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Studies on Hemicellulase System in *Aspergillus niger* (I)
— Purification and reconstitution of D-xylanase —

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*Aspergillus niger*의 Hemicellulase계 효소에 관한 연구
—D-xylanase계 효소의 정제와 재조합—

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(1982년 11월 9일 수리)

In the present study, two kinds of D-xylanases (1,4-β-D-xylan xylanohydrolase (EC 3.2.1.8) were purified and characterized from crude extract of *Aspergillus niger* KG79. Xylanase I was most active at pH 5.0, whereas xylanse II at pH 4.0 Both enzymes demonstrated their maximum activity at 45°C. They were relatively stable between pH 4.0 and 6.0 at 30°C for 6 hours. Molecular weight of xylanse I and II were 12,500 and 11,500, respectively. Michaelis-Menten constants of xylanse I and II were 0.28% and 0.26% of xylan, respectively.

Both enzymes could degrade commercial D-xylan to xylose, xylobiose, and xylotriose to the degree of about 10% of total reducing power. Xylanse I could, however, liberate arabinose from barley straw xylan in addition to xylose and xylooligasaccharides more rapidly than xylanase II. The degree of hydrolysis was about 25%. The reconstituted D-xylanase system with purified xylanases and β-xylosidase degraded commercial xylan and barley straw xylan to the degree of 28% and 54%, respectively. The limit of hydrolysis by the enzymes was suggested to be resulted from the physical structure of the substrate.

D-Xylanase (1,4-β-D-xylan xylanohydrolase EC 3,2,1,8) is a hemicellulase which hydrolyzes xylan, one of the major constituents of agricultural wastes. This enzyme is produced by various microorganisms such as *Aspergillus niger*,[1-8] *Ceratocystis paradoxa*,[9,10] *Tricoderma viride*,[11,12] *Penicillium janthinellum*,[13] *Bacillus subtilis*,[14,15] and *Streptomyces xylophagus*.[16,17] In paticular, D-xylanase from fungi has been more extensively studied than that from other sources. It has been reported that *Aspergillus niger* produces several different types of D-xylanases, which are not functionally distinguishable.[6,8,18]

Furthermore, higher enzymatic conversion of xylan into D-xylose appears to occur in the presence of β-xylosidase in addition to D-xylanase. It is likely that D-xylanase is an endo-enzyme which cleaves randomly 1 → 4-linkage as well as xylose-arabinase side chain linkage of xylan.[5,6,18] β-Xylosidase is, however, an exo-enzyme which liberates monomeric D-xylose at the non-reducing end of xylan oligomers.

$$\text{Xylan} \xrightarrow{\text{D-xylanase}} \text{oligomeric xylan (or xylobiose)} \xrightarrow{\text{ß-xylosidase}} \text{D-xylose}$$

In the course of studies on bio-degradation of D-xylan, β potent microorganism, *A. niger* KG79 producing xylanase was isolated in our laboratory. It was found that there were two kinds of xylanases in this enzyme system. The authors should like to report purification and characterization of these xylanases and relationship with β-xylosidase to maximize the degree of conversion of xylan into momeric D-xylose.

Materials and Methods

Preparation of D-xylans

D-Xylans from straw was prepared according to the procedure of Fukumoto[6] with minor modification. One kg of finely hammer milled straw (~5 mesh) was soaked in 10L of water and boiled for 3 hours at 100°C in a water bath. After excessive washing with runnin water, straw was delignified in 100L of sodium hypochlorite solution of 2% available chlorine concentration for 1 day at 20°C. Ex-

cessive washing with water was repeated. Extraction of D-xylan was carried out with 10L of 10% NaOH solution for 20 hours at 20°C with bubbling of nitrogen gas to prevent oxidative cleavage. After the removal of solid particle,pH of the extracting solution was adjusted to 4.5 with acetic acid. Equal volume of acetone was added and precipitate was collected by centrifugation. The precipitate was washed with 50% acetone, pure acetone, ethanol, and ethyl ether, successively, and dried at 40°C under vacuum to obtain crude D-xylan.

For the further refinement, the preparation of crude D-xylan was dissolved in 2% NaOH solution. After removal of the precipitate by addition of the equal volume of acetone was redissolved in 2% NaOH solution. After the repetition of the above procedure, the precipitate was washed with 50% acetone, pure acetone, ethanol, and ethyl ether, successively, and dried at 40°C under vacuum.

Isolation and culture of microorganism

Microorganisms producing high activity of xylanase was screened out with modified Czapek-Dox media containing 0.5% xylan as a sole carbon source. A strain producing the highest activity of xylanase was isolated and identified to be *Aspergillus niger* according to the description of Randall.[19]

For the purpose of the enzyme production in a large scale the solid culture process on wheat bran koji was adopted. Wheat bran was well mixed with water equivalent to 60% of the weight of wheat bran. After the sterilization and inoculation koji was evenly spread in a pan. Three days of culture at 26°C produced the maximum yield of the enzyme productivity. The crude extract of enzymes was obtained by extracting the koji with 5 volumes of 25mM acetate buffer (pH 5.0) at 40°C for 2 hours.

