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Purification and Characterization of Carboxymethyl Cellulase IV from *Penicillium verruculosum*

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*Penicillium verruculosum*으로부터 Carboxymethyl Cellulase IV의 精製 및 特性

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ABSTRACT: An endo-type cellulase, carboxymethyl cellulase(CMCase) IV, was purified from culture filtrate of cellulolytic fungus *Penicillium verruculosum*. The CMCase IV was acidic glycoprotein having carbohydrate of 13% as glucose and pI value of 4.0. The CMCase IV was 52 KDa of molecular weight in SDS-polyacrylamide gel electrophoresis and have optimum temperature and pH of 50℃ and 5.0 for enzyme activity. The CMCase IV liberated glucose and cellobiose as major products of the enzyme against carboxymethyl cellulose(CMC) and seemed to has transglycosylation activity simultaneously. Cellulase activity staining(zymogram) showed that the cellulase components of *P. verruculosum* were not aggregated in the medium. *P. verruculosum* mRNA was translated *in vitro* and translation product by the mRNA coding for CMCase IV was identified by immunoprecipitation.

KEYWORDS: *Penicillium verruculosum*, cellulase, purification of CMCase IV.

Cellulose is a polysaccharide composed of β-D-glucopyranosyl units joined by β-1,4-glycosidic bonds. Cellulose, however, is very resistant to hydrolysis due to its fully extended, flat conformation and tight packaging into microfibrils to form a fibrious, naturally crystalline, insoluble materials. Furthermore, the fibers are almost always embedded in a matrix of other compounds, such as hemicelluloses, lignin, and other polysaccharides, which further reduces its bioconversion (Aubert *et al.*, 1988; Wood, 1985).

All organisms that can degrade crystalline cellulose secrete more or less complex cellulase systems. Such systems are composed of a variety of enzymes with different specificities and modes of action, which act in synergism to hydrolyze cellulose. The widely recognized constituents for the enzymatic degradation of cellulose are based on fungal cellulases, which have been ordered into three major categories; cellobiohydrolase, endoglucanase, and β-glucosidase(Nisizawa, 1973; Ryu and Mandels, 1980; Tomme,1988). The current concept of a mode of action of the activities involved in the degradation of crystalline cellulose assumes an initial attack by endoglucanases, which hydrolyse cellulose chains in a random fashion in a amorphous region of the cellulose, thereby, converting long chains to oligosaccharides(Ryu and Mandels, 1980). The net effect is a rapid decrease in polymer length coupled with a slow increase in reducing groups. This initial attack is followed by attachment of cellobiohydrolase and hy-

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drolysis of cellobiosyl moieties from the nonreducing ends of the nicked chains. The result of this type of hydrolysis is a rapid increase in reducing sugar with little change overall chain length over the short term. Finally, β-glucosidase hydrolyse cellobiose to two glucose units.

P. verruculosum F-3, a hypercellulolytic fungus, and its cellulolytic system has been reported by Chung *et al.*(1982, 1984). It has been emphasized that *P. verruculosum* has strong activities toward natural cellulose such as cotton and Avicel and a different cellulase induction mechanism from that of *Trichoderma* species. In this study, purification of one CMCase, CMCase IV, from culture filtrate of the *P. verruculosum* and characterization of the purified enzyme are reported.

Materials and Methods

Culture condition for cellulase production

The fungal medium used for the cellulase production contained the following ingredients per liter; $(NH_4)_2SO_4$, 4.2g, KH_2PO_4, 2.0g, urea, 0.3g, $CaCl_2$, 0.3g, $MgSO_4 \cdot 7H_2O$, 0.3g, $FeSO_4 \cdot 7H_2O$, 5 mg, $MnSO_4 \cdot H_2O$, 1.6 mg, $ZnSO_4 \cdot 7H_2O$, 1.4 mg, $CoCl_2$, 2.0 mg, cellobiose octaacetate, 10g, wheat bran, 10 g, and Tween 80, 2 m*l*, pH 5.2. The fungal spore (1×10^7 spores/m*l*) harvested from 7 day old potato dextrose agar (PDA) culture was inoculated to the medium and incubated on a rotary shaker (200 rpm) at 30℃ for 14 days.

