Purification and Characterization of an *Endo*-cellulase from *Acremonium cellulolyticus*

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An *endo*-cellulase from a commercial cellulase preparation of a filamentous fungus *Acremonium cellulolyticus* was separated and extensively purified to essential homogeneity by using consecutive column chromatographies of QAE-Toyopearl 550C, DEAE-Toyopearl 650S, Butyl-Toyopearl 650M and Bio-Gel P-100, and was designated as cellulase IV. The purified enzyme was homogeneous on both Native- and SDS-PAGE, and was completely free from β -glucosidase. The enzyme showed high specific activity for CMC and an extremely low specific activity for Avicel (a microcrystalline cellulose powder), 106 and 0.08 U/mg of protein, respectively. The molecular mass of the enzyme was estimated to be about 38 kDa by SDS-PAGE. The enzyme was an acidic protein with a *pI* value of ≤ 3.4 . The N-terminal amino acid sequence from the 2nd up to the 27th residue of the purified cellulase was Gln-Ala-Ser-(Cys)-Phe-Glu-Trp-Phe-Gly-Ser-Asn-Glu-Ser-Gly-Ala-Glu-Phe-Gly-Ser-Gly-Asn-Ile-Pro-Gly-Val-Glu-. The optimum pH and temperature were 4.0 and 60°C, respectively. The enzyme was completely stable over the range of pH 5.0–12.0 at 4°C for 24 h and at temperatures below 50°C. Cellulase IV was characterized as a medium *endo*-type cellulase on the basis of its action on CMC and cellooligosaccharides. The enzyme was completely inhibited by 5 mM Hg^{2+} , Fe^{3+} and KMnO_4 . Cellulase IV split various cellulosic substrates to produce predominant cellobiose and a small amount of glucose as the final hydrolysis products.

The main deterioration of cellulose is caused by cellulolytic microbes, and the biological degradation of highly ordered native cellulose is an extremely important process for the maintenance of the carbon balance in nature. Since crystalline cellulose is an insoluble substance with a supramolecular structure, the enzymological approach to this substrate is rather complicated and difficult. In

most cellulolytic microorganisms, several cellulase [1,4-(1,3;1,4)- β -D-glucan-4-glucanohydrolase, EC 3.2.1.4] components together constitute a “cellulase system” that solubilizes native cellulose through their synergistic action.¹⁾

We have presented a series of reports on the cellulase system of *Trichoderma viride*.^{2–8)} We are at present focusing our research efforts on the complete elucidation of the mechanism involved in the enzymatic degradation of native cellulose. Recently, we found that the commercial cellulase preparation of *Acremonium cellulolyticus* possessed extremely potent activity toward native cellulose. Little is known about the cellulase system of this filamentous fungus.

This paper describes the purification, physicochemical and enzymic properties of an *endo*-cellulase from *A. cellulolyticus*.

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Abbreviations: QAE, quaternary aminoethyl; DEAE, diethylaminoethyl; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; CMC, sodium carboxymethylcellulose; PNPG, *p*-nitrophenyl β -D-glucoside; $\overline{\text{DS}}$, degree of substitution; G₁, D-glucose; G₂–G₆, cellooligosaccharides from cellobiose to cellohexaose; NBS, *N*-bromosuccinimide; EDTA, ethylenediaminetetraacetic acid; HPLC, high-pressure liquid chromatography.

MATERIALS AND METHODS

Enzyme material. The commercial cellulase preparation from the culture filtrate of *A. cellulolyticus* (*Acremonium* cellulase, lot No. AUS-0301, Meiji Seika Kaisha, Ltd., Japan) was used in this work.