Assay of enzyme Activity

a) D-xylanase activity: The D-xylanase activity was determined with 0.25% solution of D-xylan as a substrate in 50mM acetate buffer (pH 4.5) at 40°C. Reducing sugar was assayed by the method of Somogyi-Nelson method.[20]

Substrate solution was prepared by dissolution of 0.25g of D-xylan in 25ml of 0.5M NaOH solution. After neutralization with 0.5M HCl solution, pH was adjusted to 4.5 with acetate buffer to a final concentration of 50mM and the volume was made up to 100ml.

One unit of D-xylanase activity was defined as the amount of the enzyme which releases reducing sugar equivalent to 1 μmole of D-xylose per minute under the specified condition.

b) β-Xylosidase: The β-xylosidase activity was determined with 2mM of a synthetic substrate, p-nitrophenyl-β-xylopyranoside in 50mM acetate buffer, pH 4.5 at 40°C.

One unit of β-xylosidase activity was defined to be the amount of the enzyme which hydrolyzes 1 μmole of p-nitrophenol per minute under the specified condition.

Protein determination

Protein was estimated according to the method of Lowry et al.[21] using crystalline bovine serum albumin as a standard.

Gel electrophoresis

Disc gel electrophoresis on 7% polyacrylamid gel was carried out at pH 8.3 according to the procedure of Davis.[22] Polyacrylamide gel electrophoresis in the presence of SDS and β-mercaptoethanol was carried out by the procedure of Weber and Osbon.[23]

Protein staining was performed with Coomassie Brilliant Blue R250 and glycoprotein was detected with periodic-schiff reagent by the method of Segrest and Jackson.[24]

Estimation of molecular weight by gel filtration

Molecular weight of the enzymes was determined by the methods of Sephadex G-75 chromatography (1.5x90cm) and 10% SDS gel electrophoresis. The chromatography was performed in 50mM acetate buffer (pH 4.5) containing 0.1M NaCl. The flow rate was 9ml/hour and 1.5ml fraction was collected. The void volume (Vo) of the column was determined with blue dextran (M.W. 2,000,000).

pH effect

The effect of pH of the activities of D-xylanases was tested in 50mM buffer according to the standard assay procedure. The buffer solution employed were 50mM sodium phosphate over pH 6.0-7.5, and 50mM citrate-phosphate over pH 2.5-7.5.

Storage stabilities of the enzymes in various pH's were tested by measuring their residual activities at the standard assay condition after 6 hours of incubation at 30°C in 50mM buffer of various pH.

Temperature effect

Temperature effects on enzyme activities were determined according to the standard assay procedure at various temperature.

Storage stabilities of the enzymes at various temperature were tested by measuring the residual ac-

tivities under the standard assay condition after 6 hours of incubation at a specified temperature in 50mM acetate buffer (pH 4.5). Thirty minutes of preincubation at 4°C was preceded by the assay procedure.

Enzymatic hydrolysis of D-xylan

To study the digestibility of D-xylans by the enzymes, D-xylanase I, II and/or, β-xylosidase were incubated with substrates (1U of enzyme/ml of substrate) at 30°C. Reducing sugars released were determined by Somogyi-Nelson method[20] and also analyzed by paper chromatography.

Analysis of sugar components

a) Acid hydrolysis: One hundred mg of D-xylan sample was dissolved in 25ml of 1M H_2SO_4 solution. Hydrolysis was performed in a boiling water bath for 2 hours. After neutralization with $Ba(OH)_2$, the supernatant was collected by centrifugation and analyzed.

b) Paper chromatography: Sugar composition of acid hydrolyzates or enzyme reaction products of D-xylan was analyzed by paper chromatography on Whatman No. 1 filter paper. The developing solvent system was composed of n-butanol/pyridine/water = 6:4:3 (v/v).[25] The hydrolyzate was desalted by mixing with equal weight of a mixed ion exchange of IR120 and IRA400 before subjecting to chromatography. Double development was carried out for 48 hours. Sugar spots on paper were detected by anilin hydrogen phthalate method.[26] The colored spots were eluted from the chromatogram and the concentration of sugar was determined by spectrophotometry. Eluting solution was 0.7M HCl in 80% ethanol (v/v) solution.[26] The absorbance of the solution was measured at 390nm for pentoses.

c) High performance liquid chromatography: Component sugar was analyzed by the High Performance Liquid Chromatography (Varian Model 5021) with Micropak-NH_2 column. Using acetonitrile-H_2O as an eluent the effluent was detected by a UV detector at 192nm.

