Purification and Some Properties of Exo- and Endo- β-1, 3-Glucanases from Irpex lacteus (Polyporus tulipiferae)

Kazumasa WAKABAYASHI

(Received February 1, 1988)

Summary

Two major components of β-1, 3-glucanase were purified by gel filtration and ion exchange chromatography from Driselase, a commercial enzyme preparation from *Irpex lacteus (Polyporus tulipiferae)*. The purified enzymes were almost homogeneous, as judged through polyacrylamide gel disc electrophoresis. The two pure enzymes were designated as β-1, 3-glucanases L-I and -IV.

β-1, 3-Glucanases L-I and -IV were purified approximately 55- and 47-fold from the starting solution, and their molecular weights were estimated to be 58,000 and 28,000 by gel filtration on Bio-gel P-100, respectively. The two enzymes are different from each other with respect to enzymatic properties in pH and thermal stability, and those in pH and thermal optima for pachyman hydrolysis. The activities of β-1, 3-glucanases L-I and -IV are significantly inhibited by Ag^{+}, Co^{++}, Cu^{++}, Mn^{++}, Pb^{++}, or Zn^{++}, while their susceptibilities to the same metal ion are somewhat different from each other. Both the enzymes are completely inhibited by Hg^{++}.

β-1, 3-Glucanase L-IV rapidly decreases the viscosity of solution of CM-pachyman, whereas β-1, 3-glucanase L-I practically allows release of reducing sugars. β-1, 3-Glucanase L-IV attacks pachyman at random, producing smaller oligosaccharides. The enzyme attacks also laminari-oligomers at random, except for laminaribiose. On the other hand, β-1, 3-glucanase L-I produces only glucose from pachyman. These results indicate that β-1, 3-glucanases L-I and -IV are the typical exo- and endo- β-1, 3-glucanases, respectively.

Introduction

β-1, 3-Glucanases (E. C. 3. 2. 1. 6) are widely distributed in fungi, bacteria, higher plants, animals, and so on. Consequently, studies on them have been made extensively by many workers. In general, β-1, 3-glucanases play an important role in the degradation of yeast and fungal cell walls. Purification of such kinds of enzymes has been of interest for their use in the structural analysis of related polysaccharides and the preparation of yeast protoplasts.

It has been reported that *Irpex lacteus (Polyporus tulipiferae)*[1] produced a large

quantity of β−1, 3−glucanase in a medium. However, no studies have so far been reported on enzymatic properties of β−1, 3−glucanase from *Irpex lacteus* in its highly purified state, although cellulases[2-12] and polygalacturonases[13] from this fungus have been investigated in detail.

During a work with column chromatography on the purification of β−1, 3−glucanase from this fungus using Driselase, a commercial cellulase preparation obtained from *Irpex lacteus*, the author noticed that the fungus produced at least four kinds of β−1, 3−glucanase components differing from one another in chromatographic properties.

This paper describes the purification of two major β−1, 3−glucanase components of them found in preparation of *Irpex lacteus* and, some properties and modes of action of these purified β−1, 3−glucanases.

Materials and Methods

Enzyme Source. Driselase, a commercial enzyme preparation from *Irpex lacteus*, supplied by Kyowa Hakko Co., Ltd., was used as the starting material for purification of β−1, 3−glucanases.

Chemicals and Reagents. Pachyman was prepared from air−dried *Bukuryo (Poria cocos)* following the method described by Saito *et al.*[14] Sodium carboxymethyl pachyman (CM−pachyman, DS = 0.4−0.6) was prepared from pachyman by the method of Clark and Stone.[15] Laminari−oligosaccharides (G_2−G_5) were prepared from *Bukuryo (Poria cocos)* according to the method described by Whelan.[16] The preparations were found to be pure on inspection by paper chromatography. Sodium carboxymethyl cellulose (CMC, DS = 0.63) was obtained from Daiichi Industrial Pharmaceutical Co., *p*−nitrophenyl β−D−glucoside (PNPG) from Nakarai Chemical Co., and xylan from Tokyo Kasei Chemical Co., DEAE−Sephadex A−50 was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden), and Bio−gel P−100 from Bio−Rad Laboratories (Calif., U. S. A.). The other chemicals used were of reagent grade, commercially available.

Enzyme Assays and Analytical Methods. *a) Pachyman−Saccharifying Activity.* A reaction mixture (2 ml), consisting of 0.125% pachyman, 0.05 M sodium acetate buffer (pH 5.0), and enzyme, was incubated at 30°C for a given period (10 min in most cases). The reducing power produced in the reaction mixture was determined by the method of Somogyi[17] and Nelson.[18] One unit of β−1, 3−glucanase is defined as the amount of enzyme which produces a reducing power from the substrate equivalent to 1μ mol of glucose per min.

b) CMC− and Xylan−Saccharifying Activities. Assays were carried out under the same conditions as used for the pachyman−saccharifying activity, except for the use of 1% CMC or xylan in place of 0.5% pachyman.

c) β−Glucosidase Activity. The reaction mixture used consisted of 0.1 ml of 0.048 M PNPG, 0.2 ml of 0.1 M sodium acetate buffer (pH 5.0), and 0.1 ml of enzyme solution.

After incubation at 30°C for 10 min, 3 ml of 1% Na_2CO_3 was added to the mixture, and the *p*-nitrophenol liberated was estimated from the absorbance at 420 nm.

d) Measurement of Viscosity Decrease of CM-Pachyman. The reaction mixture used contained 0.25% CM-pachyman, 0.05 M sodium acetate buffer, pH 5.0, and the enzyme in a total volume of 6 ml. The mixture was incubated at 30°C in an Ostwald viscometer, and the decrease in relative viscosity (η_{rel}) was measured at intervals, and the reciprocal specific viscosity ($1/\eta_{sp}$) was calculated from the measurement, where $\eta_{sp} = \eta_{rel} - 1$ and $\eta_{rel} = t/t_0$, t and t_0 being the flow times of the CM-pachyman solution and the solvent, respectively.