Materials. Sodium carboxymethylcellulose (CMC), Cellogen WS-C ($\overline{DS}$ =0.62–0.68), was kindly supplied from Daiichi Industrial Pharmaceutical Co., Ltd., Japan, a microcrystalline cellulose powder (Avicel) was obtained from Asahi Kasei Co., Ltd., Japan, and *p*-nitrophenyl β -D-glucoside (PNPG) was a product of Tokyo Kasei Kogyo Co., Ltd., Japan. Dewaxed cotton was a product of Japanese Pharmacopoeial Standard, manufactured by Warabieizai Co., Ltd., Japan, and Whatman No.1 filter paper was from Whatman International, Ltd., England. Cellooligosaccharides (G_3 – G_6) were products of Seikagaku Kogyo Co., Ltd., Japan, and cellobiose (G_2) was from Sigma Chemical Co., Ltd., USA. Pyroglutamate aminopeptidase from *Pyrococcus furiosus* was a product of Takara Shuzo Co., Ltd., Japan. QAE-Toyopearl 550C, DEAE-Toyopearl 650S and Butyl-Toyopearl 650M were the products of Tosoh, Japan, and Bio-Gel P-100 was from Bio-Rad Laboratories, USA. Polyacrylamide slab gel (PAGE, NPU-12.5 L) was a product of Atto Co., Ltd., Japan. SDS-PAGE molecular mass standards (low range of 14.4 to 97.4 kDa) were products of Bio-Rad Laboratories, USA, and Coomassie Brilliant Blue R-250 was a product of Nakarai Chemical Ltd., Japan. Ampholine PAGplate (pH 3.5–9.5; 245×110×1 mm) and IEF Calibration Broad pI Kit (pH 3–10) were products of Amersham Pharmacia Biotech, Sweden. All other reagents were of analytical grade.

Enzyme assays.

(a) CMC-saccharification activity. CMC-saccharification activity was used as a standard assay. The reaction mixture for CMC-saccharification assay contained 0.25 mL of 1% CMC solution, 0.5 mL of 50 mM acetate buffer (pH 5.0), and 0.25 mL of the enzyme solution. The mixture was incubated at 30°C for an appropriate period. Reducing

sugars produced per 1.0 mL of the mixture were estimated by the colorimetric method of Somogyi⁹⁾ and Nelson.¹⁰⁾

(b) Avicel-saccharification activity. The reaction mixture was comprised of 40 mg of Avicel powder and 2.0 mL of enzyme solution in 50 mM acetate buffer (pH 5.0). Incubation was carried out at 40°C for an appropriate period. The reaction was stopped by the addition of 20 μ L of 2 N NaOH solution. After centrifugation of the mixture at 3500 rpm for 20 min, 1.0 mL of the supernatant was carefully withdrawn and analyzed for reducing sugars by the colorimetric method of Somogyi-Nelson.

One unit (U) of each saccharification activity was defined as the amount of enzyme liberating reducing sugars equivalent to 1.0 μ mol/min of D-glucose from the substrate under these conditions.

(c) Aryl β -glucosidase activity. The reaction mixtures, containing 0.05 mL of 13.6 mM PNPG, 0.10 mL of 50 mM acetate buffer (pH 5.0) and 0.05 mL of enzyme solution, was incubated at 30°C for an appropriate period. The reaction was stopped by the addition of 5 mL of 100 mM Na_2CO_3 to the mixture (0.20 mL), and then the amount of *p*-nitrophenol liberated was colorimetrically measured by absorbance at 420 nm. One unit (U) of the enzyme activity was defined as the amount of enzyme that produces 1.0 μ mol of *p*-nitrophenol under these assay conditions.

Measurement of protein and carbohydrate. Protein content was measured by the method of Lowry *et al.*¹¹⁾ with crystalline bovine serum albumin as a standard. Absorbance at 280 nm was used for monitoring protein in column effluents.

The total carbohydrate in the purified enzyme was determined by the phenol-sulfuric acid method¹²⁾ with D-glucose as a standard.

