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Conversion of Water-insoluble Components of the Basidiocarps of *Ganoderma lucidum* to Water-soluble Components by Hydrolyzing with Chitinase

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We investigated the optimum conditions for conversion of water-insoluble components of basidiocarps of *Ganoderma lucidum* to water-soluble components by hydrolyzing with chitinase. We also tried it with *Ganoderma lucidum* residue remaining after extracting hot water-soluble components of *Ganoderma lucidum*. After hydrolyzing under optimum conditions (20 ppm chitinase, 2% *Ganoderma lucidum* or 6% *Ganoderma lucidum* residue, at pH 3 and at 35°C), the contents of total water-soluble components (polysaccharide or protein) were measured, and it was found that the contents of water-soluble components increased to 1.5~2.7 fold. Michaelis constant, K_m and maximum rate, V_{max} calculated by Lineweaver-Burk plot for hydrolysis of *Ganoderma lucidum* were 1.75% and 0.02%/min respectively and those for hydrolysis of *Ganoderma lucidum* residue were 53.15% and 0.53%/min respectively. The protein-bound polysaccharide was isolated after hydrolysis and molecular weights were measured by Sepharose CL-4B gel filtration and compared with the molecular weights of polysaccharide before hydrolysis.

Key words : *Ganoderma lucidum*, Chitinase, Hydrolyze, Polysaccharide, Molecular weight

INTRODUCTION

The basidiocarps of *Ganoderma lucidum*, called Young Ji, which belongs to the family of Polyporaceae are used as a dietary supplement or a traditional folk medicine for the treatment of chronic human diseases (Lee *et al.*, 1990, Nogami *et al.*, 1985).

Polysaccharides with antitumor activity have been isolated from various natural sources, including higher plants (Nakahara *et al.*, 1964), fungi (Chihara *et al.*, 1970), yeasts (Bradner *et al.*, 1958) and bacteria (Kato *et al.*, 1981). Since Ringler *et al.* reported the antitumor activities of Basidiomycetes (Ringler *et al.*, 1957), studies on the antitumor polysaccharides of Basidiomycetes have been extensively carried out (Kang *et al.*, 1980, Hyun *et al.*, 1990). The antitumor polysaccharides can be extracted with various solvents but extracted components can differ depending on the choice of solvent. One common point of the active polysaccharides is their relatively high molecular weight. It is believed that these components exert their antitumor activity through the enhancement of a host-mediated immunity rather than direct cytotoxicity to tumor cells. It is widely accepted that activated macrophages, cytotoxic T cells, natural killer cells and antibody dependent cytotoxic cells usually play important roles in tumor immunity (Shin *et al.*, 1985).

Miyazaki *et al.* reported that a water-soluble, antitumor polysaccharide, GL-1, M.W.4000, was isolated from *Ganoderma lucidum*. It was suggested that the essential structure of the antitumor activity of GL-1 was a branched glucan core involving (1→3)-β-, (1→4)-β- and (1→6)-β-linkages (Miyazaki *et al.*, 1981). Miyazaki *et al.* also reported that the structure of an alkali-extracted compound of *Ganoderma lucidum* was the highly branched and involved 1,3,4-tri substituted D-mannopyranosyl and (1→4)-linked D-xylopyranosyl residues (Miyazaki *et al.*, 1982).

Mizuno *et al.* reported that a hot-water extract of *Ganoderma lucidum* inhibited the growth of sarcoma 180 and that the antitumor polysaccharide fraction was composed of a backbone of (1→3)-β-linked-D-glucosyl residue with a single branch of the (1→6)-β-linked-D-glucosyl group in every 4 to 6 residues of backbone chain. Mizuno *et al.*, also reported that

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water-insoluble antitumor polysaccharide from *Ganoderma lucidum* was isolated and involed molecular weights of 2000 kD and 30~70 kD (Mizuno *et al.*, 1985).

Kim *et al.* reported that protein-bound polysaccharide exerting antitumor activities was isolated from the fruit body or the cultured mycelia of *Ganoderma lucidum* (Kim *et al.*, 1980).

Much attention has also been paid to the low-molecular weight of biologically active constituents of *Ganoderma lucidum* and many bitter lanostane type triterpenoids and ganoderenic acids, ganoderic acids and lucidenic acids have been isolated and identified (Komoda *et al.*, 1985; Kuboda *et al.*, 1982).

Chitin composed of (1→4)-β-N-acetyl-D-glucosamine can be hydrolyzed by chitinase (Austin *et al.*, 1981; Knorr *et al.*, 1982). Endochitinase from higher plants hydrolyzes chitin to give soluble N-acetyl-chitooligosaccharides, while exochitinase from microbial sources cleaves the non-reducing terminus of chitin and yields mainly N,N'-diacetylchitobiose. (Ohtakara *et al.*, 1978; Manuel *et al.*, 1985).

It was reported that *Ganoderma lucidum* contained chitin (Mizuno *et al.*, 1985). It is assumed that the chitin contained in *Ganoderma lucidum* may interfere with the extraction of water-soluble components which might have biological activities. There are some possiblities that bioactive polysaccharide can be extracted more from *Ganoderma lucidum* hydrolyzed with chitinase. In this study, we investigated the optimum conditions for hydrolyzing water-insoluble components contained in *Ganoderma lucidum* in order to obtain water-soluble polysaccharides by hydrolyzing with chitinase. We also used the *Ganoderma lucidum* residue remaining after extracting hot water-soluble materials in *Ganoderma lucidum*.

MATERIALS AND METHODS

Materials and reagents

The dried fruit bodies of *Ganoderma lucidum* harvested in Kang Hwa-Do and *Ganoderma lucidum* residue were kindly provided by the IL-Yang Pharma. Co.. Chitinase (13.57 units/mg) from the streptomyces species, was purchased from Boehringer Mannheim. Sepharose CL-4B, molecular weight marker (dextran : MW 2×10^6~3.91×10^4), bovine serum albumin and chitin from shrimp shells were obtained from the Sigma Chem. Co.. All other reagents were analytical grade and commercially available.

