聖徳栄養短期大学紀要No. 23 (1992)

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JOURNAL OF FERMENTATION AND BIOENGINEERING
Vol. 74, No. 6, 392-394. 1992

Comparison of Xylan and Methyl β-Xyloside-Induced Xylanases from *Streptomyces* sp.

KOTOYOSHI NAKANISHI,[1]* MASAKI MARUI,[2] AND TSUNEO YASUI[2]

Department of Brewing and Fermentation, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156,[1] and Seitoku Junior College of Nutrition, 1-4-6 Nishishinkoiwa, Katsusika-ku, Tokyo 124,[2] Japan

Received 17 June 1992/Accepted 27 August 1992

Three xylanases induced by xylan from *Streptomyces* sp. no. 3137 were purified to homogeneity. The enzymatic, physicochemical, and immunological properties of the enzymes were compared with those of three xylanases induced by non-metabolizable methyl β-xyloside. It was found that each xylanase produced under different culture conditions showed very similar properties.

There are many kinds of microorganisms which can produce xylanase in the presence of xylan as a carbon source (1). It has been reported that the xylanase of *Streptomyces*, except for *Streptomyces ostreogriseus*, consists of one enzyme system (2-7). We have already found that the production of xylanase by *Streptomyces* sp. no. 3137 could be induced by a variety of non-metabolizable β-xylosides, which showed marked inducing ability in comparison with xylan and related materials (8-10). In previous papers, three xylanases induced by methyl β-xyloside from the strain, tentatively named X-I, X-II-A, and X-II-B, were purified to homogeneity. While X-I was evidently different from X-II-A and X-II-B in enzymatic, physicochemical, and immunological properties (11, 12), strong similarities were found between X-II-A and X-II-B in various properties tested. On the other hand, the information on the properties of xylanases induced by xylan from the strain is very limited. It is interesting to compare the enzyme systems induced by xylan and by methyl β-xyloside.

The present paper deals with the purification and properties of xylanases induced by xylan from *Streptomyces* sp. no. 3137. In addition, similarities in the properties of the enzyme systems induced by xylan and methy β-xyloside are discussed.

The growth medium consisted of 3 g glucose, 0.3 g urea, 1 g KH_2PO_4, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.1 g yeast extract, and 0.5 g corn steep liquor in 100 ml of tap water. This was inoculated with *Streptomyces* sp. no. 3137 and incubated at 36°C for 40 h in a shaker (120 strokes/min). The mycelia were then harvested and transferred into a 500 ml flask containing 100 ml inducing culture medium consisting of 0.45 g insoluble xylan (0.3 g as anhydroxylose) (13), 0.2 g KH_2PO_4, and 0.01 g $MgSO_4 \cdot 7H_2O$ in 100 ml distilled water. This was incubated for 24 h under the same culture conditions and the culture filtrate was used for enzyme purification.

Xylanase activity was measured by the release of reducing sugar from xylan as described previously (11). One unit of activity was defined as the amount of enzyme capable of liberating 1 μmol of xylose equivalent in 1 min. Protein concentration was measured by the Lowry method with bovine serum albumin as the standard.

The amino acid composition of xylanase was analyzed with a Durrum D-502 amino acid analyzer after hydrolysis of the enzyme with 6N HCl for 24 h at 110°C in a sealed evacuated tube. Polyacrylamide slab gel electrophoresis was done in a 10% acrylamide gel with β-alanine-acetic acid buffer (pH 4.3) at 15 mA for 45 min. The molecular weights of the purified enzymes were estimated by sodium dodecyl sulfate slab gel electrophoresis. Bovine serum albumin (68000), ovalbumin (45000), RNA polymerase from *E. coli* α-subunit (39000), chymotrypsinogen A (25000), trypsin inhibitor (21500), cytochrome C (12500) were used as marker proteins. Isoelectric focusing was carried out with a 110 cm-column (LKB Produkter AB, Bromma, Sweeden) for 48 h at 900 V and 4°C. Servalyte 6-8 or 9-11 ampholyte was employed. Experimental conditions relating to the electrophoresis and the isoelectric focusing were described in detail in a previous paper (11). Rabbit antisera against two xylanases used, X-I and X-II-B, were prepared in a previous study (12). These antisera were stored at −15°C before use.