Material

Xylan from larch wood x-3875, Lot 48C-00541, was purchased from Sigma Chemical Co. Barley straw was collected in the area of Suwon, Korea. Filter paper was obtained from Whatman Ltd., and Sephadex G-75 and DEAE-Sephadex from Pharmacia Fine Chemicals, Sweden. Other chemicals used were reagent grade.

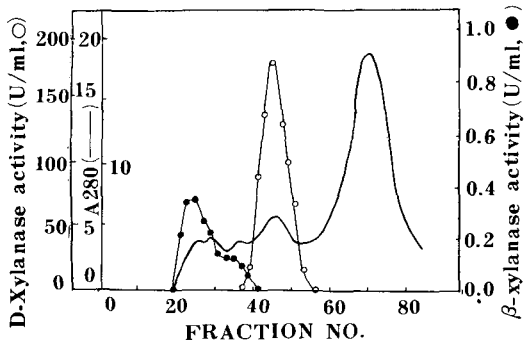

Fig. 1. Chromatogram of D-xylanase on Sephadex on Sephadex G-75.

Column dimension was 2.6×90cm and the flow rate of the buffer solution, 25mM acetate, (pH 5.0) was 36ml/hr with 6ml/tube fraction. ○ : xylanase activity, ● : β-xylosidase activity

Results

Purification of D-xylanases

Preparation of crude enzyme extract: The enzymes produced by *A. niger* KG79 in koji culture were extracted with 5 volumes of 25mM acetate buffer (pH 5.0) at 40°C for 2 hours. The extractant solution was used for the further purification of the enzymes. All procedures for the purification of the enzymes were carried out at 4°C.

Ammonium sulfate fractionation: The crude enzyme extract (800ml) was fractioned for D-xylanases by the addition of ammonium sulfate. The precipitate obtained by the saturation with ammonium sulfate between 40% and 70% was dissolved in 20ml of the acetate buffer. Insoluble precipitate was removed by centrifugation.

Gel filtration: The clear supernatant was subjected to the gel filtration on Sephadex G-75 column (2.6x90cm) previously equilibrated with the acetate buffer. Elution was carried out with the buffer at a flow rate of 36ml/hr with a fraction of 6ml/tube. A typical chromatogram is shown in Fig. 1. D-Xylanase was eluted as a single peak and β-xylosidase was eluted prior to the fraction of D-xylanase.

The fractions (tube No. 41-51) with high D-xylanase activity were pooled and was further purified.

Ion exchange chromatography: The pooled fraction obtained as described above was applied to DEAE-Sephadex A-25 column (2.8x35cm) equilibrated with the acetate buffer. Elution was carried out with the application of continuous linear salt gradient between 0 and 1.0M NaCl in the buffer. The volume of mixing chamber was 500ml. The flow rate was 30ml/hr and 6.5ml fraction was collected.

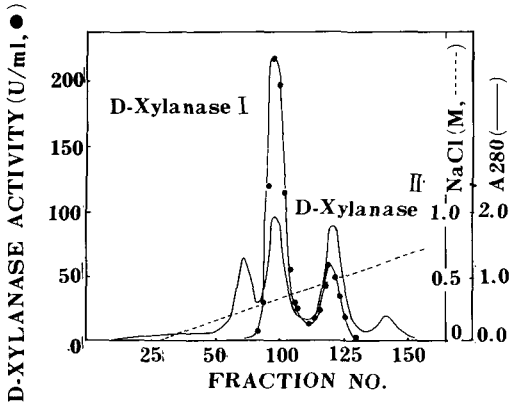

Fig. 2. Chromatogram of D-xylanases on DEAE-Sephadex A-25.

Elution was carried out with continuous lineur salt gradient between 0 and 0.1M NaCl in chromatographic buffer, 25mM acetate, pH 5.0. Column dimension was 2.8×35 cm and the flow rate was 30ml/hr with 6.5 ml/tube fraction. —: adsorbancy at 280nm, ○: xylanase activity (u/ml)

Two kinds of pure D-xylanases, D-xylanase I and II in order of elution, were separated. The fractions with high enzyme activity (fraction No. 71-77 for D-xylanase I and 92-99 for D-xylanase II) were pooled and used for the further studies. Xylanase II was crystallized by the addition of saturated ammonium sulfate solution. It was a fine needle shape as is seen in Fig. 3. According to the preliminary experiment with the crude enzyme solution, it consisted of the equal amount of D-xylanase I and II.

The purification steps of D-xylanases were summarized in Table 1. The specific activity of the pure D-xylanase I and II were 204.2 u/mg of protein and 42.4 u/mg of protein corresponding to 51- and 10.6-fold purification, respectively, over the crude extract.

Homogeniety of the enzymes: D-Xylanase I and II obtained as described above were proved to be homogeneous by 7% polyacrylamide disc gel electrophoresis. Both enzymes appeared as a single band, respectively. SDS-electrophoresis also confirmed their homogeniety.