Extraction of chitin

The Hackman method (Hackmann *et al.*, 1954) was slightly modified. The sample was boiled in 0.1 M sodium hydroxide solution for 2 hours. After filtration, this procedure was repeated three times and the residue was washed with distilled water (deproteinization). The residue was boiled in 1.0M hydrochloric acid solution for 8 hours and after filtration, this procedure was repeated three times and the residue was washed with distilled water (demineralization). The residue was washed with methanol, acetone(decolorization) and distilled water and dried at 105°C. Chitin was identified by FT-IR (Bruker IFS48) using the KBr disc method.

Hydrolysis of water-insoluble components with chitinase

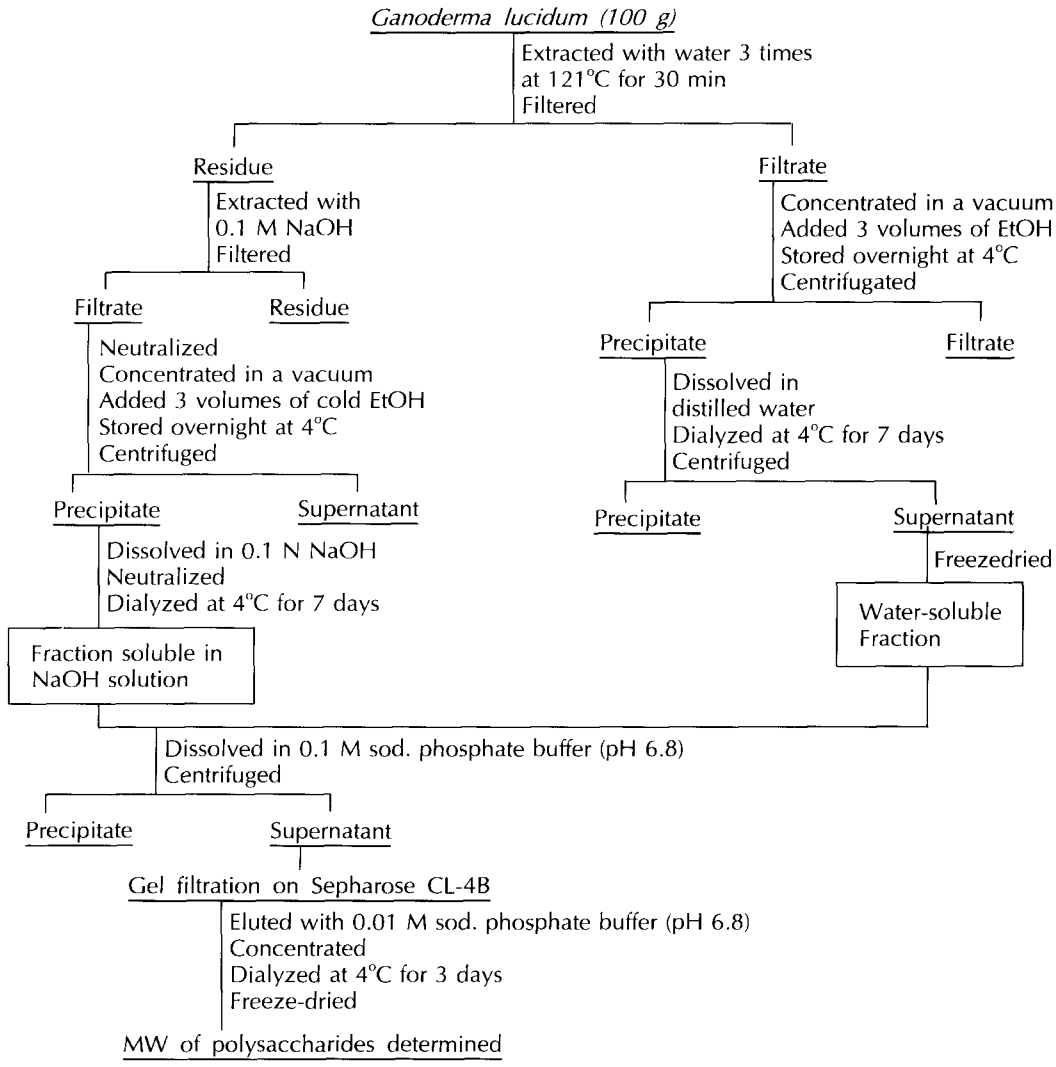
In order to determine the optimum pH, the temperature and concentration of chitinase and the concentration of the substrate used to hydrolyze the water-insoluble components contained in *Ganoderma lucidum* and in *Ganoderma lucidum* residue, the hydrolyzing procedure was performed as follows. The sample (1.0~6.0%) was hyrolyzed with chitinase (10~60 ppm) in buffer solutions (pH 3.0~11.0) at 30~50°C for 2 hours in a shaking incubater (200 rpm). To adjust the pH 0.05 M of incubating solutions, citrate-phosphate (pH 3.0~6.0), 0.01 M sodium-phosphate (pH 7.0), 0.05 M boric acid-borax (pH 8.0 and 9.0) and 0.05 M glycine-sodium hydroxide (pH 10.0 and 11.0) buffer were used respectively. 2 ml Of hydrolyzed samples were collected at intervals. Each sample was boiled at 100°C for 10 minutes and 2 ml of 0.4 M trichloroacetic acid (TCA) was added to stop chitinase action.

Measurement of the contents of polysaccharides, protein and N-acetylglucosamine

Samples were boiled at 121°C for 2 hours and centrifuged to extract water-soluble components. The content of polysaccharides in the supernatant was measured by an anthrone test (Hyun *et al.*, 1990). The content of protein in the supernatant was measured by the Lowry-Folin method (Hyun *et al.*, 1990). The content of N-acetylglucosamine of supernatant was also measured by Davidson method (Davidson, 1966).