Determination of Protein. The protein in a reaction mixture was determined by the method of Lowry *et al.*[19] or from the absorbance at 280 nm, using crystalline bovine serum albumin (from Miles Laboratories, Inc.) as a standard.

Disc Electrophoresis on Polyacrylamide Gel. Polyacrylamide gel electrophoresis was carried out in glycine-Tris buffer at pH 9.5 as determined according to the procedure described by Ornstein[20] and Davis.[21] A sample containing about 10 μg of protein was applied on the top of the gel and then subjected to an electrophoresis at 3 mA/gel. Gels were stained for enzyme protein with 0.05% coomassie brilliant blue R250.

Paper Chromatography. Hydrolysis products produced by the enzyme were detected by paper chromatography. After an appropriate period of incubation, aliquots of enzymatic hydrolyzate of substrates and solution of authentic sugars used as the standard were spotted individually on a Whatman No. 1 filter paper and chromatograms were obtained after development by the ascending technique with *n*-butanol : pyridine : water (6 : 4 : 3, v/v) at room temperature for an appropriate period. Spots of reducing sugar on the paper chromatogram were detected by the dipping procedure using silver nitrate reagents.[22]

Results

Purification of β-1, 3-Glucanase Components.

Step 1. First DEAE-Sephadex A-50 Column Chromatography.

A crude enzyme solution obtained from Driselase powder (50 g) was applied on a DEAE-Sephadex A-50 column (acetate form) previously equilibrated with 0.02 M ammonium acetate buffer (pH 5.0). Elution was carried out stepwise with 0.02, 0.1, and 0.2 M ammonium acetate buffer at the same pH. Each fraction was tested for β-1, 3-glucanase, β-glucosidase, CMCase, xylanase activities, and protein content. The results are shown in Fig. 1. Four peak fractions L-1 to -4, having pachyman-saccharifying activities were obtained, which roughly overlapped the main peaks of protein, except for peak L-4. Peak fractions L-1 and -4, which are more active than the other two toward pachyman, were subjected to further purification. These two fractions were each brought to 80% saturation by addition of solid ammonium sulfate with stirring. They were allowed to stand at room temperature overnight, and the

resulting precipitates of L−1 and −4 were each collected by centrifugation, dissolved in a small volume of water, dialyzed against tap water with a membrane bag, and lyophilized.

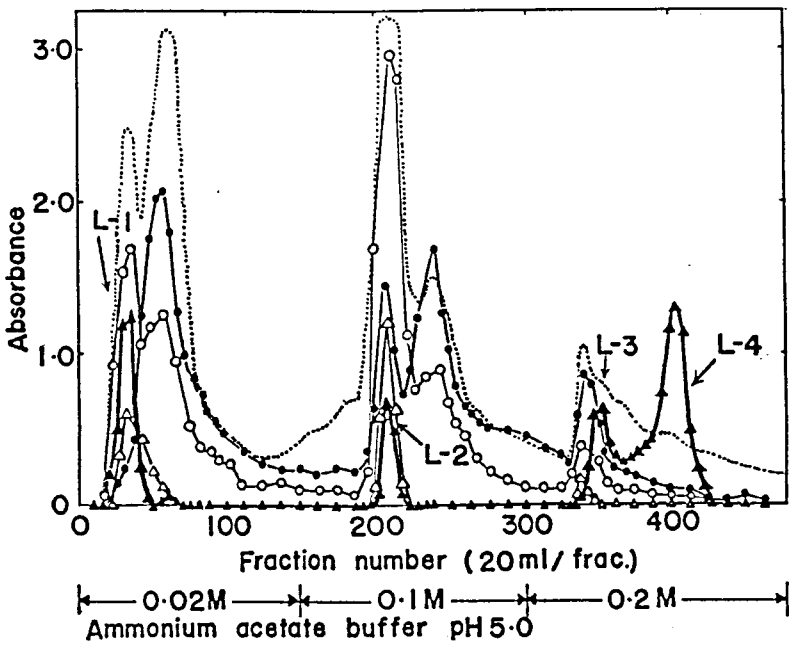

Fig. 1. Elution pattern of the crude enzyme preparation from a DEAE-Sephadex A-50 column.
−▲−, pachyman-saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5-fold; −●−, CMC-saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5-fold; −○−, xylan-saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5-fold; −△−, β-glucosidase activity (10 min incubation, A_{420}) of eluates diluted 2-fold; ·····, protein (A_{280}). Column, 5.0 × 40 cm; flow rate, 60 ml/h; fraction size, 20 ml.

Purification of L−1 Component.

Step 2a. First Bio-gel P-100 Gel Filtration. An aqueous solution of L−1 obtained above was applied on a Bio-gel P−100 column, previously equilibrated, with 0.1 M ammonium acetate buffer (pH 5.0), and eluted with the same buffer. As shown in Fig. 2, a single peak fraction (L−1−1) with a pachyman-saccharifying activity, contaminated with activities of xylanase and CMCase, was obtained. This fraction was concentrated in a collodion bag with suction and then lyophilized.