Electrophoresis. Polyacrylamide gel electrophoresis (PAGE) was done in 12.5% polyacrylamide with Tris-glycine buffer (pH 6.8) according to the method of Davis.¹³⁾ SDS-PAGE was done in 12.5% polyacrylamide containing 0.1% SDS by the method of Laemmli.¹⁴⁾ In both cases, the gels were stained with 0.05% Coomassie Brilliant Blue R-250 for 1 h and decoloured with 8.0% (v/v) acetic acid until the background be-

came clear. The *pI* value was determined by the method of Vesterberg¹⁵ using Ampholine PAG-plate (pH 3.5–9.5) and a LKB Multiphor II Electrophoresis Unit (Model 2117). Standard proteins with *pI* values ranging from 3 to 10 (Pharmacia Biotech) were run simultaneously. Gels were stained with 0.25% Coomassie Brilliant Blue R-250 for 10 min and destained with 8.0% (v/v) acetic acid until the background became clear.

Analysis of N-terminal amino acid sequence. The N-terminal amino acid sequence of the purified enzyme was analyzed by N-terminal Edman degradation employing a protein sequencer, PE Model 492 (Perkin-Elmer Co., USA).

Viscometry. The changes in viscosity during the enzymatic hydrolysis of CMC were measured at intervals with a Cannon-Fenske viscometer ($c = 0.0369$). The enzyme activity was expressed in terms of specific fluidity (ϕ_{sp}).

High pressure liquid chromatography (HPLC). Cellooligosaccharides were separated and identi-

fied by HPLC on a Shimadzu LC-10 system with a LC-10AD pump (Shimadzu Corporation), RID-6A refractive index detector (Shimadzu Corporation), and C-R7A integrator (Shimadzu Corporation) under the following conditions: column, Aminex HPX-42A (7.8 × 300 mm, Bio-Rad Laboratories); column temperature, 80°C; mobile phase, deionized water; and flow rate, 0.6 mL/min.

RESULTS

Purification of the enzyme.

Unless otherwise stated, all purification procedures for the enzyme were done in a cold room (4 °C).

Commercial cellulase powder (5 g) was mixed with 50 mL of 20 mM acetate buffer (pH 5.5) by carefully stirring overnight at 4°C. The crude enzyme extract was centrifuged at 2000 × *g* for 20 min at 4°C. The resulting supernatant was used as a starting enzyme material for purification.

Fig. 1. Elution profiles of the crude cellulase preparation on QAE-Toyopearl 550C column chromatography.

○, protein ($A_{280 \text{ nm}}$); ●, CMC-saccharification activity (U/mg protein); ▲, Avicel-saccharification activity (U/mg protein); ×, β -glucosidase activity (U/mg protein).

Step 1. QAE-Toyopearl 550C column chromatography. The starting enzyme material was put on a QAE-Toyopearl 550C column (4×28.5 cm) pre-equilibrated with 20 mM acetate buffer (pH 5.5). Elution was done stepwise with the same buffer containing 0, 40, 150 and 500 mM NaCl at a flow rate of 3 mL/min. Each 200 mL fraction was collected and assayed for cellulase activity. Four protein peaks (Peaks I–IV) were obtained (Fig. 1). Three peak fractions (Peaks I, III and IV) showed cellulase activity toward CMC (Fig. 1). Among them, the peak IV fraction had a potent cellulase activity without β -glucosidase activity. Therefore, the peak IV fraction (fraction Nos. 39–41) was collected and concentrated to about 15 mL by ultrafiltration with a Diaflo PM10 membrane (Amicon Corp., USA) and collodion bag (Sartorius GmBH), and dialyzed overnight against 20 mM acetate buffer (pH 5.5).

Step 2. DEAE-Toyopearl 650S column chromatography. The dialyzed sample was put on a DEAE-Toyopearl 650S column (4×36 cm) pre-equilibrated with 20 mM acetate buffer (pH 5.5). Elution was done stepwise with the same buffer containing 0, 100 and 150 mM NaCl at a flow rate

of 0.2 mL/min. Each 10 mL fraction was collected and assayed for cellulase activity. The active fractions were pooled, concentrated, and dialyzed overnight against 20 mM acetate buffer (pH 4.5) containing 700 mM $(\text{NH}_4)_2\text{SO}_4$.