Extraction and separation of polysaccharide with water and sodium hydroxide solution

The procedure for extraction and separation of the fraction soluble in water and sodium hydroxide solution is shown in Scheme 1. The basidiocarps of *Ganoderma lucidum* were disintegrated and extracted with distilled water at 105°C for 30 min. After filtration, this process was repeated twice. The residue was kept for extraction with sodium hydroxide solu-



Scheme 1. Procedure of isolation and extraction of protein-bound polysaccharide from *Ganoderma lucidum*.

tion and the filtrates were concentrated in a vacuum, and three volumes of 95% ethanol were added to the concentrate. The mixture was allowed to stand at 4°C overnight to complete precipitation. The precipitate was dissolved in distilled water and dialyzed at 4°C for seven days. The filtrate was concentrated and lyophilized to obtain water-soluble polysaccharides.

The residues after treatment with water were extracted with 0.1 M sodium hydroxide solution at 105°C for 30 min. After filtration, this process was repeated twice. The filtrates were concentrated in a vacuum and three volumes of 95% ethanol were added to the concentrate and the mixture was allowed to stand at 4°C overnight. After the supernatent was decanted, the precipitate was dissolved in 0.1 M sodium hydroxide solution and dialyzed at 4°C for seven days. The precipitate was removed by filtration. The filtrate was concentrated and lyophilized to obtain polysaccharides.

Molecular weight determination of the protein-bound polysaccharides

The molecular weight was determined by gel-filtration chromatography on a Sepharose CL-4B (Pharmacia-LKB) equilibrated with 100 mM sodium phosphate buffer (pH 6.8). The standards for the calibration of the column (2.5×85 cm) were blue dextran (Sigma Chem. Co., USA, MW=2×10^6), T-480 (Sigma Chem. Co., USA, MW=4.8×10^5), T-60 (Nakarai Chem. Ltd., Japan, MW=6×10^4). The blue dextran (10 mg) was dissolved in the eluent and applied to the column. It was eluted with 0.01 M sodium phosphate buffer (pH 6.8) at the flow rate of 1 ml/min (5 ml/fraction). The absorbance of each fraction was measured at 625 nm (anthrone test). Then using elution volumes and logarithm values of molecular weights of standard dextrans, the molecular weights of the polysaccharides were determined.

RESULTS AND DISCUSSION

Contents of chitin, polysaccharides and protein

The content of chitin obtained from *Ganoderma lucidum* and from *Ganoderma lucidum* residue was 18.90% (w/w) and 14.49% (w/w) respectively. The IR spectra of the chitin obtained from the sample was found to be a typical spectra similar to the spectra of standard chitin which has two bands at 1620 and 1550 cm^{-1} for characteristic stretching bands of the amide of chitin (Sannan *et al.*, 1978).

The total content of polysaccharide was calculated by adding the content of chitin obtained above and the content of polysaccharide contained in the filtrate (sodium hydroxide and hydrochloric acid fraction) remaining after obtaining chitin from the contents of the water-soluble polysaccharide measured (Table 1). The total content of protein was calculated by adding the content of protein contained in the filtrate remaining after obtaining chitin from the contents of protein measured. The total contents of the polysaccharide and protein contained in *Ganoderma lucidum* were 58.44% and 19.01% respectively. The results are similar to those of Lee *et al.* (Lee *et al.*, 1986). The contents of the polysaccharides and protein contained in *Ganoderma lucidum* residue were 2.34% and 5.58%, which were lower than the contents in *Ganoderma lucidum*. It is likely that the lower contents of *Ganoderma lucidum* residue are the result of pre-extraction of *Ganoderma lucidum* with water. But it is expected that the *Ganoderma lucidum* residue may possibly contain water-insoluble protein-bound polysaccharides which could exert biological activities.

Reaction time-activity profile of chitinase

The degree of hydrolysis was defined by the contents (% w/w) of water-soluble polysaccharide in the total polysaccharide of the sample. The degree of hydrolysis of *Ganoderma lucidum* was increased for 1~2 hrs, but afterwards decreased (Fig. 1). It is assumed that the by-product of the reaction or /and other factors inhibited the hydrolyzing reaction as it was prolonged.

Table 1. Polysaccharide and protein contents of *Ganoderma lucidum* and *Ganoderma lucidum* residue extracted with H_2O, 0.1 M NaOH and 0.1 M HCl. (g/g%)

	Ganoderma lucidum		*Ganoderma lucidum* residue	
	Polysaccharide	protein	polysaccharide	protein
H_2O	9.99	6.47	5.58	2.34
NaOH	11.63	11.54	4.84	11.30
HCl	17.92	1.00	36.54	9.86
Chitin	18.90		14.49	
Total	58.44	19.01	61.45	23.50

pH-Activity profile of chitinase

The pH-activity profile of chitinase is shown in Fig. 2. The curve was constructed from measurements of initial velocities when the reaction was carried out in

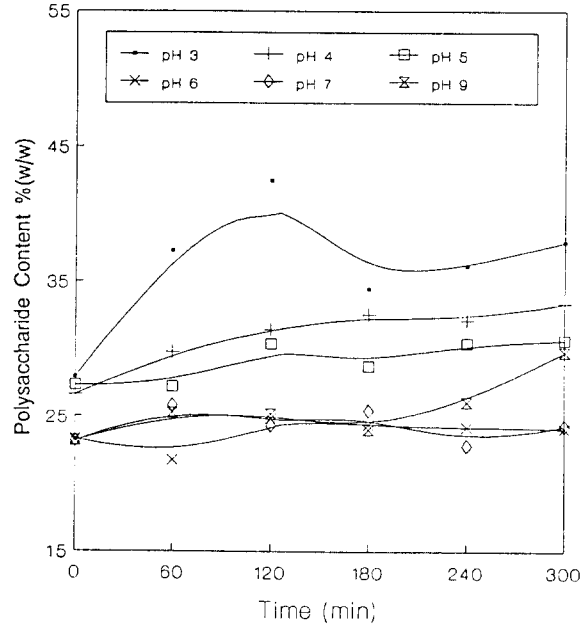


Fig. 1. Polysaccharide contents of *Ganoderma lucidum* as a function of time. *Ganoderma lucidum* was hydrolyzed with chitinase (20 ppm) at 35°C and polysaccharide content was measured.