Detection of cellulase activity on polyacrylamide gel (Zymogram)

Cellulase activity on the gel subjected to electrophoresis was detected by using polyacrylamide gel zymogram coupled with an agar replica plate containing CMC as a substrate with some modification of Beguin's procedure (Beguin, 1983). After the concentrated crude enzyme from cell culture filtrate of *P. verruculosum* was seperated by polyacrylamide gel electrophoresis (PAGE), half of this gel was stained for protein with Coomassie blue R250 and the remaining half was laid on top of a 2% agar sheet containing 0.1% CMC. If the sample protein was denatured in 2% sodium dodecyl sulfate (SDS) and seperated by SDS-PAGE, the SDS was removed by washing the gel with citrate-phosphate buffer (pH 6.3) containing 25%(v/v) isopropanol prior to enzyme reaction. After incubation at 50℃ for 30 min to 1 h in a humid chamber, the agar sheet was stained with a 0.1% Congo-red solution for 1 h, destained with 1 M NaCl, and fixed with 1% NaOH. Orange zone on a red background in the zymogram indicated cellulase activity.

Crude enzyme preparation

Extracellular protein from the fungal culture was precipitated by adding ammonium sulfate to the culture filtrate to 80% of saturation and the precipitate collected by centrifugation. The protein was dissolved in 50 mM sodium citrate buffer(pH 5.2) and ultrafiltered through UM-10 membrane (Amicon). After filtering and concentration, the protein solution was reconcentrated by lyophilization and used for further study.

Purification of CMCase

The crude enzyme was fractionated by gel filtration on a column (2.5×150 cm) packed with Ultro gel AcA44 (Bead size, 60-140 μ). The column was equilibrated with 20 mM sodium citrate buffer (pH 5.2) and eluted with the same buffer. Fractions of 8 m*l* were collected at a flow rate of 20 m*l*·h^{-1}. The U-II fraction from Ultro gel column was loaded on the top of Bio-gel hydroxyapatite (HTP) column(1.8×150 cm) and eluted with sodium phosphate buffer (pH 6.0) increasing from 5 mM to 500 mM at a flow rate of 16 m*l*·h^{-1}. The fraction volume was 6 m*l*. Subsequently, the fractions showing CMCase activity in Bio-gel HTP elutes were more purified through chromatography on DEAE-Sephadex A-50 column (2.5×150 cm) and Bio-gel P-100 column (2.5×100 cm). The H-II fraction of Bio-gel HTP was packaged in DEAE-Sephadex A-50 column and the column was step-wise eluted with acetate buffer (pH 5.2) from 5 mM to 360 mM at a flow rate of 12 m*l* · h^{-1}. After the D-I fraction of DEAE- Sephadex A-50 column was loaded on Bio-gel P-100 column equilibrated with 20 mM citrate buffer (pH 5.2), the column was eluted with the same buffer as a flow rate of 10 m*l* · h^{-1}. Finally, the CMCase protein were purified by preparative electrophoresis, and followed by excising the band from polyacrylamide gel. Purification procedure of CMCase was sum-

Crude culture broth
↓
Centrifugation (at 5,000×*g* for 30 min)
↓
Supernatant
↓
50% $(NH_4)_2SO_4$ precipitation
↓
Centrifugation (at 10,000×*g* for 30 min)
↓
Desalting by Sephadex G-25 and ultrafiltration
↓
Freeze drying
↓
Ultro-gel, pooled by U-II fraction
↓
Bio-gel HTP, pooled by H-II fraction
↓
DEAE-Sephadex A-50, pooled by D-I
↓
Bio-gel P-100
↓
Preparative gel electrophoresis
↓
CMCase IV

Fig. 1. Purification procedure of the CMCase IV from the fungal filtrate.

marized in Fig.1.

Polyacrylamide gel electrophoresis

SDS-PAGE was performed according to Laemmli (1970). A constant voltage of 60 V was applied in stacking gel, and then 120 V in the resolving gel. After running, the protein on the gel was detected with 0.25% Coomassie Blue solution.

Gel filtration for molecular weight estimation

Molecular weight of nondenatured form of purified enzyme was determined by chromatography on a column (2.5×90 cm) of Sephadex G-75 equilibrated with 50 mM sodium citrate buffer (pH 5.2).

