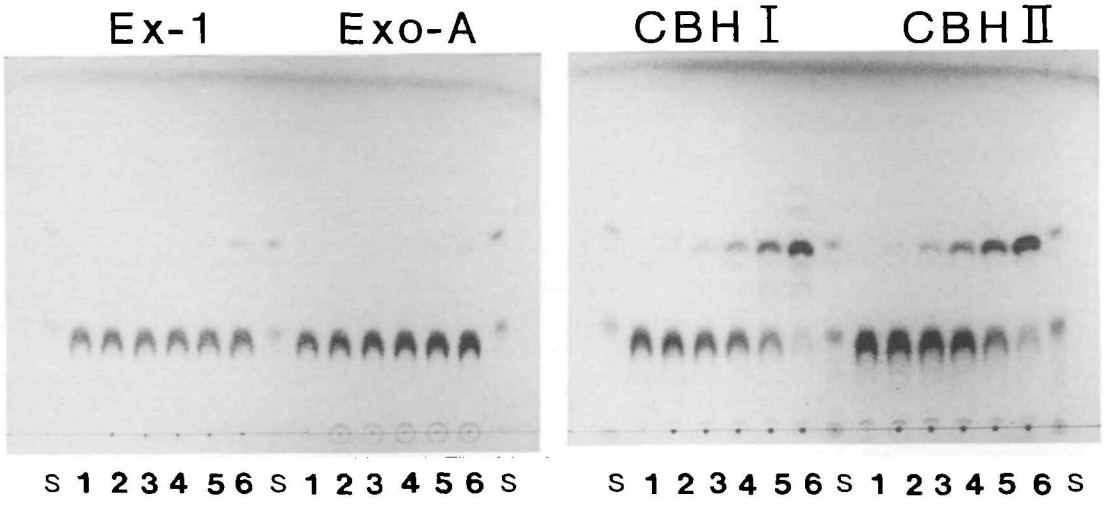

Fig. 5. **Thin layer chromatographic analysis of hydrolysis products from xyloglucan tetradecasaccharide by exo-type cellulases.** Incubation times are 0, 1, 3, 6, 12, and 24 h for 1-6, respectively. S: standard sugars of xyloglucan oligosaccharide (heptamer and tetradecamer).

G3G4G and cellotriose, respectively, and the tetrasaccharide M-3 may be identified as G4G3G4G. Gel permeation chromatography revealed the presence of oligosaccharides whose $\overline{DP}$ values are higher than tetramer in the hydrolysate of barley glucan by CBHI after incubation for 48 h (Fig. 2).

Degradation of Tamarind Xyloglucan—To examine the mode of action of exo-type cellulases upon heteroglucan in detail, we chose a tamarind xyloglucan as substrate. The xyloglucan is assumed to possess a 1,4-β-glucan backbone with α-xylosyl side chains attached to the 6-carbon atom of some β-glucosyl residues and with the 2-carbon atom of some xylosyl residues bearing a β-D-galactosyl chain (*24, 25*).

All the degradation products from xyloglucan were identified by TLC, and the result is shown in Fig. 4. Ex-1 and Exo-A were unable to attack this substrate even on prolonged incubation. However, CBHI and CBHII did attack this substrate at a later stage, CBHII showing a higher activity on the xyloglucan than CBHI.

The hydrolysis products consisted of heptasaccharide (glucose/xylose, 4 : 3; XG7), octasaccharide (glucose/xylose/galactose, 4 : 3 : 1), and nonasaccharide (glucose/xylose/galactose, 4 : 3 : 2), which were identified on the basis of the R_f values, compared with those of authentic sugars.

Next, the hydrolyzability of the tetradecasaccharide (XG14) produced from the xyloglucan by the four exo-type cellulases was investigated. As shown in Fig. 5, Ex-1 and Exo-A were unable to hydrolyze XG14. In contrast, CBHI and CBHII showed remarkably high activity on XG14, and only XG7 was detected as the hydrolysis product at an early stage.