Table 1. Purification of D-Xylanases from *Aspergillus niger*.

Fraction	Total Prot. (mg)	Total Act. (U)	Sp. Act. (U/mg)	Purification (fold)	Yield (%)
1. Crude extract	6,400	26,360	4.0	1.0	100
2. $AmSO_4$ fraction (40-70% sat.)	1,144	19,834	17.8	4.5	78.2
3. Sephadex G-75 gel filt.	441.6	17,912	40.6	10.2	70.6
4. *DEAE-Sephadex ion exchan.*					
D-xylanase I	45.9	9,373.5	204.2	51.0	37.0
D-xylanase II	61.0	2,586.4	42.4	10.6	10.2

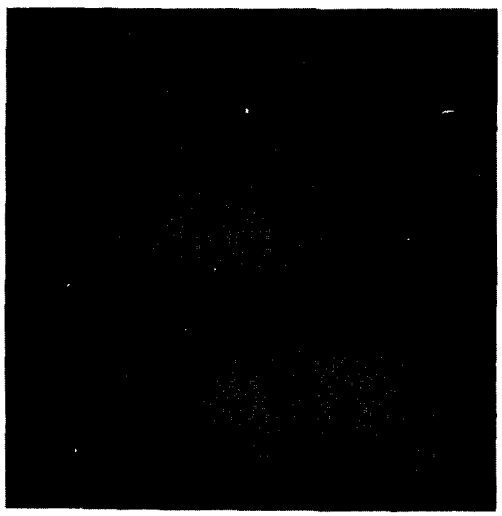

Fig. 3. Crystalline xylanase II (x 67)

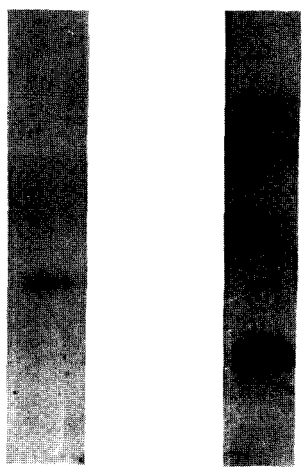

Fig. 4. Polyacrylamide disc gel electrophoresis (7 %) of purified xylanase I and II.

Characterization of D-xylanase I and II

Molecular weight: Molecular weights of D-xylanase I and II were estimated to be 12,500 and 11,800 respectively by SDS gel-electrophoresis. These values were also confirmed by the gel filtration of Sephadex G-75 column(Fig.6)

pH effect: D-Xylanase I showed its maximum activity at pH 5.0 and D-xylanase II at pH 4.0. For the reconstitueted enzyme system which contains both D-xylanases, therefore, 50mM acetate buffer (pH 4.5) was used for the subsequent experiments.

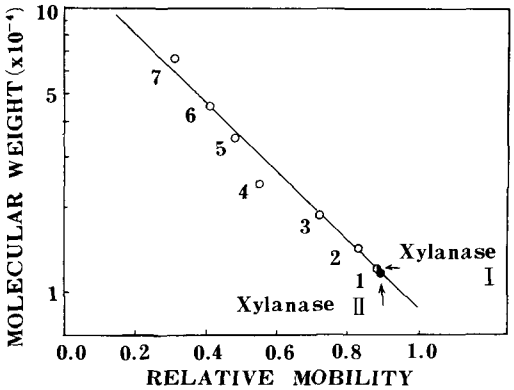

Fig. 5. Molecular weights of xylanases I and II estimated by SDS-electrophoresis. Mw stantards used were;

1. cytochrome c, 2. lysozyme, 3. β-lactoglobulin, 4. trypsinogen, 5. pepsin, 6. ovalbumin, 7. BSA

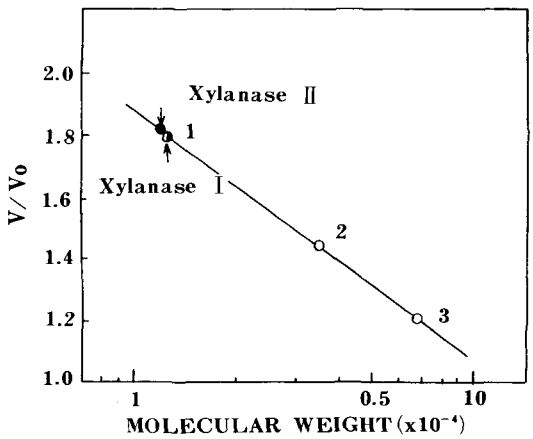

Fig. 6. Molecular weights of xylanases I and II determined by gel filtration on Sephadex G-75. Mw standards used were;