Isoelectric focusing

Analytical isoelectric focusing was carried out on a slab gel (5%) containing 6.25% Pharmalyte (pH range, 2.5-5.0) as carrier ampholyte. The experiment was run at 10℃ for 2 h using 20 mM acetic acid and 20 mM NaOH as anolyte and catholyte, respectively. 20 W constant was applied for the isoelectric focusing. Amyloglucosidase (pI, 3.55), trypsin inhibitor (pI, 4.6), and carbonic anhydrase (pI 6.6) were used as standard marker proteins.

Carbohydrate content and amino acid analysis

Carbohydrate content of purified enzymes were determined according to phenol-sulfuric acid method of Dubois (1956). To determine the amino acid composition of purified enzymes, the protein was hydrolyzed in 6 N HCl at 110℃ for 24 h and diluted in citrate buffer (pH 2.2). The hydrolyzates were analyzed in a amino acid analyzer (Hitachi, Japan).

Thin layer and paper chromatography

The hydrolysis products liberated by the purified cellulase were detected by thin layer chmatography (TLC) and paper chromatography (PC). After incubation for 18-24 h, 20 μl aliquots of the enzymatic hydrolysates against various substrates were spotted on TLC plate (Art 5745, Kiesel gel 60, Merk Co.) and Whatman paper (Whatman No. 1, England, Whatman Ltd). Chromatograms were developed by the ascending technique using n-butanol-isopropanol-water (3:12:4, v/v) in TLC and n-butanol-pyridine-water (6:4:2, v/v) in PC, respectively. Products were detected by aniline- diphenylamine reagent or nitrate-sodium hydroxide reagent.

Enzyme assays

Endo-β-1,4-glucanase (CMCase)

CMCase was assayed by measuring the amount of reducing sugar produced from CMC (D.S. 0.6-0.7, DP. 450-500, Wako Pure Chemical Co.). The reaction mixture contained 0.25 m*l* of CMC (1%, w/v) in 0.05 M citrate buffer (pH 5.0), and 0.25 m*l* of enzyme solution. After incubating the mixture at 50℃ for 30 min with shaking at 150 rpm, the reaction was stopped by cooling in an ice bath and reducing sugars were determined.

Exo-β-1,4-glucanase (β-1,4-glucan cellobiohydrolase): β-1,4-Glucan cellobiohydrolase was tested by the addition of 0.5 m*l* enzyme solution to 0.5 m*l* of 5% Avicel (w/v, Fluka Chemica, Avicel PH-101) in 0.05 M citrate buffer (pH 5.0). After the mixture was incubated at 50℃ for 1 h with shaking at 150 rpm, reducing sugars were determined.

β-Glucosidase activity: β-Glucosidase activity was measured with salicin (Wako Pure Chemical Co.) as a substrate. The reaction mixture contai-

ned 0.4 m*l* of salicin (0.5%, w/v) in 0.05 M citrate buffer (pH 5.0),and 0.4 m*l* of enzyme solution. After the mixture was incubated at 50℃ for 30 min, reducing sugars were determined.

Determination of reducing sugar

Reducing sugars were determined by the method of Miller *et al.* (1960). One unit of cellulose (CMC, Avicel and salicin) hydrolyzing activity was defined as the amount of enzyme that produced 1 μmole of reducing sugar per min under the given condition.

Determination of protein

Protein was determined according to Lowry *et al.* (1951) with bovine serum albumin as a standard.

Immunoprecipitation

For the identification of precursor of the purified CMCase IV in cell-free translation products, immunoprecipitation was performed as described elsewhere (Kim *et al.*, 1992a).

Results and Discussion

Detection of cellulase activity on polyacrylamide gel (Zymogram)

Cellulase distribution of *P. verruculosum* was illustrated by detecting the cellulase bands on the polyacrylamide gel using zymogram. About ten zones showing CMCase activity were detected on agar replica plate, which also contained Avicelase bands because Avicelase has some CMCase activity simultaneously (Fig.2). However, SDS-denatured protein sample, prepared by treating the protein in 2% SDS solution at 60℃ for 45 min, showed only one CMCase activity band (data not shown). *P. verruculosum* cellulase seems, therefore, to be not aggregated in the culture medium as does in other fungi (Coughlan, 1985). These results were different from that of *Clostridium thermocellum* (Beguin, 1983), which showed that a number of bands showing cellulase activity were detected, even after preincubation of the samples at 60℃ in the presence of 2% SDS, due to aggregation of high molecular weight cellulase. It was, in fact, recognized from the fact that *C. thermocellum* cellulase contained at least 14-18 different