Purification of xylanases induced by xylan was done according to the previously outlined procedure (11). The results are summarized in Table 1. The culture filtrate obtained from inducing culture (2,000 ml) was concentrated to about one-tenth of the original volume by ultrafiltration with DIAFLO UM-10. The concentrated solution (235 ml) was applied on a DEAE-Sephadex A-25 column (2.6× 45 cm) equilibrated with McIlvaine buffer (pH 5.5). The eluate (330 ml) was decolorized by this column and then concentrated to 30 ml by rotary evaporator at 35°C. Ten

TABLE 1. Purification of xylanases from *Streptomyces* sp. no. 3137

Step	Total protein (mg)	Total activity (units) × 10^2	Specific activity (units/ml)	Yield (%)
Culture filtrate	2350	960	40	100
UM-10	1070	791	74	82
DEAE Sephadex A-25	875	722	83	75
Bio Gel P-100				
Xn-1	218	404	186	42
Xn-2	125	165	132	17
(sum)				(59)
Isoelectric focusing				
Xn-1	165	317	192	33
Xn-2-a	14	16	114	2
Xn-2-b	53	75	143	8
(sum)				(43)

* Corresponding author.

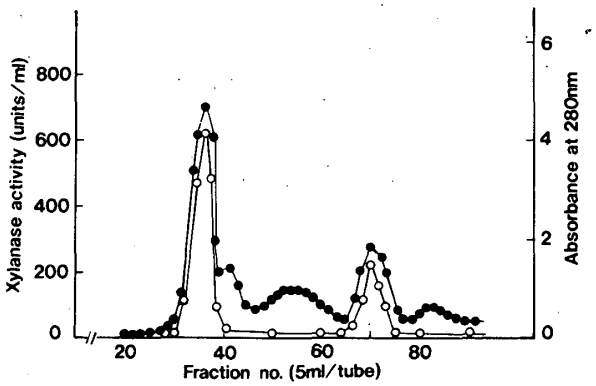

FIG. 1. Bio Gel P-100 column chromatography. Experimental conditions are described in the text. Symbols: ●, 280 nm; ○, enzyme activity.

ml of the concentrated eluate was applied on a Bio Gel P-100 column (2 × 90 cm) equilibrated with the same buffer. The gel filtration was done with the same buffer at 4°C at the rate of 10 ml/h.

Xylanase activities were recovered in two fractions and were tentatively named Xn-1 (fraction no. 31–39) and Xn-2 (fraction no. 66–75) (Fig. 1). Each active fraction was separately dialyzed against distilled water at 4°C and concentrated to about 1 mg of protein/ml by an evaporater at 35°C. Ten ml of the concentrated Xn-1 or Xn-2 was applied on a column of 110-ml capacity for isoelectric focusing. Using Servalyte 6–8, the Xn-1 fraction gave only one protein peak which was positive for xylanase activity. The isoelectric point of this fraction was 7.1. In the case of the Xn-2 fraction using Servalyte 9–11, xylanase activity was recovered in two fractions, the isoelectric points of which were 10.1 and 10.3, and which were named Xn-2-a and Xn-2-b, respectively. The total activity of recovered Xn-2-b was five times as great as that of Xn-2-a. Each of the purified enzymes gave a single protein band on polyacrylamide slab gel electrophoresis (photograph not shown).