1. cytochrome c, 2. β-lactoglobulin, 3. BSA

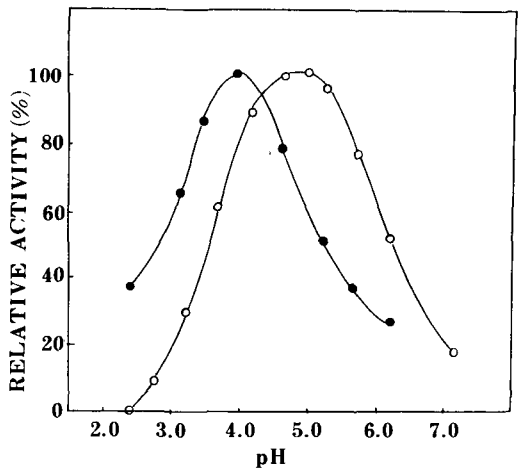

Fig. 7. Relative activities of xylanases at various pH's.

○ : xylanase I, ● : xylanase II

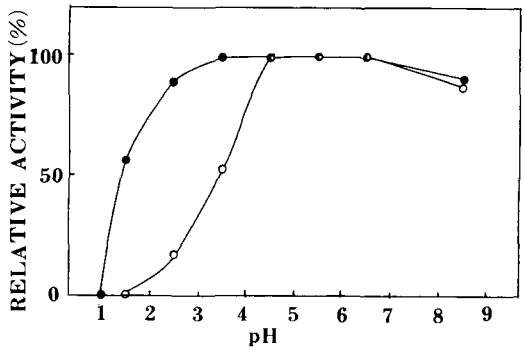

Fig. 8. Storage stabilities at various pH's for 6 hours at 30℃.

○ : xylanase I, ● : xylanase II

D-xylanase II was relatively stable between pH 2.5 and 8.5 whereas D-xylanase I retained 50% of the residual activity after incubation of 6 hours at pH 3.5, 30°C.

Temperature effect: Both D-xylanases were most active at 45°C and demonstrated the identical activity profiles as a function of temperature. The Arrhenius plots are shown in Fig. 9. The activation energy of D-xylanase reaction was calculated to be 0.5Kcal/mole at lower temperature range while the heat of inactivation[27] was -58.7Kcal/mole at higher temperature range.

D-xylanse II was stable at 40°C for 6 hour in 50mM acetate buffer, pH 4.5 whereas D-xylanase I lost about 20% of its initial activity.

Michaelis-Menten constant: Both enzymes showed similar kinetic patterns on both commercial and barley

straw D-xylan. From the Lineweaver-Burk polts, the Km values of D-xylanase I and II were determined to be 0.28% and 0.26%, respectively.

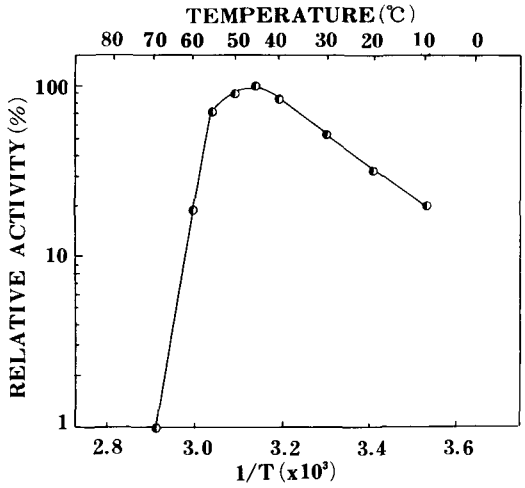

Fig. 9. Temperature effects on xylanase activity at pH 4.5.
○ : xylanase I and II

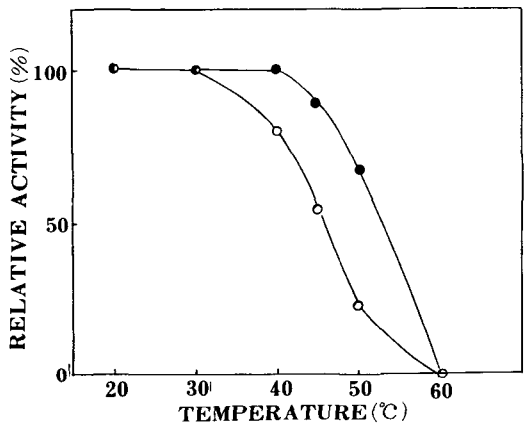

Fig. 10. Storage stabilities at various temperature in 50mM acetate buffer (pH 4.5).
○ : xylanase I, ● : xylanase II.

Hydrolysis of D-xylans by the reconstutituted D-xylanase system

Chemical composition of barley straw xylan: Xylan from barley straw was analyzed to be composed of xylose, arabinose, and glucose with the ratio of 85.4:11.2:3.4 as is seen in Fig. 12.