DISCUSSION

β-D-Glucans consisting of β-1,3- and β-1,4-mix-linked glucose units are hydrolyzed by several glycanases, including β-1,3;1,4-glucanases (lichenases), some β-1,3-glucanases and cellulases. Most of those enzymes are of endo-type. Further, it was reported that exo-β-1,3-glucanase from *Trichoderma pseudokoningii* (*26*) and exo-cellulases from *Trichoderma reesei* (*12-14*) and *Irpex lacteus* (*15*) could hydrolyze barley glucan with an endo-type mechanism. Henrissat *et al.* (*12*) reported that CBHI purified from *T. reesei* degraded barley glucan in a typical endo-wise pattern, but they did not examine the degree of hydrolysis or the products in detail. Penttila *et al.* (*13*) reported that recombinant CBHII hydrolyzed barley glucan, but CBHI did not. We found that the four exo-type cellulases from fungi degrade barley glucan and lichenan, although the hydrolytic modes are different from each other; the hydrolyzing activity of CBHI is lower than those of the other exo-type cellulases (Fig. 1). Thus, the fine specificity of these enzymes was examined in detail using four mix-linked oligosaccharides (Table I). G3G4G was not hydrolyzed by these cellulases, while G4G4G3G was rapidly hydrolyzed by all of them. Ex-1 and Exo-A hydrolyzed G4G3G and G4G3G4G, while CBHII was unable to hydrolyze these substrates, and CBHI hydrolyzed only G4G3G4G. Thus, it seems that G4G3G and G4G3G4G are better substrates to examine the mode of action of the four exo-type cellulases. Although the capacity to degrade these mix-linked oligosaccharides was different in each case, the sites of binding seem to be similar with all four exo-type cellulases because of their easy production of cellobiose from the substrates. However, this speculation seems inconsistent with the hydrolysis of G4G3G by Ex-1, though its hydrolyzability was very low. CBHII was different from the other exo-type cellulases, particularly in its inability to hydrolyze the β-1,3-linkage of G4G3G4G. CBHII, therefore, seems to require the presence of a cellotriosyl unit in substrates for activity, as reported by Claeyssens *et al.* (*27*).

Nearly 90% of barley glucan molecule has been established to be composed of cellotriosyl and cellotetraosyl residues separated by single β-1,3-linkages (*28*). Gardner and Blackwell (*29*) proposed that the glucan chains should bend at a (1→4)-β-D-glucosyl residue located next to a (1→3)-β-D-glucosyl residue. This kind of structure, therefore, may fit well with the stereochemical features around the active center of the enzymes. This is illustrated in Diagram 1. Since the C-4 carbon of the second glucosyl residue

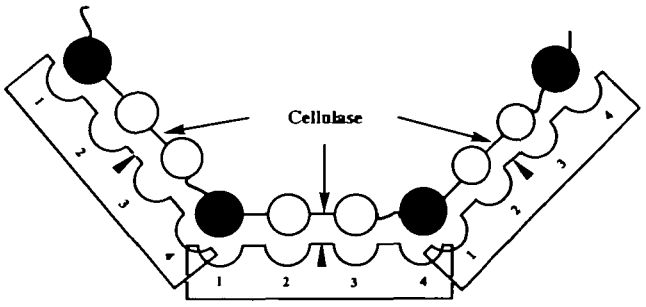

Diagram 1. ●, glucose units which are 3-*O*-substituted; ○, glucose units which are 4-*O*-substituted; −, (1→4)-β-D-glucosidic linkages; ∼, (1→3)-β-D-glucosidic linkages. Arrows show main cleavage sites by exo-type cellulases. Arabic numerals indicate the subsites of the enzyme.

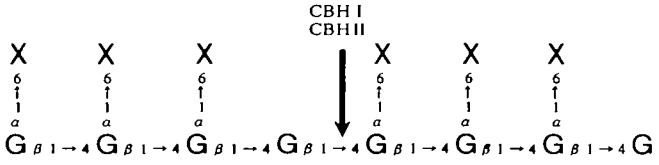

Diagram 2. Arrow shows the cleavage sites.

linked by a β-1,3-linkage in the barley glucan molecule is free, the enzyme seems to be able to differentiate the cellobiosyl unit from glucosyl residues which are 3-*O*-substituted, as in the cellobiosyl residues at the non-reducing end of ordinary cellulosic substrates. This internal cellobiosyl unit recognition in the barley glucan molecule was especially strong with Ex-1, as shown by gel permeation chromatographic analysis of the hydrolysate (Fig. 2). This may be supposed to be a reflection of minor differences in the structure in the active center and in the affinities of the subsites of the individual cellulases.