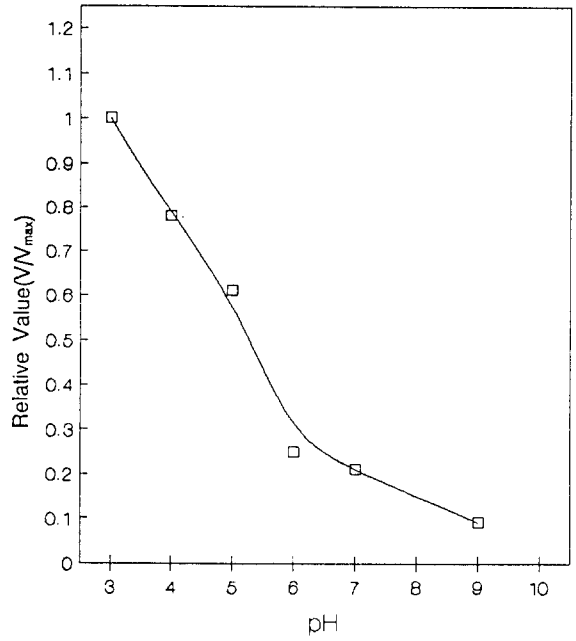


Fig. 2. pH-Activity profile of chitinase (20 ppm). The curve was plotted as relative values (V/V_{max}; as a ratio of initial velocities(V) and maximum velocities of hydrolysis) as a function of reaction pH. The velocities were calculated from the data shown in Fig. 2.

buffers of different pH. The curve was plotted in relative values (V/V_{max}; as a ratio of initial velocities (V) and maximum velocities of hydrolysis) as a function of the reaction pH. The velocity of hydrolysis was highest at pH 3 and decreased gradually as the pH increased to 9.

Temperature-activity profile of chitinase

Fig. 3 shows the temperature-activity profile of chitinase plotted relative values (V/V_{max}) as a function of reaction temperature. The reaction temperature significantly influenced the hydrolysis, and optimum temperature was 35°C. The hydrolyzing reactions significantly decreased below and over the optimum temperature. The decrease of hydrolysis is presumably due to the thermal instability of chitinase.

Enzyme concentration-activity profile of chitinase

Fig. 4 shows the rate of hydrolysis of water-insoluble contents in *Ganoderma lucidum* with chitinase at varied concentrations of pH 3 and at 35°C. There is a linear relationship between the rate of hydrolysis and enzyme concentration and 20 ppm of chitinase (13.57 units/mg) was chosen to hydrolyze water-insoluble contents contained in basidocarps of *Ganoderma lucidum* and *Ganoderma lucidum* residue.

Substrate concentration-activity profile of chitinase

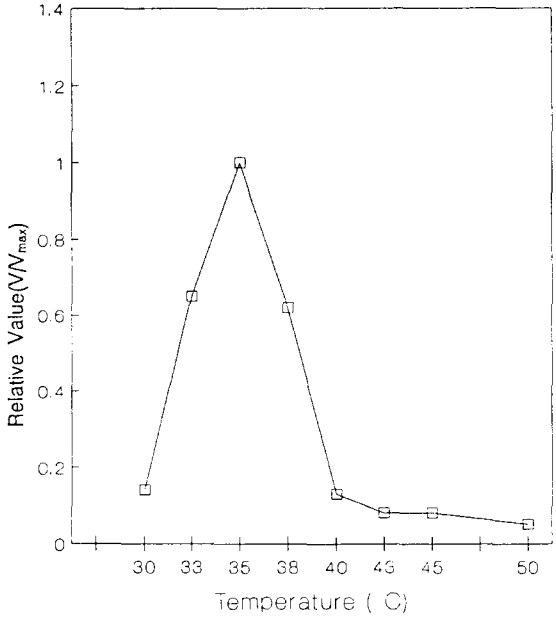


Fig. 3. Temperature-activity profile of chitinase (20 ppm). The curve was plotted as relative values (V/V_{max}) as a function of reaction temperature. *Ganoderma lucidum* was hydrolyzed with chitinase at pH 3 and polysaccharide content was measured.

One of the fundamental key factors affecting the rate of a reaction by an enzyme is the amount of substrate present. The degree of hydrolysis at different substrate concentrations was measured as a function of the hydrolyzing time. As shown in Fig. 5 and Fig. 6, the degrees of hydrolysis increased gradually for 1 hour (basidocarps of *Ganoderma lucidum*) or 2 hours

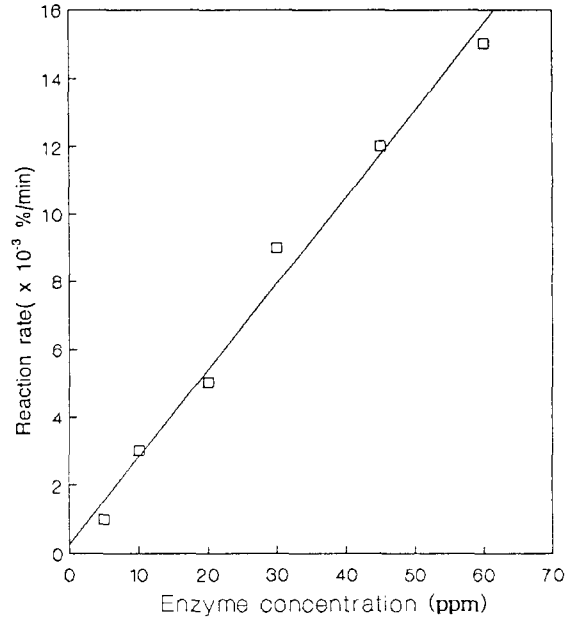


Fig. 4. Enzyme concentration-activity profile of chitinase. *Ganoderma lucidum* was hydrolyzed with chitinase at pH 3 at 35°C and polysaccharide content was measured.