Hydrolysis pattern: The enzymatic activity of D-xylanase I and II on commercial larchwood D-xylan

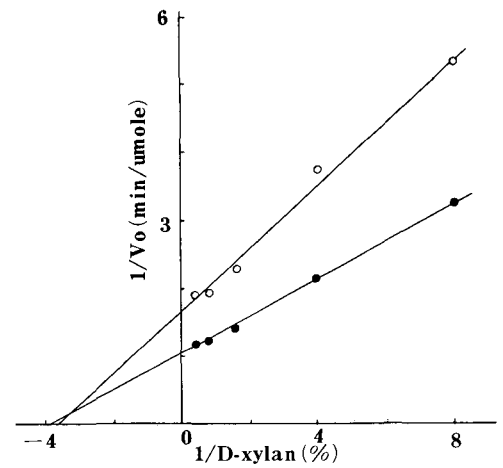

Fig. 11. Lineweaver-Burk plots for hydrolysis of xylan by xylanase I and II in 50mM acetate buf-fer, (pH 4.5).
○ : xylanase I, ● : xylanase II

showed little difference in their hydrolytic pattern.

They liberated about 10% of reducing sugar equivalent to D-xylose in 1% D-xylan solution at the end of the prolonged incubation for 72 hours at 30°C with the formation of xylan precipitate. D-xylanase I and II produced, xylose, xylobiose, and xylotriose to the ratio of 5:7:1 and 4:7:1, respectively. β-Xylosidase hydrolyzed the commercial D-xylan with the liberation of only D-xylose to the degree of 14%.

On the contrary to the larchwood D-xylan which was

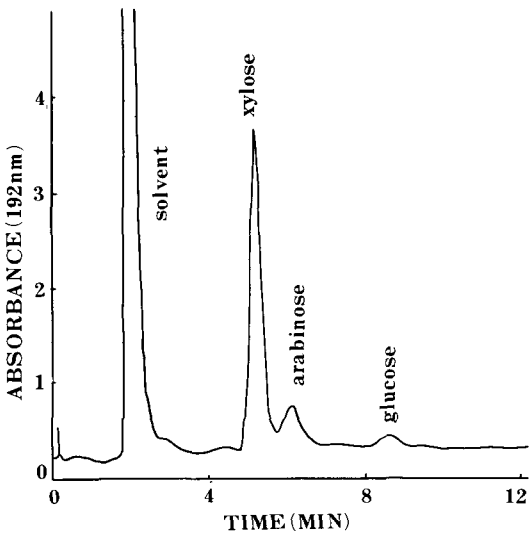

Fig. 12. High performance liquid chromatogram of acid hydrolyzed barley straw xylan. xylose :arabinose:glucose=85.4:11.2:3.4

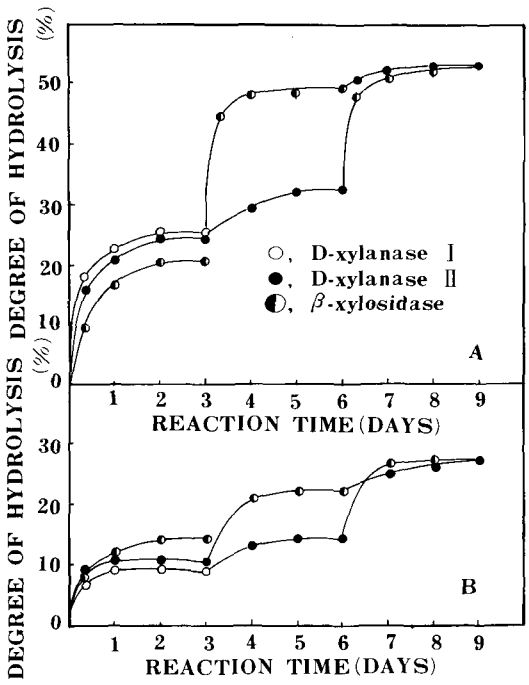

Fig. 13. Hydrolytic mode of xylanase system on barley straw xylan(A) and commercial larchwood xylan(B).

D-xylose homopolymer, D-xylan extracted from barley straw released L-arabinose by the enzymes in addition to D-xylose. D-xylanase I could liberate L-arabinose far more rapidly than D-xylanase II. At the early stage of the reaction, moreover, L-arabinose was liberted more rapidly than D-xylose by D-xylanase I. Both D-xylanases hydrolyzed

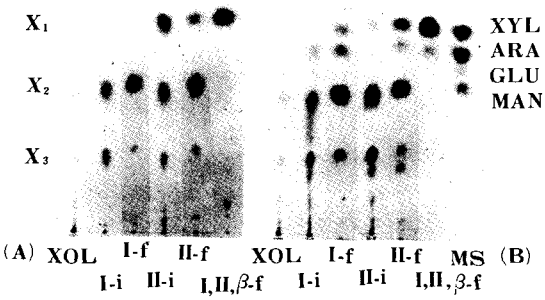

Fig. 14. Paper chromatogram of reaction products of larchwood homoxylan(A) and barley straw xylan(B).