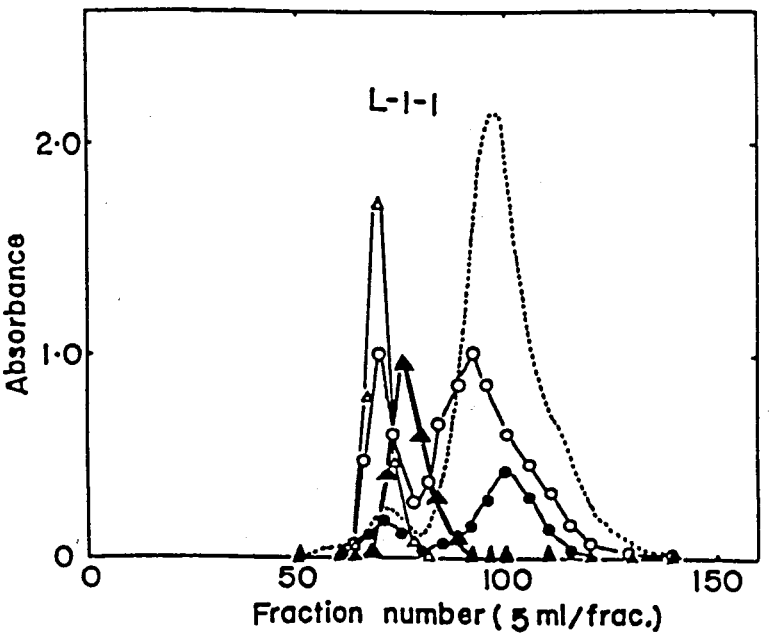

Fig. 2. Elution pattern of L-1 on a Bio-gel P-100 column.
—▲—, pachyman-saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5-fold; —●—, CMC-saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5-fold; —○—, xylan-saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5-fold; —△—, β-glucosidase activity (10 min incubation, A_{420}) of eluates diluted 2-fold; ·····, protein (A_{280}). Column, 2.0 × 45 cm; flow rate, 13 ml/h; fraction size, 5 ml.

Step 3a. Second DEAE-Sephadex A-50 Column Chromatography. Fraction L-1-1 was again subjected to a DEAE-Sephadex A-50 column chromatography under the same conditions with the first DEAE-Sephadex A-50 chromatography, except for the column size. As shown in Fig. 3, a pachyman-saccharifying activity fraction was eluted as a sigle peak (L-1-1-1) which corresponded to a protein peak. This fraction was concentrated and lyophilized in the same way as described above.

Step 4a. Second Bio-gel P-100 Gel Filtration. In an attempt to remove the accompanying xylanase, fraction L-1-1-1 was again subjected to a gel filtration under the same conditions with the first Bio-gel P-100 Gel filtration, except for the column size. Fig. 4 shows the profile of the enzyme elution from the column. The peak with a pachyman-saccharifying activity overlapped with the protein one. However, xylanase was not fully eliminated from this peak fraction. The fractions Nos. 14 to 18 having pachyman-saccharifying activities in Fig. 4 were combined and concentrated as fraction L-1-1-1-1 by use of a collodion bag.

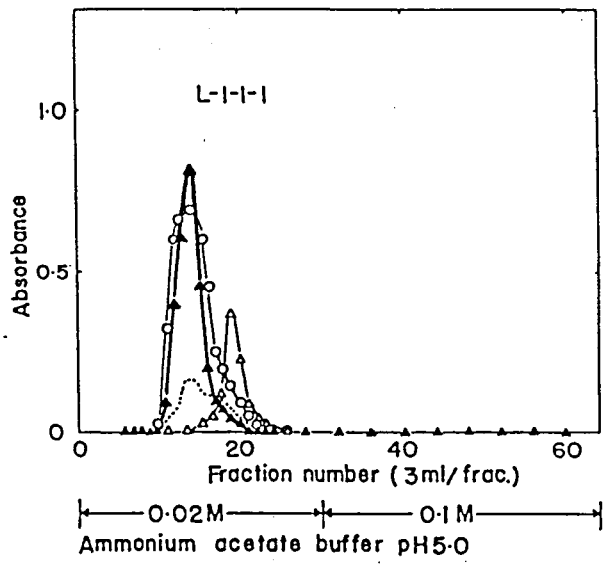

Fig. 3. Elution pattern of L-1-1 on a DEAE-Sephadex A-50 column. —▲—, pachyman-saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5-folde; —○—, xylan-saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5-fold; —△—, β-glucosidase activity (10 min incubation, A_{420}) of eluates diluted 2-fold; ·····, protein (A_{280}). Column, 2.0 × 27 cm; flow rate, 7.5 ml/h; fraction size, 3 ml.

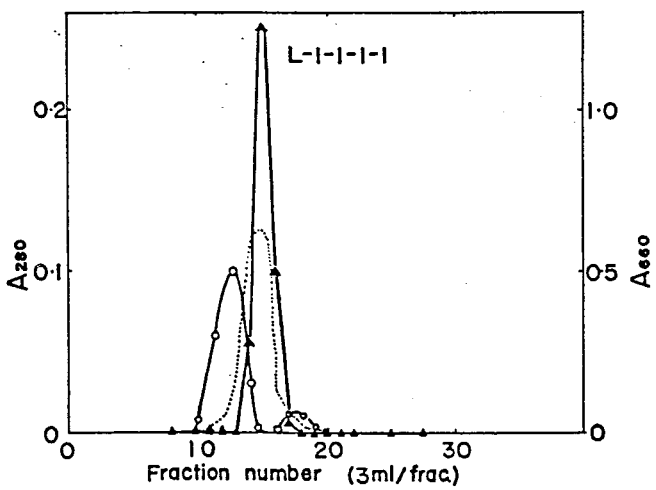

Fig. 4. Elution pattern of L-1-1-1 on a Bio-gel P-100 column. —▲—, pachyman-saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5-fold; —○—, xylan-saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5-fold; ·····, protein (A_{280}). Column, 1.0 × 137 cm; flow rate, 3.0/h; fraction size, 3 ml.