In the previous report (11), the xylanase activities induced by methyl β-xyloside were recovered from two fractions (X-I and X-II) on a Bio Gel P-100 column. With electrofocusing, the X-II fraction of MW 25,000 was fractionated into two fractions (X-II-A and X-II-B). No significant differences in the elution patterns by gel chromatography and isoelectric focusing were observed between xylan-induced xylanases and methyl β-xyloside-induced xylanases. However, the ratio of xylanase activity between the Xn-1 and Xn-2 fractions was about 3 : 1, while that of the Xn-2-a and Xn-2-b fractions was about 1 : 5. This result was very different from that obtained in the previous paper (the ratio of activity between X-I and X-II, and X-II-A and X-II-B were about 1 : 1 and 1 : 3, respectively). In addition, the total xylanase activity of the crude preparation, shown in Table 1 (96,000 units/2,000 ml culture filtrate), was lower than that obtained in the inducing culture medium containing methyl β-xyloside (200,000 units/2,500 ml culture filtrate), as reported previously (11).

We have already reported that the amount of xylanase produced with the inducing culture was dependent on the initial rate of xylanase synthesis, which was in turn dependent on the inducer concentration. To keep up the xylanase synthesis at the maximal rate in the inducing culture, a saturated concentration of intracellular inducer was always needed (10). In other words, a decrease of inducer concentration in the inducing culture medium during the induction resulted in a decrease in the rate of xylanase synthesis and the amount of xylanase produced. Although xylan added to the inducing culture medium was used by the cell as an energy source and inducer at the early stage of the induction, the intracellular concentration of non-metabolizable methyl β-xyloside was nearly constant throughout the induction. Therefore, methyl β-xyloside and other various β-xylosides were more effective in induction than metabolizable xylan and its related materials (8–10).

In consideration of these findings, the difference in both cases of xylan and methyl β-xyloside described above may be closely associated with the difference in the property of inducers used. However, the difference in the quantitative ratio of the three xylanases induced by xylan and methyl β-xyloside cannot be explained satisfactorily on the basis of the difference in the property of the inducers. In order to clarify this aspect, a detailed investigation of the genetic regulation system of xylanase synthesis in *Streptomyces* sp. is required.

The enzymatic and physicochemical properties of the purified enzymes (Xn-1, Xn-2-a, and Xn-2-b) were compared with those of xylanases (X-I, X-II-A, and X-II-B) purified as described previously. The optimal pH and temperature, pH and thermal stabilities, molecular weights, apparent K_m values for xylotriose and xylotetraose, and isoelectric points of these six enzymes are summarized in Table 2. There are no significant differences between xylan-induced and methyl β-xyloside-induced xylanases. The

TABLE 2. Some properties of xylanases induced by xylan (Xn) and methyl-β-xyloside (β-MX)

	Xn-induced xylanases			β-MX-induced xylanases[a]		
	Xn-1	Xn-2-a	Xn-2-b	X-I	X-II-A	X-II-B
Optimum pH	5.5–6.5	5–6	5–6	5.5–6.5	5–6	5–6
Optimum temperature	60–65°C	60–65°C	60–65°C	60–65°C	60–65°C	60–65°C
pH stability[b]	5.5–6.5	5–6	5–6	5.5–6.5	5–6	5–6
Thermal stability[c]	55°C	55°C	55°C	55°C	55°C	55°C
K_m value						
for Xylotriose	33.3	—	40.0	30.1	—	33.0
for Xylotetraose	9.1	—	11.2	9.0	—	9.1
Molecular weight	50000	25000	25000	50000	25000	25000
Isoelectric point	7.1	10.1	10.3	7.10	10.06	10.26

[a] See reference (11).
[b] The enzyme solution was maintained at various pHs at 35°C for 30 min and the residual activity was measured at the optimum pH.
[c] The enzyme solution was maintained at various temperatures for 30 min, followed by rapid cooling in ice-cold water, after which the residual activity was measured.