XOL : xylose, xylobiose, xylotriose standard
MS: xylose, arabinose, glucose, mannose standard.
The initial stage(i) and the final stage(f) of the hydrolytic reaction with xylanase I, II, and β-xylosidase (β) mixtures.

barley straw D-xylan to about 25% in 1% solution after the incubation of 72 hours at 30°C. β-Xylosidase hydrolyzed barley straw xylan to the degree of about 20% with the liberation of only D-xylose.

The subsequent addition of D-xylanase II and β-xylosidase to the hydrolyzate by D-xylanase I increased the degree of hydrolysis to 28% for commercial D-xylan and 54% for barley straw D-xylan with the liberation of only monomeric sugars. The order of the addition of each enzymes did not affect the degree of hydrolysis.

Degree of hydrolysis of D-xylan in different concentrations: As is seen in Fig. 15, commercial and barley straw

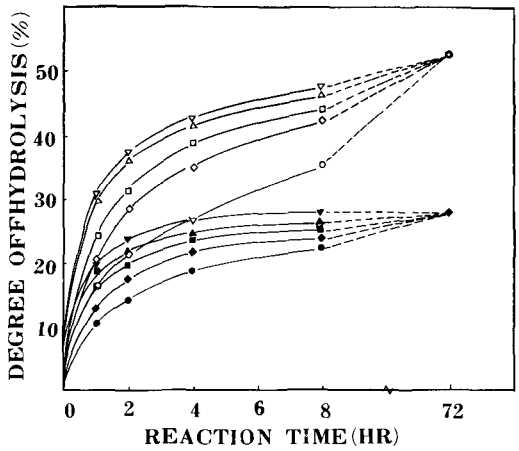

Fig. 15. Degree of hydrolysis according to various substrate concentration.

0.1%(▽), 0.25%(△), 0.5%(□), 1.0%(◇) and 2.5%(○), solution of barley straw xylan and 0.1%(▽), 0.25%(▲), 0.5%(■), 1.0%(◆), and 2.5%(●) solution of commercial xylan.

D-xylan were hydrolyzed to the degree of 28% and 54%, respectively, by the reconstituted D-xylanase system composed of equal amounts of D-xylanase I, II, and β-xylosidase The varied substrate concentration from 0.1% to 2.5% tested did not affect the degree of hydrolysis of the substrates by the reconstitueted D-xylanase system.

Degree of hydrolysis with varied ratio of β-xylosidase to D-xylanase I and II: Commercial and barley straw D-xylan were hydrolyzed to the degree of no more than 15% and 34%, respectively, by D-xylanase mixture containing the equal amount of each enzyme. Addition of β-xylosidase to the reaction mixture increased their digestibility to the degree of 28% and 54%, respectively, in 1% substrate solu-

tion. The initial velocity of the degradation of D-xylan was proportionally increased with the increased amount of β-xylosidase added to the xylanase mixture. However, the ratio of β-xylosidase to D-xylanase from 0.01 to 1.0 tested did not affect the degree of hydrolysis of the substrates.

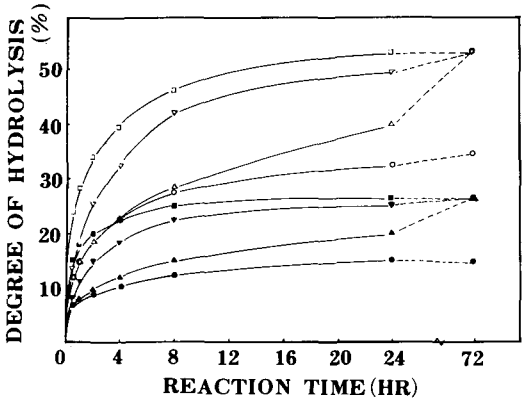

Fig. 16. Degree of hydrolysis according to various ratios of β-xylosidase to D-xylanase I and II.

0 (○, ●), 0.01(△, ▲), 0.1(▽, ▼), and 1.0u/ml(□, ■) of β-xylosidase was added to 1.0% solution of barley straw xylan(open marks) or commercial xylan(closed marks) which contains 1.0u/ml of D-xylanase I and II, respectively.

Discussion

Due to the limitation of natural resources, the efficient utilization of agricultural wastes as a renewable resource has become a great concern. In general, agricultural wastes are constituted with cellulose, hemicellulose, and lignin. Among these constituents, cellulose has been recognized as an important resource for the development of the substitute of fossil energy. However, hemicellulose was paid less attention than cellulose, although it occupies about 20-25% of the constituent of agricultural wastes. It is, thus, no doubt that proper utilization of hemicellulose is an important factor that determines the economy of the utilization of agricultural wastes (or biomass).