Step 5a. Third Bio-gel P-100 Gel Filtration. Fraction L-1-1-1-1 was further subjected to a gel filtration on a Bio-gel P-100 column under the same conditions as described in Step 2a, except for the column size. As shown in Fig. 5, a single peak fraction with a pachyman-saccharifying activity coincided entirely with the protein peak, suggesting its homogeneity. This fraction was then lyophilized after concentration and named L-I.

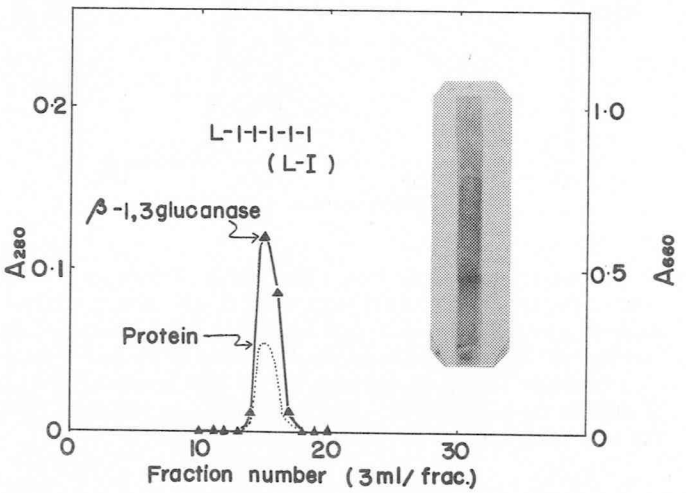

Fig. 5. Elution pattern of L-1-1-1-1 on a Bio-gel P-100 column, and disc electrophoresis of L-I on polyacrylamide gel.
—▲—, pachyman-saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5-fold; ·····, protein (A_{280}). Column, 1.0 × 131 cm; flow rate, 3 ml/h; fraction size, 3 ml. The disc electrophoresis of the purified enzyme (L-I) was carried out by the method of Ornstein[20] and Davis;[21] as described in the "Materials and Methods" section.

Purification of L-4 Component.

Step 2b. First Bio-gel P-100 Gel Filtration. As aqueous solution of L-4 obtained in Step 1 was subjected to a gel filtration with a Bio-gel P-100 column in the same manner as described in Step 2a, except for the column size. The results are shown in Fig. 6. A pachyman-saccharifying activity fraction was eluted as a broad peak (L-4-1), accompanied by CMCase and xylanase activity components. This peak fraction was concentrated by use of a collodion bag and then lyophilized.

Step 3b. Second DEAE-Sephadex A-50 Column Chromatography. Fraction L-4-1 was again subjected to a DEAE-Sephadex A-50 column chromatography under the same conditions with the first DEAE-Sephadex A-50 column chromatography, except for the column size. As shown in Fig. 7, a single broad peak fraction with a pachyman-saccharifying activity, overlapping with the protein was obtained.

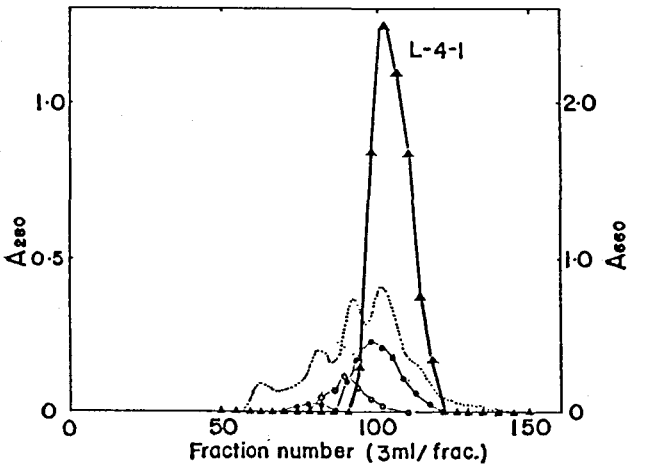

Fig. 6. Elution patern of L–4 on a Bio–gel P–100 column. –▲–, pachyman–saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5–fold; –●–, CMC–saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5–fold; –○–, xylan–saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5–fold; ·····, protein (A_{280}). Column, 2.7 × 130 cm; flow rate, 15 ml/h; fraction size, 3 ml.

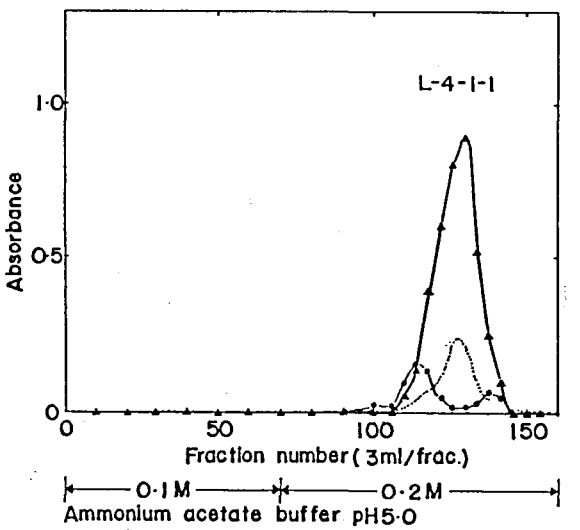

Fig. 7. Elution pattern of L–4–1 on a DEAE–Sephadex A–50 column. –▲–, pachyman–saccharifying activity (10 min incubation, A_{660}) of eluates diluted 10–fold; –●–, CMC–saccharifying activity (10 min incubation, A_{660}) of eluates diluted 5–fold; ·····, protein (A_{280}). Column, 2.0 × 30 cm; flow rate, 9 ml/h; fraction size, 3 ml.