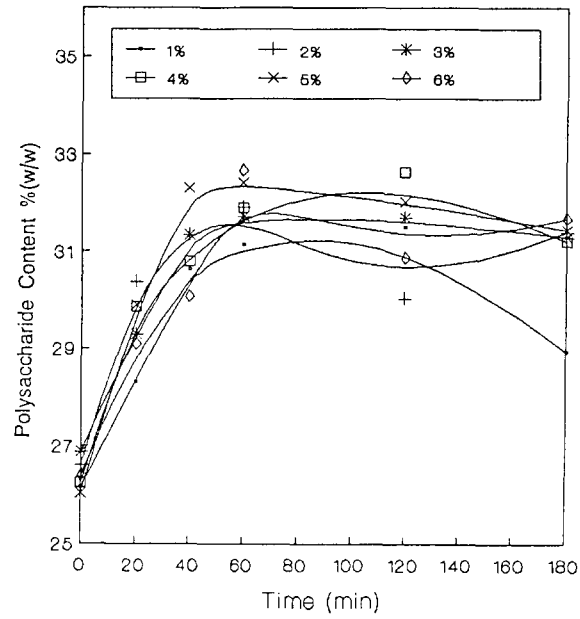


Fig. 5. Polysaccharide contents of *Ganoderma lucidum* as a function of time. *Ganoderma lucidum* was hydrolyzed with chitinase (20 ppm) at pH 3 at 35°C and polysaccharide content was measured.

(*Ganoderma lucidum residue*), but afterwards the degree of hydrolysis decreased. Substrate concentration influenced more significantly the hydrolysis of *Ganoderma lucidum* residue than the hydrolysis of *Ganoderma lucidum* and this implies that *Ganoderma lucidum* residue may contain more water-insoluble polysaccharide to be hydrolyzed by chitinase than

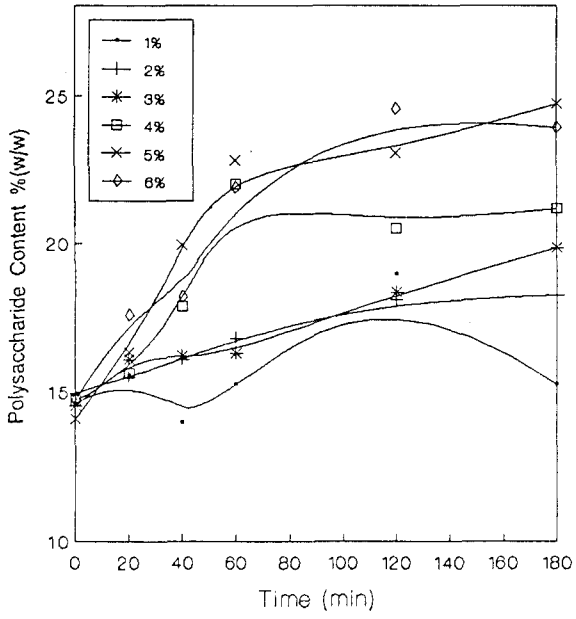


Fig. 6. Polysaccharide contents of *Ganoderma lucidum* residue as a function of time. *Ganoderma lucidum* residue was hydrolyzed with chitinase (20 ppm) at pH 3 at 35°C and polysaccharide content was measured.

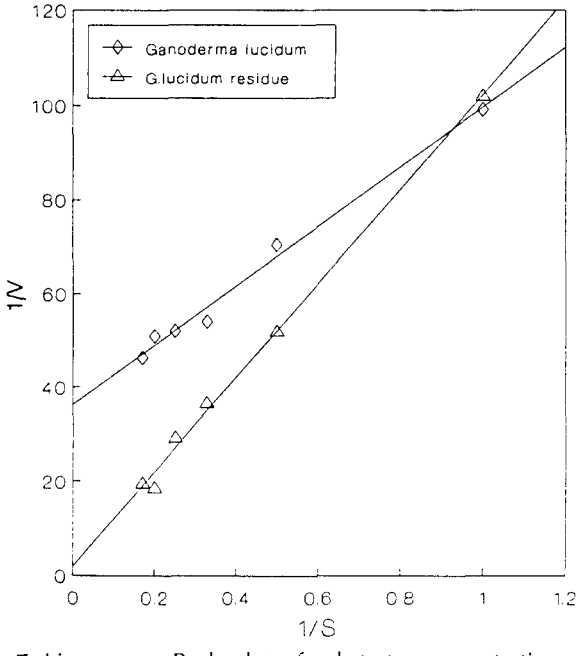


Fig. 7. Lineweaver-Burk plot of substrate concentration and initial velocity. The initial velocities were calculated from the data shown in Fig. 5 and Fig. 6.

Ganoderma lucidum contains.

Fig. 7 shows Lineweaver-Burk plots from the experimental data. A K_m value for the samples of *Ganoderma lucidum* was estimated to be 1.75 %, while the value was 53.15 % for the samples of *Ganoderma lucidum* residue. The great difference of the K_m value implies that the process of extraction of the *Ganoderma lucidum* significantly alter the substrate-binding affinity of the enzyme.

Contents of polysaccharide after hydrolysis

From the above results, it was determined that hydrolysis was conducted under the following conditions; 20 ppm of chitinase and 1~2% of *Ganoderma lucidum* or 6~7% of *Ganoderma luidum* residue at pH 3 at 35°C. The concentrations of total water-soluble polysaccharide in *Ganoderma lucidum* and *Ganoderma lucidum* residue are 17.0% and 9.1%, respectively, and the concentrations of total water-soluble polysaccharides of *Ganoderma lucidum* and *Ganoderma lucidum* residue after hydrolyzing with chitinase are 31.9%, 24.5% respectively. The contents of water-soluble components increased 1.9 fold for *Ganoderma lucidum* and 2.7 fold for *Ganoderma lucidum* residue respectively (Table 2).