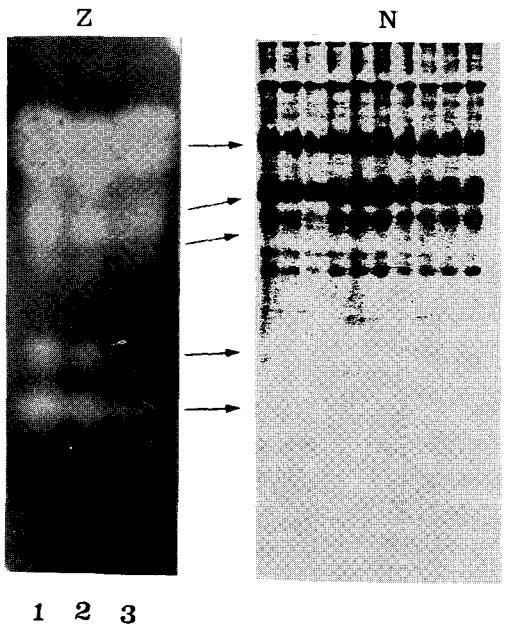

Fig. 2. Distribution of cellulase activity in the fungal culture filtrate. After nondenaturing (native) PAGE, the gels (12.5%) were stained for protein with Coomassie brilliant blue R(N) or analyzed for cellulolytic activity by means of a CMC-polyacrylamide zymogram(Z) as described in Materials and Methods. Protein concentrations; 1, 2 μg, 2, 1 μg, 3, 0.5 μg.

polypeptides forming a very stable extracellular cellulose-binding multienzyme complex termed cellulosome (Bayer and Lamed, 1986; Wu *et al.*, 1988).

Elution patterns of carboxymethyl cellulase

One endoglucanase, CMCase IV, was purified through ion exchange chromatography, gel filtration, and preparative electrophoresis sequentially. The CMCase active fractions were collected, concentrated by ultrafiltration and, thereafter, separated on each column subsequently; the U-II fraction of Ultro-gel column (Fig. 3), the H-II fraction of Bio-gel HTP column (Fig. 4), the D-I fraction of DEAE-Sephadex A-50 ion exchange column (Fig. 5), and the single fraction of Bio-gel P-100 gel column (Fig. 6). After the elutes of Bio-gel P-100 column contaminated with some other proteins were purified by preparative electrophoresis (Fig. 7), the desired band was cut and eluted with electroeluter. Purity of the eluted protein was de-

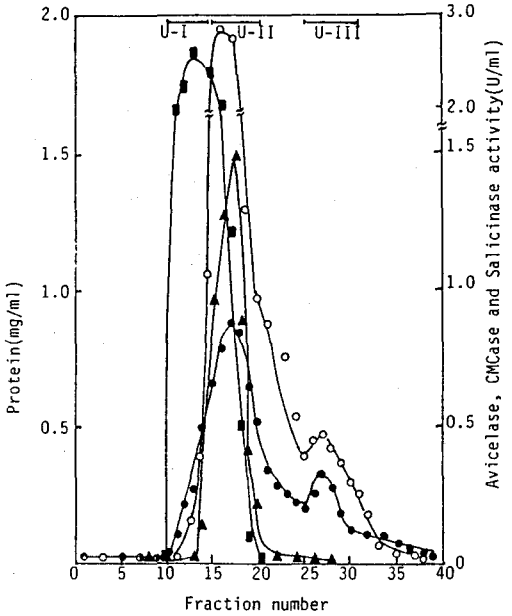

Fig. 3. Ultro-gel AcA-44 chromatogram of the concentrated fungal culture filtrate. The column (2.5×150 cm) was eluted with 20 mM citrate buffer, pH 5.2 at a flow rate of 20 $ml \cdot h^{-1}$ and a fraction volumn was 8 *ml*. Symbols: ▲, Avicel saccharifying activity; ●, CMC saccharifying activity; ■, salicin saccharifying activity; ○, protein.