This peak fraction (L-4-1-1) was practically free from xylanase but still contaminated with a reduced quantity of CMCase. The fraction was lyophilized after concentration in the same way as described before.

Step 4b. Second Bio-gel P-100 Column Chromatography. An aqueous solution of L-4-1-1 obtained above was again subjected to a gel filtration on a Bio-gel P-100 column in the same way as described in Step 2b, except for the column size. As shown in Fig. 8, a single peak fraction with a pachyman-saccharifying activity coincided fully with the protein peak, suggesting its homogeneity. After concentration, it was lyophilized and named L-IV.

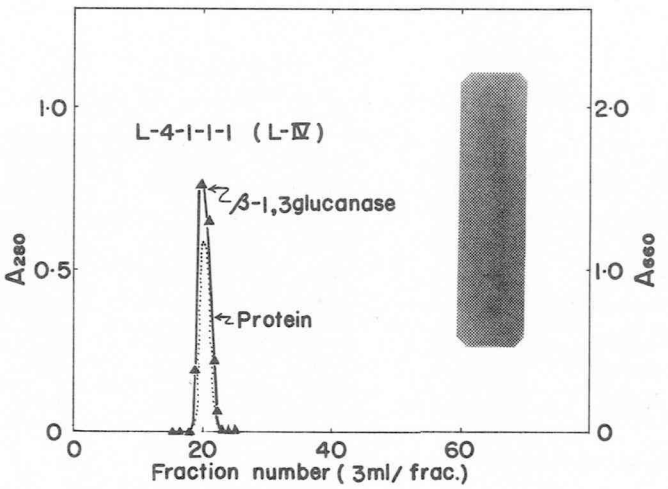

Fig. 8. Elution pattern of L-4-1-1 on a Bio-gel P-100 column and disc electrophoresis of L-IV on polyacrylamide gel.
—▲—, pachyman-saccharifying activity (10 min incubation, A_{660}) of eluates diluted 10-fold; ·····, protein (A_{280}). Column, 1.0 × 135 cm, flow rate, 3 ml/h; fraction size, 3 ml. The disc electrophoresis of the purified enzyme (L-IV) was carried out under the same conditions as in Fig. 5.

The final preparations of L-I and -IV gave each a single protein band on disc gel electrophoresis at pH 9.5 (Figs. 5 and 8). Tables 1 and 2 summarize the specific activities and recoveries during the purification course for L-I and -IV from the starting solution. The specific activities of L-1 and -IV toward pachyman were increased approximately 55- and 47-fold over those of the starting solutions, respectively.

Table 1. Recoveries and activities of L-I during the purification steps.

Purification step	Volume (ml)	Protein (mg)	Total units	Specific activity[a] (units/mg)	Recovery (%)
Starting solution	245	7640	1508	0.197	(100)
1st DEAE-Sephadex	335	442	150.0	0.339	10
$(NH_4)_2SO_4$ fractionation	18.3	188	74.8	0.398	5.0
1st Bio-gel P-100	40.5	5.4	10.4	1.93	0.7
2nd DEAE-Sephadex	10.6	1.7	7.6	4.47	0.5
2nd Bio-gel P-100	5.4	0.6	4.8	8.00	0.3
3rd Bio-gel P-100	5.0	0.3	3.2	10.7	0.2

a) Specific activity is defined as activity units/mg of enzyme protein. One unit is the pachyman-saccharifying activity which produces a reducing power equivalent to 1.0 μmol of glucose in one min under the reaction conditions employed in the present work.

Table 2. Recoveries and activities of L-IV during the purification steps.

Purification step	Volume (ml)	Protein (mg)	Total units	Specific activity[a] (units/mg)	Recovery (%)
Starting solution	28.0	15200	1896	0.125	(100)
1st DEAE-Sephadex	435	204	154.8	0.759	8.2
$(NH_4)_2SO_4$ fractionation	8.0	57.1	111.6	1.95	5.9
1st Bio-gel P-100	39.0	14.8	39.6	2.68	2.1
2nd DEAE-Sephadex	30.0	6.8	28.8	4.24	1.5
2nd Bio-gel P-100	6.0	3.8	22.4	5.89	1.2

a) Specific activity and one unit are the same as in Table 1.

Molecular Weight by Gel Filtration. L-I and -IV were each applied on a Bio-gel P-100 gel column equilibrated with 0.1 M ammoniun acetate buffer (pH 5.0). Elution was carried out with the same buffer. Bovine serum albumin (M. W. 67,000), ovalbumin (M. W. 43,000), chymotrypsinogen A (M. W. 25,000), and ribonuclease A (M. W. 13,700), were used as standards. The void volume was determined using blue dextran 2,000. The molecular weights of L-I and -IV were estimated to be 58,000 and 28,000, respectively (Fig. 9).

Effects of Metal Ions and Inhibitor. Effects of metal ions on the enzyme activity were examined at a concentration of 0.2 mM of various metal compounds under the standard conditions. As shown in Table 3, the activities of β-1, 3-glucanases L-I and -IV are significantly inhibited by Ag^+, Co^{++}, Cu^{++}, Mn^{++}, Pb^{++}, or Zn^{++}, while their susceptibilities to the same metal ion are somewhat different from each other.

The enzyme activities were a little affected by the presence of metal chelater (EDTA). Both the enzymes were completely inhibited by Hg^{++}.