D-xylan occupies one half of hemicellulose and is distributed through all land plants with the contents of 30% for annual plant, 20-25% for hard wood, 7-12% for soft wood, 25% for corn cobs, and 15% for wheat bran.[28-31] many D-xylans from land plants have been shown to have a basal structure of backbone of (1-4)-linked β-D-xylopyranoside residue. But linear, simple homoxylan is rare except from esparto grass,[32] tamarind seed,[33] and cotton shell.[1] They differ in the structural arrangements of other component sugar residues, commonly, L-arabinose, D-glucuronic acid, and its 4-methyl ester, as a single unit of side chain or multiple units of branches. Recently, with the aids of various hemicellulases, the chemical structure of xylan became understood. The degradation product of xylan is D-xylose, which is an important substrate for ethanol as well as acetone-butanol fermentation[34,35] and SCP Production.[36] Xylitol, a non-cariogenic artificial sweetner, can also be produced from xylose by direct hydrogenation.[37] Xylose can also be converted to furfural with high yield which is a starting material for Nylon 66.[38]

Thus, the establishment of the bioconversion process of hemicellulose in biomass appears to be important as much as that for cellulose. Recently, studies on the hemicellulase system have been attracted and appeared in several publications.[39,40] However, there is controvercy in the mechanism of D-xylanse system for the degradation of xylan. In particular, the multiplicity of D-xylanase from fungal origins has been reported.[8-10,41] *Aspergillus niger*, for example, produced multiple numbers (1 to 10 kinds of xylanases) of xylanases according to the strains studied.[42]

As a result of three stages of purification procedures we obtained two kinds of pure D-xylanase I and II which were purified 510- and 106-fold as compared with the crude enzyme extract, respectively. Their molecular weights were relatively small (12,500 and 11,500) and these enzymes appeared to be acidic ones.

Considering the physicochemical properties of these enzymes, xylanase I and II purified from *A. niger* KG-79, it is likely that they resemble with xylanase II and III reported by Fukumoto et al.[6,8] We also have separated three kinds of D-xylanase from another strain *Aspergillus niger* KTS-321. But the degree of hydrolysis of xylan by these enzymes were the same as the two enzyme system.

D-Xylanase degrades xylan to xylose, xylobiose, and xylotriose, to the degree of about 15% as reducing sugar for commercial xylan and about 34% for barley straw xylan. Takenish et al.[8] hydrolyzed rice straw xylan to the degree of about 60% by the combination of three kinds of xylanase.

With the addition of β-xylosidase to the xylan hydrolyzate by xylanase, the liberation of monomeric sugar increased to the degree of conversion of 54% and

28% for barley and larchwood xylans, respectively. This limit of hydrolysis did not appear to be caused by the product inhibition of the enzymes, but caused by the structural properties of the substrates, isolated pure xylans. It was found that the unconverted xylan became precipitated after hydrolysis process.

The different types of xylanases as mentioned previously did not affect the degree of hydrolysis. Neither variation of substrate concentration nor varied ratio of β-xylosidase to D-xylanases affected the degree of hydrolysis. After the removal of the soluble product by centrifugation, the unhydrolyzed precipitate was further hydrolyzed by the addition of fresh xylanases. However, the enzymes couldn't attack the precipitate as it was, whereas precipitate retreated with solvent such as acetone or direct drying was degraded again by the enzyme to the same degree as before. Consequently, it is concluded that the structural properties of the substrate appears to be the limiting factor for the determination of the degree of hydrolysis of the isolated xylan as a substrate. It was observed that the degree of conversion of xylan in barley straw *in situe* was more than 80%.[43]

요 약

본 연구에서는 ***Aspergillus niger*** KG79에서 두 종류의 D-xylanase를 분리·정제하여 그 특성을 규명하였다. 이 두 종류의 D-xylanase의 물리화학적 및 동력학적 특성은 큰 차이가 없었다. 이들 Xylanase는 D-Xylan으로부터 Xylose, Xylobiose와 Xylotriose를 분해 생성하였다. 그러나 보리짚 Xylan을 기질로 사용했을 경우에는 Xylanase I은 II보다 측쇄 arabinose를 상당히 빨리 분해하였다. 이들 효소에 의한 Xylan의 분해도는 기질의 종류에 따라 차이가 나서 보리짚 Xylan과 larchwood Xylan의 분해도는 각각 10%와 25%(환원당량) 정도로 나타났다.

순수정제된 Xylanase와 β-Xylosidase를 사용하여 재조합한 Xylanase계의 기질분해력을 비교한 결과 최적조건에서 보리짚과 larchwood Xylan은 각각 28%와 54%씩 분해 전환되었다. 이러한 결과는 Xylan의 효소분해도의 제한요소는 기질의 물리적 특성의 차이에 기인함을 추정할 수 있었다.

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