Isolation of protein-bound polysaccharide residue

As shown in Table 3, 1.15g of brown powder (water-soluble protein-bound polysaccharide) was extracted from the basidocarps (100 g) of *Ganoderma lucidum* with water. It was found that a significantly increased amount (6.29 g) of polysaccharide was extracted from *Ganoderma lucidum* (100 g) after hydrolyzing with chitinase and 4.32g was extracted from *Ganoderma lucidum* residue (100 g) after hydrolyzing with chitinase. It was also found that 0.85 g of N-acetylglucosamine was extracted from *Ganoderma lucidum* (100 g) and 0.62 g of N-acetylglucosamine was extracted from *Ganoderma lucidum* residue (100 g) after hydrolyzing with chitinase.

The increased amount is regarded as the result of the conversion of water-insoluble polysaccharide to

Table 2. Concentrations of water-soluble polysaccharides contained in *Ganoderma lucidum* and *Ganoderma lucidum* residue before and after hydrolyzing with chitinase at optimum conditions; 20 ppm of chitinase and 2% of *Ganoderma lucidum* for 1 hour and 6% of *Ganoderma lucidum* residue for 2 hours at pH 3 at 35°C %(w/w)

	Before hydrolysis	After hydrolysis	Increasing Rates
Ganoderma lucidum	17.0	31.9	1.9
Ganoderma lucidum residue	9.1	24.5	2.7

Table 3. Contents (g) of water-soluble protein-bound polysaccharide (A) and N-acetylglucosamine (B) in the basidocarps (100 g) of *Ganoderma lucidum* or *Ganoderma lucidum* residue (100 g) extracted with water or 0.1 M NaOH solution. Samples were also hydrolyzed with chitinase and extracted in the same method

Hydrolysis with Chitinase	Solvents for Extraction	*Ganoderma lucidum*		*Ganoderma lucidum* residue	
		A	B	A	B
No	H_2O	1.15	-	-	-
No	NaOH	1.43	0.07	3.66	0.14
Yes	H_2O	6.29	0.85	4.32	0.62
Yes	NaOH	1.63	0.23	1.89	0.24

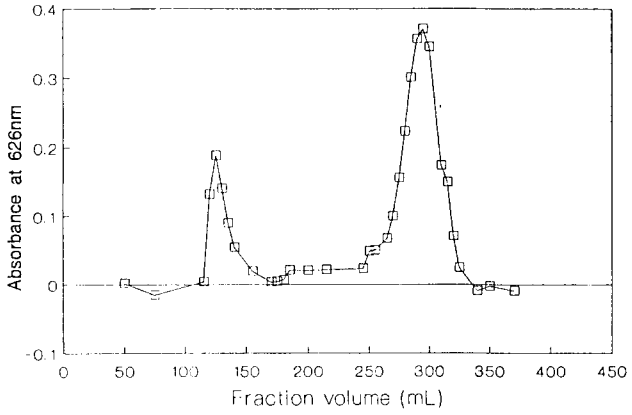


Fig. 8. Elution diagram of polysaccharide of *Ganoderma lucidum* extracted with distilled water by Sepharose CL-4B gel filtration. Sample (5 mg) was dissolved in 0.01 M sodium phosphate buffer solution (3 ml) and loaded.

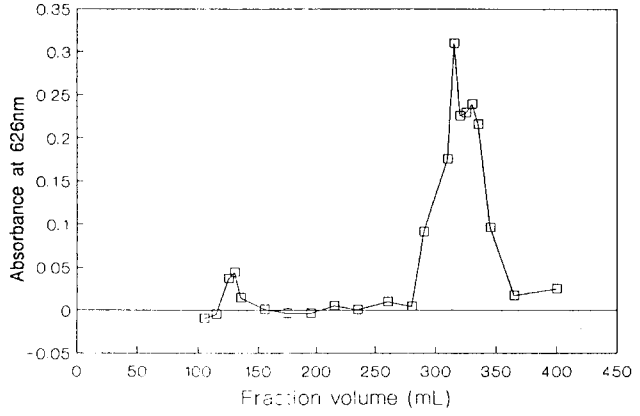


Fig. 9. Elution diagram of polysaccharide of *Ganoderma lucidum* extracted with 0.1 M sodium hydroxide solution by Sepharose CL-4B gel filtration. Sample (5 mg) was dissolved in 0.01 M sodium phosphate buffer solution (3 ml) and loaded.

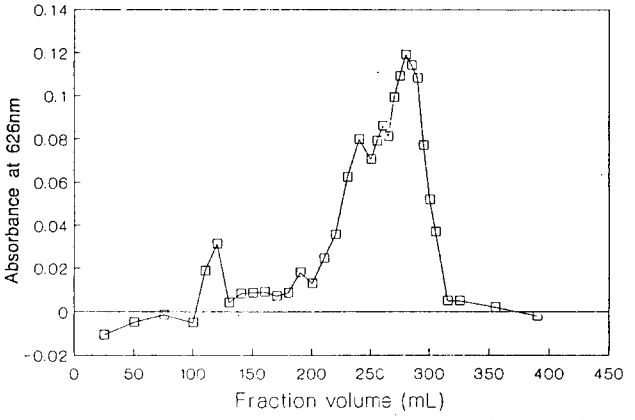


Fig. 10. Elution diagram of polysaccharide of *Ganoderma lucidum* residue extracted with 0.1 M sodium hydroxide solution by Sepharose CL-4B gel filtration. Sample (3 mg) was dissolved in 0.01 M sodium phosphate buffer solution (3 ml) and loaded.