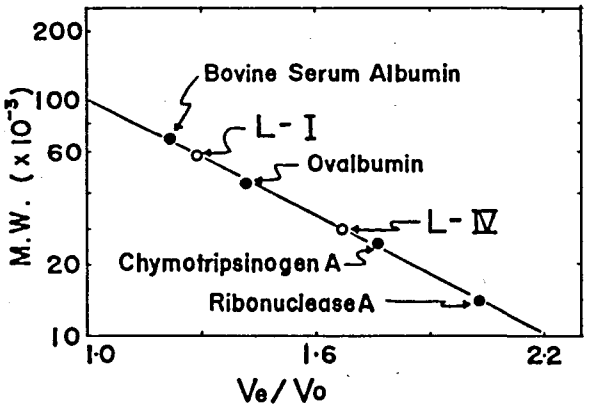

Fig. 9. Determination of the molecular weights of two purified enzymes, L-I and -IV, by Bio-gel P-100 gel filtration.
Ve and *Vo* are the elution volume and void volume, respectively.
Column, 1.0 × 145 cm; flow rate, 3 ml/h; fraction size, 3 ml; elution buffer, 0.1 M ammonium acetate buffer, pH 5.0.

Table 3. Effect of metal ions on enzyme activity.[a)]

Metal ion	Conc. (mM)	Residual activity (%) L-I	L-IV
None	—	100	100
$AgNO_3$	2.0	72	52
$AlCl_3$	2.0	82	83
$BaCl_2$	2.0	82	96
$CoCl_2$	2.0	68	73
$CuSO_4$	2.0	47	40
$FeSO_4$	2.0	89	89
$HgCl_2$	2.0	0	0
KCl	2.0	76	99
$MgSO_4$	2.0	76	77
$MnSO_4$	2.0	27	59
NaCl	2.0	95	91
$Pb(AcO)_2$	2.0	79	69
$ZnCl_2$	2.0	61	67
EDTA	2.0	82	87
Tris	2.0	95	94

a) Enzyme activity was determined as the pachyman-saccharifying activity.

Effects of pH and Temperature on Enzyme Activity and Stability. The pH dependence of the activity of the purified enzyme was studied under the standard assay conditions with Britton-Robinson's wide range buffers (pH 2 to 8). As shown in

Fig. 10, the optimal pHs of L–I and –IV are 6.0 and 5.0, respectively. The effect of pH on the stability of the enzymes was examined after preincubation for 24 hr at 30°C at various pH values. Remaining activities were then assayed at pH 5.0. The L–I activity was stable in the relatively wide pH range from 2.0 to 8.0, while the L–IV activity was stable only in the narrow range of pH 3.0 to 7.0. The effect of temperature on the stability was studied by heating the enzymes at various temperatures for 10 min in acetate buffer (pH 5.0), cooling them quickly in ice water, and determining residual activities at 30°C by measuring the increase in the amount of reducing sugar. The optimum temperature and heat stability of each enzyme activity are shown in Fig. 11. The L–I and –IV activities showed maxima at 50° and 60°C, respectively. The enzyme activities are stable at temperatures lower than 40°C but L–I loses its activity remarkably at 60°C under the conditions employed.

Viscometric Assay of L–I and –IV Activities. The relationship between the decrease in the viscosity of CM–pachyman, expressed as the increase in reciprocal specific viscosity ($1/\eta_{sp}$), and the increase in the reducing power of the reaction mixture caused by L–I or –IV was examined under the standard conditions, as shown in Fig. 12. The slope of the graph for L–IV is steep, while that for L–I is almost horizontal.

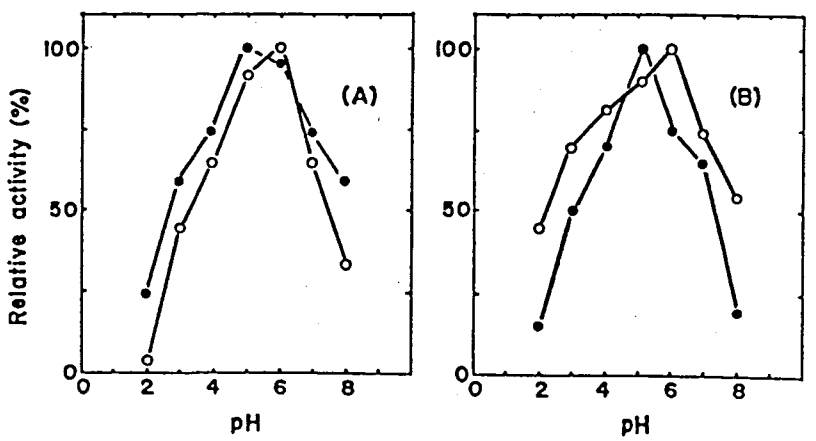

Fig. 10. Optimum pH and pH–stability curves for the pachyman–saccharifying activities of two purified enzymes.
(A), optimum pH; (B), pH–stability.
–○–, L–I; –●–, L–IV.

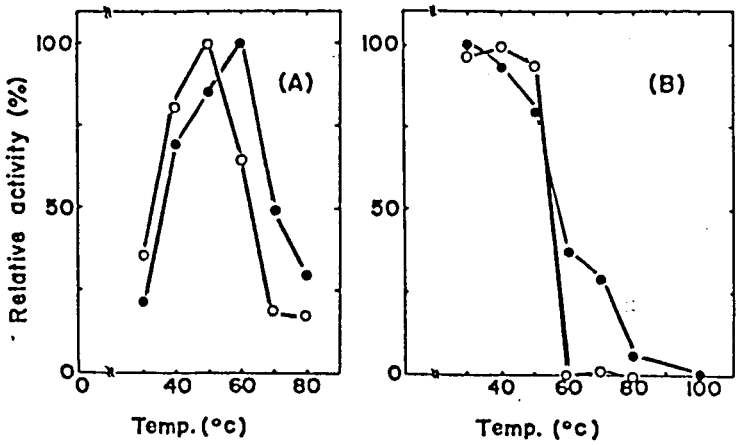

Fig. 11. Optimum temperature and thermal-stability curves for the pachyman-saccharifying activities of two purified enzymes. (A), optimum temperature; (B), thermal-stability. —○—, L-I; —●—, L-IV.