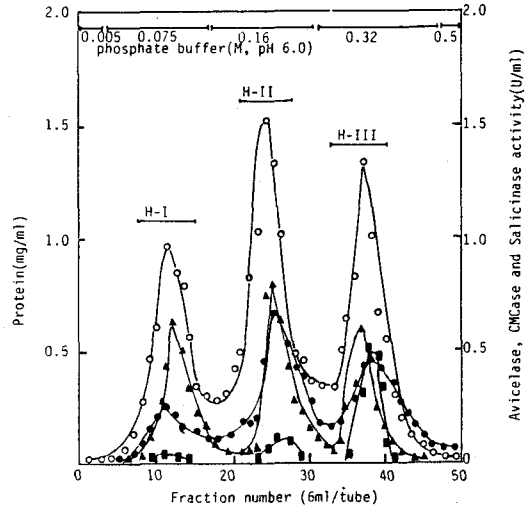

Fig. 4. Bio-gel HTP (Hydroxyapatite) chromatogram of U-II fraction from Ultro-gel chromatography. The column (1.8×150 cm) was step-wise eluted with sodium phosphate buffer, pH 6.0, increasing from 5 mM to 500 mM at a flow rate of 16 $ml \cdot h^{-1}$. The fraction volumn was 6 *ml*. Symbols are the same as Fig. 3.

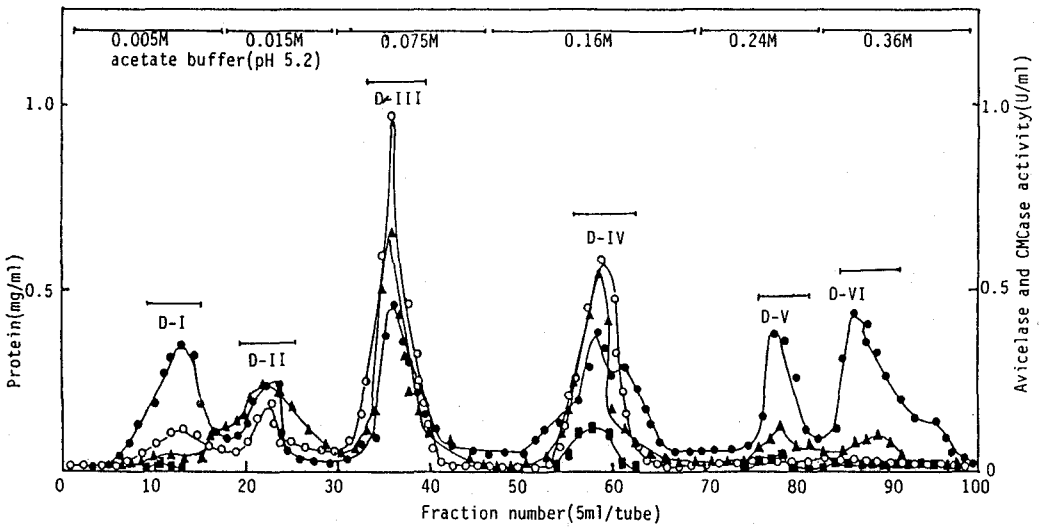

Fig. 5. Elution patterns of the H-II fraction from Bio-gel HTP chromatogram on DEAE-Sephadex A-50 column chromatography. The column (2.5×150 cm) was step-wise eluted with acetate buffer, pH 5.2, increasing from 5 mM to 360 mM at a flow rate of 12 $ml \cdot h^{-1}$. The fraction volumn was 4 *ml*. Symbols are the same as Fig. 3.

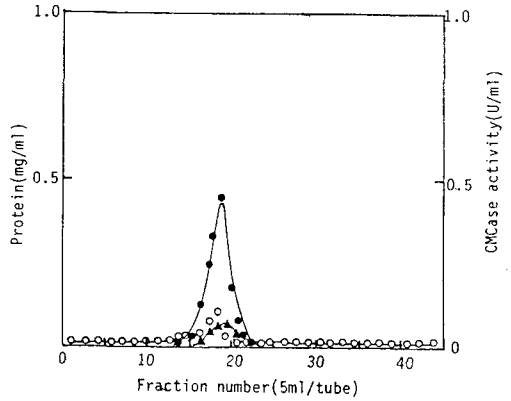

Fig. 6. Bio-gel P-100 chromatogram of D-I fraction from DEAE-Sephadex A-50 chromatography. The column (2.5×100 cm) was eluted with 20 mM citrate buffer, pH 5.2 at a flow rate of 10 $ml \cdot h^{-1}$. The fraction volume was 3 *ml*. Symbols are the same as Fig. 3.