In the enzymatic hydrolysis of xyloglucan and its oligosaccharide, we obtained different results from those with barley glucan. CBHI and CBHII from *T. reesei* can degrade XG14 and produce two XG7 molecules, as illustrated in Diagram 2. These enzymes seemed to recognize an internal glucosyl unit which is not substituted by an α-xylosyl residue. As with XG14, CBHI, and CBHII were also able to degrade xyloglucan to produce hepta-, octa-, and nona-saccharides as main products. However, the hydrolysis rate of xyloglucan was slower than that of XG14, apparently because xyloglucan has galactose residues in place of some xylosyl residues. The results would suggest that CBHI and CBHII are able to permit various binding modes of soluble substrates because of flexibility of their in active sites. The cleft size of the active site of both enzymes may be larger than in the other two exo-type enzymes, because xyloglucan has many side chains consisting of xylose and galactose residues (*24, 25*).

The modes of action of the four exo-type cellulases in the hydrolysis of crystalline cellulose are roughly similar to each other (*5*), but differ depending on the solubility or molecular size of the substrate, as well as the crystallinity. Depending on the stereochemical or chemical structure of the substrates, even exo-type cellulases may change their hydrolysis mode to an endo-type one.

We thank Dainippon Pharmaceutical Co., Ltd., for providing xyloglucan of tamarind and Novo Nordisk Bioindustry Ltd. for providing barley glucan. We wish to thank Dr. Yasushi Mitsuishi (National Institute of Bioscience and Human Technology) for supplying xyloglucan oligosaccharides.

REFERENCES

1. Wood, T.M. (1985) Properties of cellulolytic enzyme systems. *Biochem. Soc. Trans.* **13**, 407-410
2. Klyosov, A.A. (1990) Trends in biochemistry and enzymology of cellulose degradation. *Biochemistry* **29**, 10577-10585
3. Henrissat, B., Claeyssens, M., Tomme, P., Lemesle, L., and Mornon, J.-P. (1989) Cellulase families revealed by hydrophobic cluster analysis. *Gene* **81**, 83-95
4. Beguin, P. and Aubert, J.-P. (1994) The biological degradation of cellulose. *FEMS Microbiol. Rev.* **13**, 25-58
5. Kanda, T., Amano, Y., Shiroishi, M., and Hoshino, E. (1994) Mode of action of exo- and endo-type cellulases from fungi in the hydrolysis of various substrates (in Japanese). *Oyo Toshitsu Kagaku* **41**, 273-282
6. Parrish, F.W., Perlin, A.S., and Reese, E.T. (1960) Selective enzymolysis of poly-β-D-glucans, and the structure of the polymers. *Can. J. Chem.* **38**, 2094-2104
7. Clarke, A.E. and Stone, B.A. (1966) Enzymic hydrolysis of barley and other β-glucans by a β-(1→4)-glucan hydrolase. *Biochem. J.* **99**, 582-588
8. Marshall, J.J. (1975) Degradation of barley glucan by a purified (1→4)-β-D-glucanase from the snail, *Helix pomatia*. *Carbohydr. Res.* **42**, 203-207
9. Anderson, M.A. and Stone, B.A. (1975) A new substrate for investigating the specificity of β-glucan hydrolases. *FEBS Lett.* **52**, 202-207
10. Anderson, M.A. and Stone, B.A. (1978) Enzymic degradation of chemically modified extracellular polysaccharides from *Rhizobia*. *Carbohydr. Res.* **61**, 479-492
11. Woodward, J.R. and Fincher, G.B. (1982) Substrate specificities and kinetic properties of two (1→3),(1→4)-β-D-glucan endohydrolases from germinating barley (*Hordeum vulgare*). *Carbohydr. Res.* **106**, 111-122
12. Henrissat, B., Driguez, H., Viet, C., and Schulein, M. (1985) Synergism of cellulases from *Trichoderma reesei* in the degradation of cellulose. *Bio/Technology* **3**, 722-726
13. Penttila, M.E., Andre, L., Lehtovaara, P., Bailey, M., Teeri, T.T., and Knowles, J.K.C. (1988) Efficient secretion of two fungal cellobiohydrolases by *Saccharomyces cerevisiae*. *Gene* **63**, 103-112
14. Henriksson, K., Teleman, A., Suortti, T., Reinikainen, T., Jaskari, J., Teleman, O., and Poutanen, K. (1995) Hydrolysis of barley (1→3),(1→4)-β-D-glucan by a cellobiohydrolase II preparation from *Trichoderma reesei*. *Carbohydr. Polym.* **26**, 109-119
15. Kanda, T., Yatomi, H., Makishima, S., Amano, Y., and Nisizawa, K. (1989) Substrate specificities of exo- and endo-type cellulases in the hydrolysis of β-(1→3)- and β-(1→4)-mixed D-glucans. *J. Biochem.* **105**, 127-132
16. Hayashi, T. (1991) Biochemistry of xyloglucans in regulating cell elongation and expansion in *The Cytoskeletal Basis of Plant Growth and Form* (Lioyd, C.W., ed.) pp. 131-144, Academic Press, New York
17. Mitsuishi, Y. and Kosugi, Y. (1993) A fungal new endo-1,4-β-glucanase from *Penicillium* sp. M451 hydrolysing only xyloglucan in *Genetics, Biochemistry and Ecology of Lignocellulose Degradation* (Shimada, K., Hoshino, S., Ohmiya, K., Sakka, S., Kobayashi, Y., and Karita, S., eds.) pp. 516-523, Uni Publishers, Tokyo
18. Hayashi, T., Wong, Y.-S., and Maclachlan, G. (1984) Pea xyloglucan and cellulose II. Hydrolysis by pea endo-1,4-β-glucanases. *Plant Physiol.* **75**, 605-610
19. Kanda, T., Nakakubo, S., Wakabayashi, K., and Nisizawa, K. (1978) Purification and properties of an exo-cellulase of Avicelase type from a wood-rotting fungus, *Irpex lacteus* (*Polyporus tulipiferae*). *J. Biochem.* **84**, 1217-1226