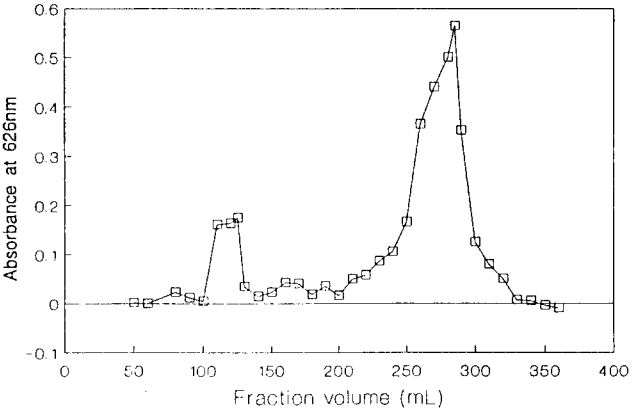


Fig. 11. Elution diagram of polysaccharide of *Ganoderma lucidum* hydrolyzed with chitinase and extracted with distilled water by Sepharose CL-4B gel filtration. Sample (4 mg) was dissolved in 0.01 M sodium phosphate buffer solution (3 ml) and loaded.

water-soluble polysaccharide by a hydrolyzing reaction with chitinase. It is expected that this water-soluble material obtained by hydrolysis can exert biological activities.

The content of polysaccharide from *Ganoderma lucidum* and *Ganoderma lucidum* residue extracted with 0.1 M sodium hydroxide solution was not increased by treating with chitinase.

Molecular weight determination of protein-bound polysaccharide

Molecular weights of protein-bound polysaccharides were determined by Sepharose CL-4B gel filtration using blue dextran, T-480 and T-60 as standards. The elution patterns of fraction from the basidocarps of *Ganoderma lucidum* and *Ganoderma*

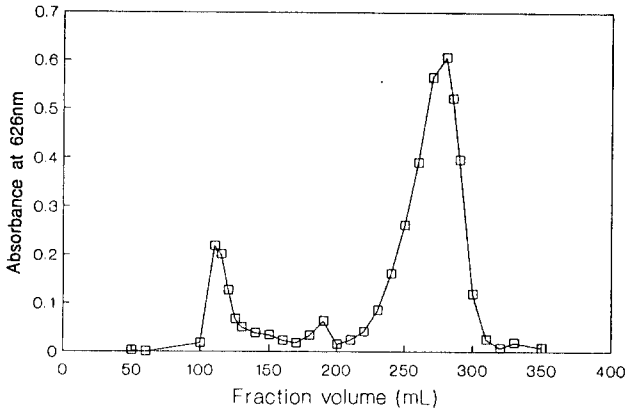


Fig. 12. Elution diagram of polysaccharide of *Ganoderma lucidum* residue hydrolyzed with chitinase and extracted with distilled water by Sepharose CL-4B gel filtration. Sample (5 mg) was dissolved in 0.01 M sodium phosphate buffer solution (3 ml) and loaded.

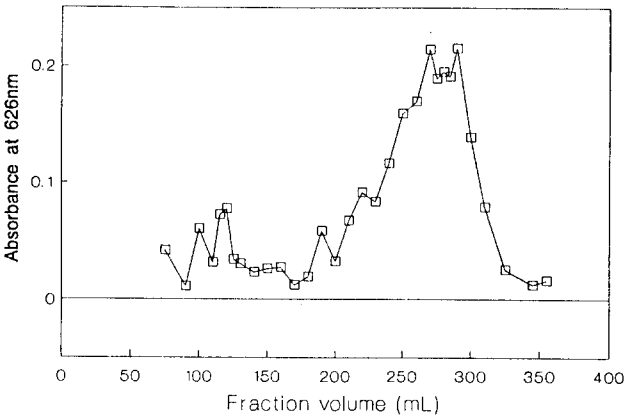


Fig. 13. Elution diagram of polysaccharide of *Ganoderma lucidum* hydrolyzed with chitinase and extracted with 0.1 M sodium hydroxide solution by Sepharose CL-4B gel filtration. Sample (5 mg) was dissolved in 0.01 M sodium phosphate buffer solution (3 ml) and loaded.

lucidum residue extracted with water and NaOH solution are shown in Fig. 8~Fig. 10.

The elution patterns of the fraction from the sample treated with chitinase and extracted with water are shown in Fig. 11 and Fig. 12 and the patterns of the fraction from the sample treated with chitinase and extracted with NaOH are shown in Fig. 13 and Fig. 14 respectively. The molecular weights of each fraction are shown in Table 4 and the results are similar to that reported by Mizuno *et al.* (*i.e.* 2000 kD and 30~70 kD) (Mizuno *et al.*, 1985). It was observed that the high molecular weight (2,020 KD) of polysaccharides contained in *Ganoderma lucidum* residue extracted with sodium hydroxide solution was disapeared after hydrolyzing with chitinase and it is presumed that the polymer bond of the polysaccharide was possibly cleaved by chitinase.

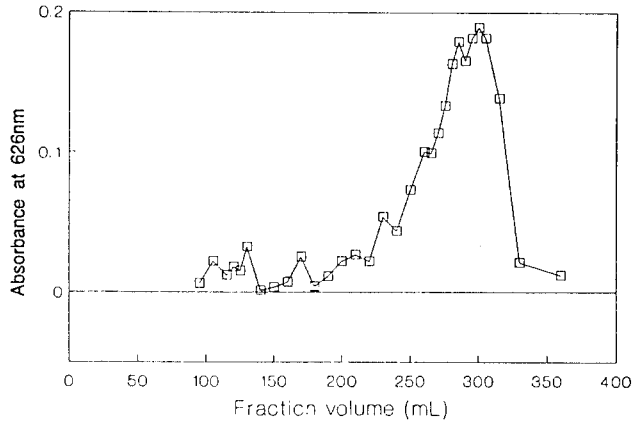


Fig. 14. Elution diagram of polysaccharide of *Ganoderma lucidum* residue hydrolyzed with chitinase and extracted with 0.1 M sodium hydroxide solution by Sepharose CL-4B gel filtration. Sample (5 mg) was dissolved in 0.01 M sodium phosphate buffer solution (3 ml) and loaded.