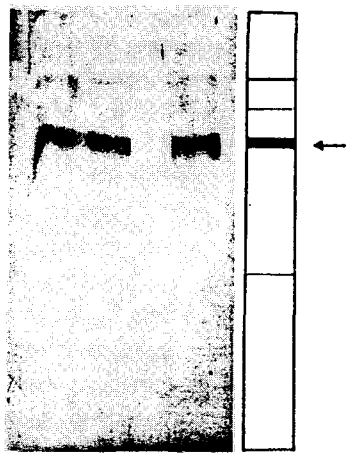

Fig. 7. Protein profiles differentiated by native gel electrophoresis. Effluent of Bio-gel P-100 single fraction was separated by native-PAGE. Arrow indicates band to be cut and extracted.

monstrated by SDS-PAGE (Fig. 8) and the specific activity of CMCase IV was 7.5 U/mg.

Characteristics of purified enzyme

Determination of molecular weight: Molecular weight of the CMCase IV was estimated to be approximately 52 KDa from the plot of the relative mobilities of the standard proteins *versus* their molecular weight by SDS-PAGE (Fig. 8).

pI value, carbohydrate content and amino acid composition: The CMCase IV was acidic protein

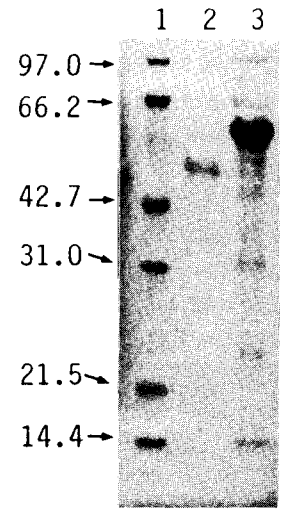

Fig. 8. Purity and estimation of mlecular weight of the purified enzyme, CMCase IV, by SDS-polyacrylamide gel electrophoresis. Lanes; 1, Mr markers (KDa); 2, purified CMCase IV; 3, crude enzyme from the fungal culture filtrate. Molecular weight markers: 1, Phosphorylase B (92,500); 2, Bovine serum albumin (66,200); 3, Ovalbumin (45,000); 4, Carbonic anhydrase (31,000); 5, Soybean trypsin inhibitor (21,500); 6, Lysozyme (14,400).

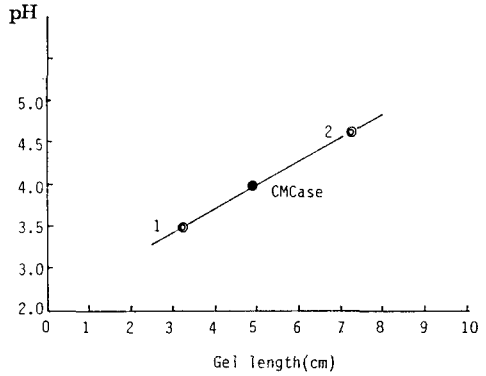

Fig. 9. Analytical isoelectrofocusing of purified CMCase IV on acrylamide gel. Proteins were focused for 2 h at 10℃ and 25 W constant power on slab gel containing 6.25% pharmalyte(pH range, 2.5-5.0) as a carrier ampholyte. pI markers: 1, Amyloglucosidase (3.55); 2, Trypsin inhibiter (4.6).

having pI value of 4.0 (Fig. 9) and glycoprotein containing carbohydrate of 13% as glucose. The enzyme was rich in acidic and neutral amino acid such as aspartic acid, glutamic acid, serine, and

Table 1. Amino acid composition of CMCase IV.

Amino acid	Molar ratio(%)
Cysteine	1.8
Methionine	2.0
Lysine	2.1
Histidine	1.8
Arginine	1.0
Aspartic acid	14.5
Threonine	8.4
Serine	8.5
Glutamic acid	13.0
Proline	6.5
Glycine	7.5
Alanine	6.4
Valine	6.0
Isoleucine	5.2
Leucine	4.7
Tyrosine	5.2
Phenylalanine	4.3

thereonine (Table 1).

Analysis of hydrolysates by TLC: The enzyme CMCase IV liberated glucose, cellobiose and cellooligosaccharides against CMC from the results of TLC (Fig. 10). However, this enzyme seemed to have transglycosylation activity by showing high contents of cellooligosaccharides in late stage of the reaction.