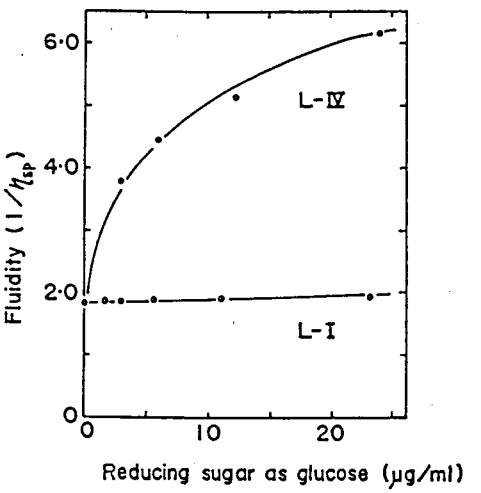

Fig. 12. Relationship between the increase in fluidity and the increase in reducing power during hydrolysis of CM-pachyman (DS = 0.4) by L-I or -IV.

Hydrolysis Products from Pachyman. Hydrolysis of pachyman by L—I or —IV was first carried out under the standard conditions, and the products were identified by paper chromatography. Fig. 13 shows paper chromatograms in the time-course of hydrolysis of pachyman by the L-I or —IV. The products formed during the entire course of hydrolysis by L-I were only glucose. On the other hand, in the earlier stage for L-IV it was hydrolyzed into low saccharides ranging from glucose to

pentaoligo-saccharides in which laminaribiose appeared to be most dominant. The laminaritriose, -tetraose, and -pentaose were decomposed gradually with prolonged incubation, and glucose and laminaribiose supposedly remained as the end products.

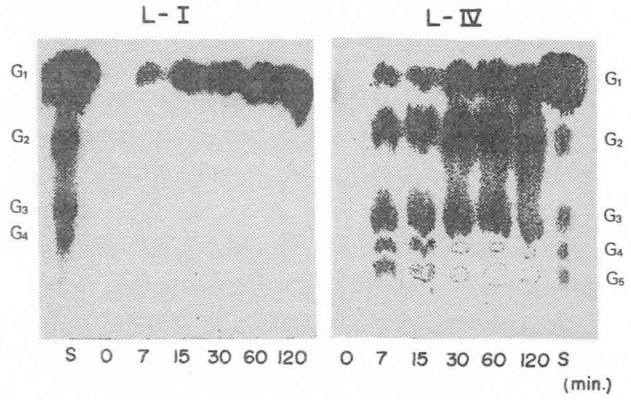

Fig. 13. Paper chromatograms showing products formed in course of hydrolysis of pachyman by two purified enzymes.
S, standard; G_1, glucose; G_2, laminaribiose; G_3, laminaritriose; G_4, laminaritetraose; G_5, laminaripentaose.
Development time, 24h.

Discussion

In the present study, two β-1, 3-glucanase fractions (L-I and -IV) were obtained from Driselase, a commercial product of *Irpex lacteus (Polyporus tulipiferae)*, through several steps of purification such as salting out with ammonium sulfate, gel filtration, and chromatography on Sephadex ion exchanger. These β-1, 3-glucanases L-I and -IV were purified approximately 55- and 47-fold from their initial preparations, respectively. The purified enzymes were almost homogeneous on polyacrylamide disc electrophoresis.

The approximate average molecular weights of the two β-1, 3-glucanases which were estimated by the gel filtration method, were considerably different from each other (58,000 and 28,000 for L-I and -IV, respectively).

The activities of β-1, 3-glucanases L-I and -IV were significantly inhibited by Ag^+, Co^{++}, Cu^{++}, Mn^{++}, Pb^{++}, or Zn^{++}. The enzyme L-I was more susceptible to Mn^{++} than L-IV, while their susceptibilities to Ag^+ were in the reverse relationship. Metal chelater (EDTA) had no significant effect on the enzyme reactions. Both the enzymes were completely inhibited by Hg^{++}.

The two enzymes are different from each other with respect to enzymatic properties in pH and thermal stability, and those in pH and thermal optima for pachyman hydrolysis.

β-1, 3-Glucanase L-IV of this fungus causes a rapid decrease in viscosity of

CM-pachyman, with an increase in the simultaneous production of reducing power in the reaction mixture. On the other hand, β-1, 3-glucanase L-I causes little decrease in the viscosity, although the reducing power in the mixture increases (Fig. 12). From this relationship between the increase in fluidity ($1/\eta_{sp}$) and the production of reducing power during the hydrolysis, it is clear that the hydrolysis of CM-pachyman by L-IV is random, whereas that by L-I is of exo-action. L-I and -IV also hydrolyzed pachyman rapidly, with reducing sugars as products. Moreover, the paper chromatographic analysis of the reaction products (Fig. 13) showed that L-IV attacked at random the inner glycosidic bond of pachyman, producing smaller-oligosaccharides at early stages. The products formed from pachyman after 2 hr incubation by L-IV, were identified mainly with laminaritriose, -biose, and glucose. The laminaritriose was decomposed gradually with prolonged incubation into laminaribiose and glucose, but laminaribiose was not further hydrolyzed. On the other hand, the analysis showed that the products in the entire course of pachyman hydrolysis by L-I were almost all glucose. From these results, it is concluded that L-I may be classified into an exo-β-1, 3-glucanase, and L-IV into an endo-β-1, 3-glucanase of a smaller oligo-saccharides-producing type.[23-25]

The endo-β-1, 3-glucanase (L-IV) from this fungus is capable of transfer action, like enzymes from *Rhizopus arrhizus*[26] and *Rizoctonia solani*[27] which have a significant transglycosylase activity. The action patterns of the exo- and endo-β-1, 3-glucanases (L-I and -IV) from *Irpex lacteus* on some polysaccharides such as laminarin, barley glucan, and lickenan will be further reported in detail.