Step 3. Butyl-Toyopearl 650M column chromatography. The dialyzed sample was put on a Butyl-Toyopearl 650M column (2×30 cm) pre-equilibrated with 20 mM acetate buffer (pH 4.5) containing 700 mM $(\text{NH}_4)_2\text{SO}_4$. Stepwise elution was done with the same buffer containing 700 mM $(\text{NH}_4)_2\text{SO}_4$, 20 mM acetate buffer (pH 4.5) and distilled water, respectively. Each 6 mL fraction was collected at a flow rate of 0.5 mL/min and assayed for cellulase activity. The active fraction was eluted only with 20 mM acetate buffer (pH 4.5). Fractions containing cellulase activity were pooled, concentrated and dialyzed overnight against 20 mM acetate buffer (pH 5.5).

Step 4. Bio-Gel P-100 column chromatography.

The dialyzed sample was loaded on a Bio-Gel P-100 column (2×42 cm) pre-equilibrated with 20 mM acetate buffer (pH 5.5). The enzyme was eluted with the same buffer at a flow rate of 0.1 mL/min. A symmetrical protein peak containing

Fig. 2. Gel filtration profiles of the cellulase fractions on Bio-Gel P-100 after Butyl-Toyopearl 650M column chromatography.

○, protein ($A_{280 \text{ nm}}$) ; ●, CMC-saccharification activity (U/mg protein) ; ▲, Avicel-saccharification activity (U/mg protein).

Table 1. Summary of the purification of cellulase IV from *Acremonium cellulolyticus*.

Purification step	Total protein ^a (mg)	Total activity (U)	Specific activity ^b (U/mg)	Yield (%)	Purification (fold)
1. Crude cellulase extract	3750.0	29325.0	7.8	100.0	1.0
2. QAE-Toyopearl 550C	407.9	7520.7	18.4	25.6	2.4
3. DEAE-Toyopearl 650S	132.4	6829.7	51.6	23.3	6.6
4. Butyl-Toyopearl 650M	99.1	6698.8	67.6	22.8	8.7
5. Bio-Gel P-100	61.9	6557.1	105.9	22.4	13.6

^aMeasured by the method of Lowry *et al.* ¹¹⁾ ^bCMC was used as a substrate.

only cellulase activity was obtained (Fig. 2). The active fractions were then combined as the purified enzyme. The purified enzyme gave a single protein band on both Native- and SDS-PAGE, and was designated as cellulase IV.

Table 1 summarizes the yield and specific activity of cellulase IV during the purification procedure. Cellulase IV had a high level of specific activity, about 106 U/mg of protein in a final yield of about 22%, and was used for the subsequent characterization.

Physicochemical properties of the purified enzyme.

The molecular mass of cellulase IV was found to be about 38 kDa by comparison of its relative mobility on SDS-PAGE with those of standard proteins (Fig. 3). About 1.3% carbohydrate moiety seemed to be associated with the enzyme protein. The isoelectric point of the enzyme determined by isoelectric focusing was ≤ 3.4 . Concerning the N-terminal amino acid sequence of the purified enzyme, the first amino acid by Edman degradation was not detected, indicating the presence of a modified amino acid residue at the N-terminus. Pyroglutamic acid was strongly suggested as the N-terminus amino acid judging from the result of pyroglutamate aminopeptidase treatment. The N-terminal amino acid sequence from the 2nd up to the 27th residue of cellulase IV was determined to be: Gln-Ala-Ser-(Cys)-Phe-Glu-Trp-Phe-Gly-Ser-Asn-Glu-Ser-Gly-Ala-Glu-Phe-Gly-Ser-Gly-Asn-Ile-Pro-Gly-Val-Glu-.

Fig. 3. Native- and SDS-PAGE of the purified enzyme.