Substrate specificity of CMCase IV: The substrate specificity of CMCase IV were examined using cellulosic substrates such as filter paper, Avicel, phosphate swollen cellulose, and cellobiose. As shown in Table 2, the rate of hydrolysis of these substrate were in order filter paper>phosphate swollen cellulose>Avicel.

Some phycochemical properties of four CMCase components produced from *P. verruculosum* was summarized in Table 3. The optimum temperature and pH for the activity of CMCase IV were 50℃ and pH 5.0, respectively (data not shown). The enzyme was also stable in range of pH 4.5-5.5, and retained 80% original activity even on heating at 60℃ for 30 min (data not shown).

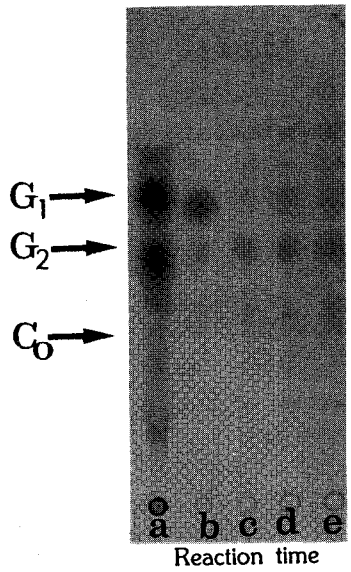

Fig. 10. Thin layer chromatogram of CMCase IV lysates against CM-cellulose. Ascending separation of products on Silica gel G-60 was carried out using n-butanol-isopropanol-water (v/v, 3:12:4) eluent. G_1; glucose, G_2; celobiose, C_6; cellooligosaccharides. Reaction times: a, 72 h; b, 48 h; c, 16 h; d, 8 h; e, 4 h.

Two endo-1,4-β-glucan glucanohydrolase (C_x enzyme) were purified from *T. viride* by Bergham *et al.* (1976), in which endoglucanase I had molecular weight of 12,500, pI of 4.65, and 21% of carbohydrate content, while endoglucanase II had molecular weight of 50,000, pI of 3.39, and 12% carbohydrate content. On the other hand, Okada (1975) purified two cellulase components derived from Meicelase, prepared from *T. viride*, of which molecular weight were 30,000 and 43,000, respectively. Both enzymes contained 12-14% carbohydrates as glucose and showed optimum pH range of 4.5-5.0. Five components of endo-1.4-β-glucanases were also purified from rot fungus *Sporotrichum pulverulentum* (*Chrysosporium lignorum*) by Eriksson *et al.* (1975). Molecular weight of the components were varied between 28,300 and 37,500. The pI values and carbohydrate contents were also varied from 4.20 to 5.32 and from 0% to 10.5 %, respectively. In addition, many fungal endoglucanases were reported, including *Aspergillus acu-*

Table 2. Degradation activity of purified CMCase IV toward different substrates

Enzyme	Specific activity (U/mg)							
	CMC	FP	Avicel	PSC	Salicin	Cellobiose	Xyln	Lamin
CMCase IV	7.60	1.10	0.37	2.57	—[a]	—	9.00	0.16

[a]Not detected.

Abbreviation: CMC, carboxymethyl cellulose; FP, filter paper; PSC, phosphate swollen cellulose; Xyln, xylan; Lamin, laminarin.

Table 3. Some properties of purified CMCase I and IV.

CMCase and EG	MW		Carbohydrate content (%)	Optimun	
	SDS-PAGE	Native		pH	Temp(℃)
CMCase I[a]	70,000	—[b]	8.5	5.0	50
EG II[c]	40,000	120,000	3.2	4-5	50-55
EG III[c]	58,000	140,000	7.7	4-5	50-55
CMCase IV	52,000	45,000	13.0	5.0	50

[a]Ref. Kim *et al.*, 1992a.
[b]Not determined.
[c]Ref. Kim *et al.*, 1992b.

leatus (MW, 25,000 and 38,000), *Fusarium lini* (MW, 28,000), *Pellicularia filamentosa* (MW, 26,000-68,000), *Lenzites trabea* (MW,29,000) and *T. koningii* (MW,22,000).