The β-1, 3-glucanase of this fungus seems to be composed of at least four kinds of β-1, 3-glucanase components (Fig. 1), although two (L-2 and -3) of them have not yet been isolated in highly purified form. The existence of similar multiple components in this enzyme complex has already been reported for *Alternaria solani*[28] and *Flavobacterium dormitator*.[29] However, it is not clear at present whether all the β-1, 3-glucanase components of *Irpex lacteus* are formed *in vivo* under individual genetic control or whether only some of the components are produced by secondary modification under the action of some factors discussed on cellulase components from *Trichoderma viride* by Nakayama *et al.*[30]

Acknowledgments. The author wishes to thank Kyowa Hakko Co., Ltd. for the generous gift of Driselase. The author is also grateful to Messrs. Ken Kobayashi and Koichi Tabuchi, Facult. of Eng., Shinshu Univ., for their cooperation to this work.

References

1) M. Kawai, *Agric. Biol. Chem.* (in Japanese), **47**, 473 (1973).
2) K. Wakabayashi and K. Nisizawa, *J. Ferment. Technol.* (in Japanese), **42**, 347 (1964).
3) K. Wakabayashi, T. Kanda, and K. Nisizawa, *J. Ferment. Technol.* (in Japanese), **43**, 739 (1965).
4) K. Wakabayashi, T. Kanda, and K. Nisizawa, *J. Ferment. Technol.* (in Japanese), **44**, 669 (1966).

5) T. Kanda, K. Wakabayashi, and K. Nisizawa, *J. Ferment. Technol.* (in Japanese), 48, 607 (1970).
6) K. Nisizawa, Y. Tomita, K. Kanda, H. Suzuki, and K. Wakabayashi, *Proc. IV IFS: Ferment. Technol., Today,* 719 (1972).
7) T. Kanda, K. Wakabayashi, and K. Nisizawa, *J. Biochem.,* **79**, 977, 989, and 997 (1976).
8) K. Nisizawa, T. Kanda, S. Shikata, and K. Wakabayashi, *J. Biochem.,* **83**, 1625 (1978).
9) T. Kanda, S. Nakakubo, K. Wakabayashi, and K. Nisizawa, *J. Biochem.,* **84**, 1217 (1978).
10) T. Kanda, S. Nakakubo, K. Wakabayashi, and K. Nisizawa, *Advances in Chemistry Series,* No. **181**, 211 (1979) (Ross D. Brown, Jr. and Lubo Jurasek, editors), American Chemical Society, Washington, D. C.
11) T. Kanda, K. Wakabayashi, and K. Nisizawa, *J. Biochem.,* **87**, 1625 and 1635 (1980).
12) T. Kanda, I. Noda, K. Wakabayashi, and K. Nisizawa, *J. Biochem.,* **93**, 787 (1983).
13) K. Wakabayashi, T. Kanda, and H. Fuchu, *J. Faculty of Eng., Shinshu Univ.,* No. **57**, 11 (1985).
14) H. Saito, A. Misaki, and T. Harada, *Agric., Biol. Chem.,* **32**, 1261 (1968).
15) A. E. Clarke and B. A. Stone, *Phytochemistry* **I**, 175 (1962).
16) W. J. Whelan, *Methods in Carbohydrate Chemistry,* **I**, 330 (1962).
17) M. Somogyi, *J. Biol. Chem.,* **195**, 19 (1952).
18) N. Nelson, *J. Biol. Chem.,* **153**, 375 (1944).
19) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.,* **193**, 265 (1951).
20) L. Ornstein, *Ann. N. Y. Acad. Sci.,* **121**, 321 (1964).
21) B. J. Davis, *Ann. N. Y. Acad. Sci.,* **121**, 404 (1964).
22) W. E. Trevelyan, D. P. Procter, and J. S. Harrison, *Nature,* **166**, 444 (1950).
23) S. Nagasaki, K. Saito, and S. Yamamoto, *Agric. Biol. Chem.,* **41**, 493 (1977).
24) Y. Iwamuro, M. Aoki, and Y. Mikami, *J. Ferment. Technol.,* **63**, 61 (1985).
25) S. Kusama, I. Kusakabe, and K. Murakami, *Agric. Biol. Chem.,* **50**, 1101 (1986).
26) D. R. Clark, J. Johnson, Jr., K. H. Chung, and S. Kirkwood, *Carbohydr. Res.,* **61**, 457 (1978).
27) A. Totsuka and T. Usui, *Agric. Biol. Chem.,* **50**, 543 (1986).
28) S. Yamato, T. Yadomae, and T. Miyazaki, *J. Ferment. Technol.,* **52**, 706 (1974).
29) H. Mori, S. Yamamoto, and S. Nagasaki, *Agric., Biol. Chem.,* **41**, 611 (1977).
30) M. Nakayama, Y. Tomita, H. Suzuki, and K. Nisizawa, *J. Biochem.,* **79**, 955 (1976).