Lane 1, standard protein mixture containing phosphorylase *b* (97.4 kDa), bovine serum albumin (66.2), ovalbumin (45.0), carbonic anhydrase (31.0), soybean trypsin inhibitor (21.5), and lysozyme (14.4); Lanes 2 and 3, purified enzyme on SDS- and Native-PAGE, respectively.

Effects of pH and temperature on the enzyme activity and stability.

The effects of pH and temperature on the activity of the purified enzyme were studied under standard assay conditions at 30°C for 10 min using 50 mM McIlvaine buffer at pH 2.7–8.2 and at different reaction temperatures for 10 min. Cellulase IV showed a single maximum at pH 4.0 and 60°C.

On the other hand, each solution containing an equal amount of the purified enzyme (0.01 U) was individually adjusted to a pH from 2.7 to 12.0 by adding 50 mM McIlvaine or Britton-Robinson buffer. After the buffered enzyme solutions had been kept at 4°C for 24 h, they were diluted 25-fold with 50 mM McIlvaine buffer (pH 4.0). Each 0.25 mL enzyme sample was then examined for

remaining CMC-saccharification activity by the standard assay at 30°C for 10 min. Cellulase IV was completely stable over the range of pH 5.0–12.0 at 4°C for 24 h.

The purified enzyme in 50 mM McIlvaine buffer (pH 4.0) was heated at various temperatures for 10 min, then cooled immediately in an ice-bath. The remaining CMC-saccharification activity was then measured with 0.25 mL of each enzyme solution by the standard assay at 30°C for 10 min. The enzyme was completely stable at temperatures below 50°C. The enzyme retained about 25 and 5% of its original activity on heating at 80 and 90°C, respectively. However, cellulase IV was completely inactivated by heating at 100°C under the conditions used.

The effects of pH and temperature on the activity and stability of cellulase IV are shown in Fig. 4A and B.

Effects of various metal ions and several enzyme inhibitors on the activity of the enzyme.

The enzyme was incubated with an equal volume of each metal ion or inhibitor solution (10 mM, pH 6.0) at 30°C for 30 min. Then the mixtures were diluted 25-fold with 50 mM McIlvaine buffer (pH 4.0), and the remaining enzyme activity per milliliter of each reaction mixture containing 0.34 U of enzyme was examined by the

standard assay at 30°C for 10 min. Inactivation of the enzyme was found to be partial with 5 mM Ba^{2+} , Cu^{2+} , Sr^{2+} and Ag^{+} being about 10, 15, 20 and 25% inhibition, respectively and with 5 mM NBS being about 15% inhibition. On the other hand, 5 mM Hg^{2+} , Fe^{3+} or KMnO_4 inactivated the enzyme completely. Pb^{2+} , EDTA and the sulfhydryl reagents tested had no inhibitory effect on the activity of the enzyme.

Mode of action of the enzyme toward CMC.

The mode of action of cellulase IV toward CMC was analyzed by a viscometer. The reaction mixture was composed of 6 mL of 50 mM acetate buffer (pH 4.0) containing 0.5% CMC and 6 mL of the enzyme solution (0.09 U). The mixture was incubated in a Cannon-Fenske viscometer at 30°C. The decrease in viscosity of the incubation mixture and formation of reducing sugars were measured every 5 min. Then the increases in specific fluidity (ϕ_{sp}) were plotted against the increases in reducing power. As can be seen in Fig. 5, a straight line with the slope of an angle of about 45° was obtained for the enzyme. This result indicates that the purified enzyme is a medium *endo*-type cellulase. The differences in slopes produced by cellulase IV and the crude enzyme suggest that the crude enzyme preparation contains other cellulase components other than the present one.

Fig. 4. Effects of pH and temperature on the activity and stability of the purified enzyme.

(A) ○, pH activity; ●, pH stability. (B) □, thermal activity; ■, thermal stability. Experimental details are given in the text.

Action of the purified enzyme on cellooligosaccharides and insoluble substrates.