20. Bhikhabhai, R., Johansson, G., and Pettersson, G. (1984) Isolation of cellulolytic enzymes from *Trichoderma reesei* QM9414. *J. Appl. Biochem.* **6**, 336-345
21. Perlin, A.S. and Suzuki, S. (1962) The structure of lichenin: Selective enzymolysis studies. *Can. J. Chem.* **40**, 50-56
22. Somogyi, M. (1952) Notes on sugar determination. *J. Biol. Chem.* **195**, 19-23
23. Nelson, N. (1944) A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* **153**, 375-380
24. York, W.S., van Halbeek, H., Darvill, A.G., and Albersheim, P. (1990) Structural analysis of xyloglucan oligosaccharides by ^{1}H-NMR spectroscopy and fast-atom-bombardment mass spectrometry. *Carbohydr. Res.* **200**, 9-31
25. Gidley, M.J., Lillford, P.J., Rowlands, D.W., Lang, P., Dentini, M., Crescenzi, V., Edwards, M., Fanutti, C., and Reid, J.S.G. (1991) Structure and solution properties of tamarind-seed polysaccharide. *Carbohydr. Res.* **214**, 299-314
26. Matsuzawa, T., Amano, Y., Kubo, M., and Kanda, T. (1996) Mode of action of exo-β-1,3-glucanase from *Trichoderma pseudokoningii* TM37 on various β-D-glucans (in Japanese). *Seibutsu-Kogaku Kaishi* **74**, 261-267
27. Claeyssens, M., van Tilbeurgh, H., Tomme, P., Wood, T.M., and McCrae, S.I. (1989) Comparison of the specificities of the cellobiohydrolases isolated from *Penicillium pinophilum* and *Trichoderma reesei*. *Biochem. J.* **261**, 819-825
28. Staudte, R.G., Woodward, J.R., Fincher, G.B., and Stone, B.A. (1983) Water-soluble (1→3),(1→4)-β-D-glucans from barley (*Hordeum vulgare*) endosperm. III. Distribution of cellotriosyl and cellotetraosyl residues. *Carbohydr. Polym.* **3**, 299-312
29. Gardner, K.H. and Blackwell, J. (1974) The structure of native cellulose. *Biopolymers* **13**, 1975-2001