Immunoprecipitation

To identify proteins synthesized by CMCase mRNAs, the translation products were precipitated with each anti-CMCase IgG. The precipitants were also analyzed by fluorography. The fluorographic result indicated that the immunoprecipitated products exhibited radioactive band corresponding to molecular weight of purified CMCases estimated using several standard proteins (Fig.11). The immunoprecipitated protein, therefore, was proven immunologically and electrophoretically to be identical with purified CMCase.

Molecular biology of cellulase genes and their products have been extensively studied in recent year, and it is possible to understand the organization of cellulase genes, their regulation at the molecular level, and structural features required for enzyme activity. The strategy based on the efficient induction of cellulolytic enzymes has been used to isolate genes coding for both exo- and endoglucanases from filamentous fungi. After differential hybridization using cDNA probes syn-

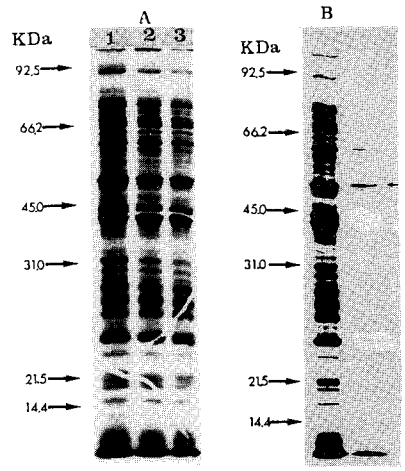

Fig. 11. Fluorograph of total polypeptides *in vitro* synthesized from purified poly(A^+) RNA and immunoprecipitant with CMCase IV.
A; Purified poly(A^+) RNA was *in vitro* translated in rabbit reticulocyte lysate and [^{35}S]methionine system, separated by SDS-PAGE (10%), and fluorographed. Protein concentrations: 1, 20 μg; 2, 10 μg; 3, 5 μg. B; In immunoprecipitation experiment, cell-free translation products of poly(A^+) RNA were bound with anti-CMCase IV IgG and fluorographed. Molecular weight markers: 1, Phosphorylase B(92,500); 2, Bovine serum albumin(66,200); 3, Ovalbumin(45,000); 4, Carbonic anhydrase(31,000); 5, Soybean trypsin inhibitor(21,500); 6, Lysozyme(14,400).

thesized from two distinct mRNAs; cellulase induced-mRNA and cellulase-repressed mRNA, the genes coding for several different endo- and exoglucanases were identified by hybrid selection of the corresponding mRNAs and subsequent immunoprecipitation of the proteins synthesized *in vitro*. Two cellobiohydrolase gene (*cbh*1 and *cbh*2) and two endoglucanase gene(*egl*1 and *egl*3) of *T. reesei* were sequenced and expressed in yeast (Chen, 1987; Penttila *et al.*, 1986, Saloheimo *et al.*, 1988; Shoemaker *et al.*, 1983; Teeri *et al.*, 1983; Van Arsdell *et al.*, 1987).

Four CMCase were purified from *P. verruculosum* to prepare CMCase-specific antibody for immunological screening in cDNA library (Kim *et al.*, 1992a; Kim *et al.*, 1992b). The components were not aggregagated in culture filtrate and they also seemed to have each different precursors in immunoprecipitation (Kim *et al.*, 1992a). The fluorographic result of cell-free translation product represented that almost full length of mRNAs were extracted from the fungus, because proteins with high molecular weight were synthesized by the mRNA, so that the mRNA might be good material for cDNA synthesis.

적 요

섬유소 분해균인 *P. verruculosum*의 배양 여액으로부터 endo형 cellulase 인 CMCase IV를 정제하였다. CMCase IV는 13%의 탄수화물과 4.0의 pI값을 가진 산성, 당단백질이었다. CMCase IV의 SDS-PAGE 상에서 분자량은 52 KDa이었으며, 효소 활성을 위한 최적 pH 및 온도는 5.0과 50℃ 였다. CMCase IV를 CMC에 반응시 대부분 glucose와 cellobiose가 생산되었으며, 또한 동시에 transglycosylation 작용을 함께 갖는 것으로 사료되었다. Cellulase 활성 염색법(zymogram)을 통해서 *P. verruculosum*의 cellulase component가 배지 내에서 aggregation 되어있지 않음을 알 수 있었다. *P. verruculosum* mRNA의 *in vitro* 번역을 통하여 CMCase IV를 coding하는 번역산물이 동정 되었다.

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