The hydrolysis products from cellooligosaccharides by cellulase IV were analyzed by HPLC. Table 2 shows the degree of hydrolysis and product distribution due to the action of cellulase IV at the initial and late stages of incubation. The enzyme hydrolyzed G₆, G₅ and G₄ to give G₂ as the major end product. However, G₃ was considerably hard to hydrolyze compared to G₄–G₆. The enzyme did not act entirely on G₂.

Avicel, filter paper and dewaxed cotton were used as insoluble substrates. The reaction mixture

contained 40 mg of each substrate and 2 mL of purified enzyme (2.7 U) in 50 mM acetate buffer (pH 4.0). Incubation was done at 40°C for an appropriate period. The reaction was stopped by adding 20 μ L of 2 N NaOH solution. Reducing sugars produced were measured as in the case of Avicel-saccharification activity. Glucose formed in the reaction mixture was measured by the method of glucose oxidase.^{16,17)}

As shown in Fig. 6, it is clear that cellulase IV produces a high level of reducing sugars and a small amount of glucose from both Avicel and filter paper. On the other hand, dewaxed cotton was hard to hydrolyze using this enzyme in a short-time incubation. HPLC analysis indicated that most reducing sugars produced were G₂. After prolonged incubation (40°C, 7 days), the enzyme split insoluble substrates tested to give predominant G₂ and a small amount of G₁ as the final hydrolysis products (data not shown).

DISCUSSION

An *endo*-cellulase from a commercial cellulase preparation of *Acremonium cellulolyticus* was extensively purified by consecutive column chromatographies using QAE-Toyopearl 550C, DEAE-Toyopearl 650S, Butyl-Toyopearl 650M and Bio-Gel P-100. The purified enzyme (Cellulase IV) was homogeneous on both Native- and SDS-PAGE, and was completely free from β -glucosidase. Cellulase IV showed a high specific activity

Fig. 5. Relationship between increases in fluidity and reducing power during the hydrolysis of CMC by the purified enzyme and crude enzyme.

●, purified enzyme; ○, crude enzyme.

Table 2. Degree of hydrolysis and product distribution due to the action of cellulase IV at the initial and late stages of incubation.

Substrate (4 mM)	Degree of hydrolysis ^a (%)	Time (min)	Product distribution (mM)					
			G ₆	G ₅	G ₄	G ₃	G ₂	G ₁
Cellohexaose	97.0	2	0.12	0.10	1.94	1.74	4.46	0.14
	100.0	240	—	0.06	0.59	1.85	7.11	0.73
Cellopentaose	95.0	2	—	0.20	0.80	2.02	4.56	0.10
	100.0	240	—	—	0.27	1.83	6.13	0.54
Cellotetraose	9.3	10	—	—	3.63	0.19	0.34	0.09
	72.3	240	—	—	1.11	1.00	3.61	0.85
Cellotriose	6.5	240	—	—	—	3.74	0.30	0.05

^aCalculated from the amount of residual substrate.

Fig. 6. Courses of reducing sugar and glucose formation due to the action of the purified enzyme on insoluble substrates. ●, reducing sugar from dewaxed cotton; ○, glucose from dewaxed cotton; ■, reducing sugar from filter paper; □, glucose from filter paper; ▲, reducing sugar from Avicel; △, glucose from Avicel.

for CMC and extremely low specific activity for Avicel, 106 and 0.08 U/mg of protein, at pH 4.0, 30°C for CMC and 40°C for Avicel, respectively.

Our purified cellulase is extremely close in many points to FV CM-cellulase from *Aspergillus aculeatus*¹⁸⁾ which possesses the following properties: molecular mass, 38 kDa; *pI*, 3.4; optimum pH, 4.0; optimum temperature, 65°C; pH stability, pH 3.5–9.0; and thermal stability, $\leq 50^\circ\text{C}$. The present enzyme also fairly closely resembles an *endo*-type cellulase from *Aspergillus niger*¹⁹⁾ which has the characteristic properties of molecular mass, 31 kDa; *pI*, 3.67; specific activity for CMC, 117 U/mg of protein; optimum pH, 4.0 and pH stability, pH 5.0–8.0. However, the purified cellulase was extremely stable in the alkaline region (Fig. 4 A) compared to the above two *Aspergillus* cellulases.