Table 4. Molecular weights (KD) of protein-bound polysaccharide extracted from *Ganoderma lucidum* and *Ganoderma lucidum* residue extracted with water or NaOH solution. Samples were also hydrolyzed with chitinase and extracted in the same method

Hydrolysis with Chitinase	Solvents for Extraction	*Ganoderma lucidum*	*Ganoderma lucidum* residue
No	H_2O	1,570, 25	-
No	NaOH	1,570, 15, 10	2,020, 60, 36
Yes	H_2O	1,780, 32	2,590, 36
Yes	NaOH	2,020, 349 164, 46, 28	576, 128

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REFERENCES CITED

Austin, P. R., Brine, O.J., Castle, J. E., Zikakis, J.P. Chitin: New facts of research, *Science*, 212(15) May, 749-753 (1981).

Bradner, W.T., Clarke, D.A. and Stock, C.C. Stimulation of host defence against experimental cancer. I. zymosan and sarcoma 180 in mice, *Cancer Res.*, 18, 347 (1958).

Chihara, G., Hamuro, T., Maeda, y., Arai, Y. and Fukuoka, F. Antitumor polysaccharide derived chemically from natural glucan (pachyman), *Nature*, 225, 943 (1970).

Davidson, E.A. Analysis of sugars found in mucopolysaccharides, Methods Enzymol. 8. 52 (1966).

Hackmann, R.H. Studies on chitin. 1.Enzyme degradation of chitin and chitin esters, *Austr. J. Biol. Sci.*, 7, 168-178 (1954).

Hyun. J. W., Choi, E. C., Kim, B. K. Studies on constituents of the higher fungi of Korea(LXVII) Antitumor components of the basidiocarp of *Ganoderma lucidum., Kor. J. Mycol.*, 18(2), 58 (1990).

Kang, C. Y., Shin, M. J., Choi, E. C., Lee, Y. N. and Kim, B. K. Studies on antineoplastic components of Korean basidiomycetes. Mycelial culture and an antineoplastic component of *Ganoderma lucidum, Korean Biochem. J.*, 14, 101 (1980).

Kato, I., Kobayashi, S., Yokokura, T. and Mutai, M. Antitumor activity of *Lactobacillus casei* in mice. *Gann*, 72, 517 (1981).

Kim, B. K., Chung, H. S., Chung, K. S. and Yang, M. D. Studies on the antineoplastic components of Korean basidiomycetes, *Kor. J. Mycol.*, 8, 107 (1980).

Knorr. D. Functional properties of chitin and chitosan, *J. of Food Sci.* 47, 593-595 (1982).

Komoda, Y., Nakamura, H., Ishihara, S., Uchida, M., Kohda, H. and Yamasaki, K. Structures of new terpenoid constituents of *Ganoderma lucidum* (Fr.) Karst (polyporaceae), *Chem. Pharm. Bull.* 33(11), 4829-4835 (1985).

Kubota, T., Asaka, Y., Miura, I. and Mori, H. Structure of ganoderic acid A and B, two new lanostane type bitter triterpenes from *Ganoderma lucidum* Karst (Fr.), *Helv. Chim. Acta.*, 65, 611 (1982).

Lee, K. H., Jeong, H. , Kim, Y. I.and Kim, B. K. Production of antihypertensive constituents from *Ganoderma lucidum* IY005 by fermentation using industrial wastes, *Kor. J. Mycol.*, 19(1), 79 (1990).

Lee, M.H., Kim, H.W., Shim, M.J., Toh, S.H., Choi, E. C. and Kim, B.K., Studies on constituents of the higher fungi of Korea(LVI) General constituents and immuno-stimulation of *Ganoderma lucidum, Kor. J. Mycol.* 14(2), 149- (1986).

Manuel, E. Y. and Richard, I.B., Kinetics of chitinase production. 1. Chitin hydrolysis, *Biotech. and Bioeng.* 27 June, 769-775 (1985).

Miyazaki, T. and Nishijima, M. Studies on fungal polysaccharides (XXVII), structural examination of a water-soluble, antitumor polysaccharide of *Ganoderma lucidum, Chem. Pharm. Bull.*, 29, 3611 (1981).

Miyazaski, T. and Nishijima, M. Structural examination of an alkali-extracted, water-soluble heteroglycan of the fungus *Ganoderma lucidum, Carbohydr. Res.*, 109, 290 (1982).

Mizuno, T., Suzuki, E., Maki, K. and Tamaki, H. Fractionation, chemical modification and antitumor activity of water-soluble polysaccharides of the fruiting body of *Ganoderma lucidum, Nippon Nokeikagaku Kaishi.*, 59, 1143 (1985).

Nogami, M., Tsuji, Y., Kubo, M., Takahashi, M., Kimura, H. and Matsuike, Y. Studies on *Ganoderma lucidum* V1. Anti-allergic effect(1), *Yakugakuzasshi*, 106(7), 594-599 (1985).

Nakahara, W., Fukuoka, F., Maeda, Y. and Aoki, K. The host mediated antitumor effect of some plant polysaccharide. *Gann*, 55, 283 (1964).

Ohtakara, A., Uchida, Y., and Misutomi, M., in Processings of the first international conference on chitin/chitosan, Muzzarelli and Pariser, E., R., Eds. (MIT Sea Grant report MITSG 78-7), 587-600(1978).

Ringler, R.L., Byerrum, R.U., Steven, T.A., Clark, P.A. and Stock, C.C. Studies on antitumor substances produced by Basidomycetes. *Antibiot. Chemother.*, 7, 1 (1957).

Sannan, T., Kurita, K. and Iwakura, Y., Studies on chitin: 7. IR spectroscopic determination of degree of deacetylation, *Polymer*, 19, April, 458-459 (1978).

Shin, H. J., Kim, H. W. , Choi, E. C., and Kim, B. K. Studies on constituents of the higher fungi of Korea (XLII), inorganic components of *Ganoderma lucidum. Kor. J. Mycol.*, 13, 53 (1985).