TABLE 3. Amino acid composition of xylanases

Amino acid	Molar ratio (%)			
	Xn-1	Xn-2-b	X-I[a]	X-II-B[a]
Aspartic acid	12.9	12.3	13.1	11.5
Threonine	7.4	12.8	7.7	11.6
Serine	6.4	11.2	7.9	10.4
Glutamic acid	9.0	6.1	8.8	5.7
Proline	2.4	2.7	2.5	3.3
Glycine	13.1	14.6	12.4	15.9
Alanine	9.6	3.6	9.4	3.8
Valine	6.7	5.7	6.0	6.1
Cysteine[b]	2.5	1.0	2.7	1.3
Methionine	1.9	1.4	1.3	1.6
Isoleucine	3.7	2.5	3.5	2.2
Leucine	5.2	4.1	5.9	4.0
Tyrosine	3.8	8.5	3.4	8.2
Phenylalanine	2.4	3.0	2.8	3.3
Histidine	1.5	1.6	1.9	1.5
Lysine	3.8	2.4	3.1	2.7
Arginine	5.0	3.5	5.1	3.5
Tryptophan[c]	2.8	3.1	2.6	3.1

0.5 mg of the purified enzyme was used for the analysis of amino acids.

[a] See reference.

[b] Not corrected for destruction.

[c] Measured spectrophotometrically (15).

amino acid compositions of fractions Xn-1 and Xn-2-b were compared with those of X-I and X-II-B, as shown in Table 3; no significant differences in amino acid composition between fractions Xn-1 and X-I, and between fractions Xn-2-b and X-II-B were revealed.

In immunological studies, each of the antisera against X-I (anti-X-I-serum) and X-II-B (anti-X-II-B serum) was used in all combinations with the four xylanases (Xn-1, X-I, Xn-2-b, and X-II-B) in double diffusion experiments. These results are shown in Fig. 2. On Ouchterlony double diffusion plates (14), anti-X-I serum formed precipitation bands with Xn-I and X-I. The precipitation patterns with Xn-1 and X-I showed the converging arc of a homogenous reaction. In the case of precipitation patterns with Xn-2-b and X-II-B, a similar result was observed (Fig. 2). No precipitation band was observed with combinations of anti-X-I serum and Xn-2-b, nor with anti-X-II-B serum and Xn-1. Neutralization of xylanase activity with antisera was examined. The reaction mixture containing McIlvaine buffer (pH 5.5), purified xylanase, and antiserum was incubated at 40°C for 1 h. The concentration of xylanase and antiserum was defined by considering the result obtained previously (10). The residual activity was measured by the standard assay. Almost all of the activities of Xn-1 and Xn-2-b were neutralized with 200 μl anti-X-I serum or 400 μl anti-X-II-B serum, respectively. These results indicated that Xn-1 and X-I, or Xn-2-b and X-II-B, were very similar in immunological properties.

The several results obtained in this study suggested that the xylanases induced by xylan were identical with those induced by methyl β-xyloside, and that the enzyme systems induced by xylan and by methyl β-xyloside were the same.

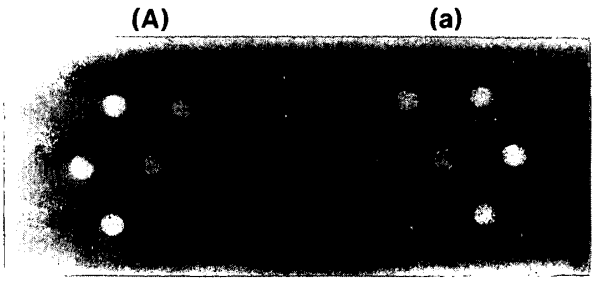

FIG. 2. Ouchterlony double immunodiffusion. The gel, containing 0.01 M potassium phosphate buffer (pH 7.2), 0.15 M NaCl and 1% agar, was incubated at room temperature in a wet box. Outside wells contained 5 μl of xylanase (2 mg/ml), X-I (I), Xn-1 (1), X-II-B (IIB), or Xn-2-b (2b). Center wells contained rabbit anti-X-I serum (A) or anti-X-II-B serum (a). The precipitation band was stained with Coomassie brilliant blue R 250.

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