Inactivation of the enzyme by the metal ions tested was found to be partial with 5 mM Ba^{2+} , Cu^{2+} , Sr^{2+} and Ag^+ , corresponding to about 10, 15, 20, and 25% inhibition, respectively. However, no inhibition of the purified enzyme was observed with EDTA or the sulfhydryl reagents tested. This suggests that the metals and sulfhydryl groups are not essential for the cellulolytic action of the en-

zyme, as is also the case with highly purified cellulases from *T. viride*.^{3,5)}

During the hydrolysis of CMC by cellulase components, there is usually a characteristic pattern in the relationship between loss of viscosity and increase in reducing power. Consequently, when the increases in specific fluidity (ϕ_{sp}) in the enzymic reaction are plotted against increases in reducing power, the slopes of the resulting curves are different. It has been reported that the highly purified cellulase components from *T. viride*^{3,5)} and *Irpex lacteus*²⁰⁾ have the different degrees of randomness during the hydrolysis of CMC. In comparison with these data, cellulase IV was characterized as a medium *endo*-type cellulase.

The enzyme was incapable of attacking either G_2 or PNPG, but hydrolyzed various cellulosic substrates such as cellooligosaccharides (G_3 – G_6), CMC, Avicel, filter paper, and dewaxed cotton to various extents. G_3 was only slightly attacked using a fairly large amount of enzyme. G_4 was hydrolyzed much more slowly by the enzyme than G_5 and G_6 . As can be seen in Table 2, cellulase IV split G_3 to give G_2 and G_1 . However, the amount of G_2 is 6 times higher than that of G_1 . This strongly suggests that the catalytic reaction is not a simple hydrolysis but a reaction involved in transglycosylation. In either case, the mode of action of cellulase IV on cellooligosaccharides was extremely similar to that of an *endo*-cellulase from *A. niger*.¹⁹⁾

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糸状菌 *Acremonium cellulolyticus* 起源の エンドセルラーゼの精製と基本性質

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糸状菌 *Acremonium cellulolyticus* の市販酵素製剤から、エンドセルラーゼの一成分を QAE-Toyopearl 550C, DEAE-Toyopearl 650S, Butyl-Toyopearl 650M および Bio-Gel P-100 のカラムクロマトグラフィーを順次行い、電気泳動的に単一になるまで精製し、セルラーゼ IV と呼称した。精製酵素は CMC に対し高い比活性値 (106 U/mg) を、また Avicel に対しきわめて低い比活性値 (0.08 U/mg) を示した。当該酵素の分子質量および等電点は、それぞれ約 38 kDa および 3.4 以下であった。セルラーゼ IV の N 末端側第 2-第 27 アミノ酸残基の配列は、Gln-Ala-Ser-(Cys)-Phe-Glu-Trp-Phe-Gly-Ser-Asn-Glu-Ser-Gly-Ala-Glu-Phe-Gly-Ser-Gly-Asn-Ile-Pro-Gly-Val-Glu-であった。本酵素の反応至適 pH および至適温度は、それぞれ 4.0 および 60℃ であった。セルラーゼ IV は各種の pH で 4℃, 24 時間処理した場合には pH 5.0–12.0 の範囲で、また 50℃ 以下においてそれぞれ安定であった。CMC および各種のセロオリゴ糖に対する作用パターンから、当該酵素の作用様式は、中位のエンド型であることが判明した。本酵素の活性発現は、5 mM の Hg²⁺, Fe³⁺ および KMnO₄ により完全に阻害された。セルラーゼ IV を各種の不溶性セルロースに作用させると、多量のセロビオースおよび少量のグルコースが、最終生成糖として得られた。