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Studies on hemicellulose-hydrolyzing enzymes from Neurospora sp.

II. Water soluble substrate, glycol hemicellulose

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In the preceding paper, the authors reported that ***Neurospora*** sp. produces at least four types of hemicellulose-hydrolyzing enzymes (HHE). Each of these enzymes was partially purified by column chromatography. However, the mode of action of each enzyme toward substrate hemicellulose was not studied.

A convenient definition of hemicellulose was given by Whistler and Smart,[1] who stated that hemicelluloses are plant cell wall polysaccharidcs, which are insoluble in water and soluble in a dilute alkali solution. Chemically, the term hemicellulose covers a large class of compounds, which contain a glycoside chain consisted of D-xylose, D-mannose, D-galactose and other minor sugar residues.

The structural complexity of hemicellulose is different from each other, according to the sources from which hemicellulose was extracted. Thus, the structural complexity and insolubility in aqueous medium of hemicellulose make a difficulty for establishing a standard method of assay of the activity of HHE. It is, therefore, necessary to study hemicellulose itself previous to characterizing the mode of action of each HHE isolated from ***Neurospora*** sp. The first problem to be solved will be solubilization of substrate hemicellulose without a loss of digestibility by the enzyme.

Experimental

Materials

A commercial crude hemicellulose-hydrolyzing enzyme preparation

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from ***Neurospora*** sp. was purchased from Nagase Sangyo Co. Ltd. A crude enzyme preparation from ***Tricoderma koningi,*** which contains HHEs, was kindly donated by Meiji Seika Co. Ltd.

Methods

Preparation of glycol hemicellulose

Corn seed hemicellulose (34 g)[2] was suspended in 180 ml of 14 % sodium hydroxide solution in a pressure-proof bottle with 500 ml volume, and the suspension was allowed to stand under reduced pressure* for 2 hours at a room temperature. After cooling the suspension to 0°C, ethylene oxide (30 g) was added under stirring. The temperature of the reaction mixture was raised gradually to 30°C in one hour. The resulting syrup was poured into 95 % ethanol to precipitate glycol hemicellulose. The precipitate was washed several times with 95 % ethanol until the sodium hydroxide was almost completely removed. Glycol hemicellulosc was then dissolved in a small amount of water and the solution was neutralized with 2 N hydrochloric acid and dialyzed against five changes of water for 3 days. After insoluble material was removed by centrifugation, the supernatant was dried by lyophilization.

Assay of the activity

To 1 ml of 0.5 % glycol hemicellulose solution, 1 ml of the enzyme solution was added and the reaction mixture was incubated at 40°C for 60 minutes. Reducing power produced was analyzed by Somogyi-Nelson method. In the case of hemicellulose, insoluble material in the substrate solution was removed previous to the addition of the enzyme solution." Enzyme unit represents the enzyme amount which can produce the reducing power equivalent to 1 μmole of xylose in 60 minutes at 40°C.

Results

Some properties of glycol hemicellulose

Glycol hemicellulose is pale brown powder, being easily soluble in water and insoluble in organic solvents. Since the change in the viscosity due to the cleavage of glycoside linkage was extremely small, the viscometry could not be applied for the assay of the activity. Only xylobiose was formed by hydrolysis of glycol hemicellulose by 0.3 N hydrochloric acid at 100°C for 5 hours, but not xylose and substituted

* To accelerate the penetration of the sodium hydroxide into the micelle of hemicellulose.

sugars. Therefore, the extent of substitution at hydroxyl group of xylopyranose by β-hydroxyethyl group could not be estimated by analysis of hydrolyzed product.

The pH-dependence of activity toward glycol hemicellulose

The pH-dependence of the activity of crude enzyme preparation from ***Neurospora.*** sp. toward glycol hemicellulose is shown in Fig. 1. The maximum activity appeared near pH 6.0.

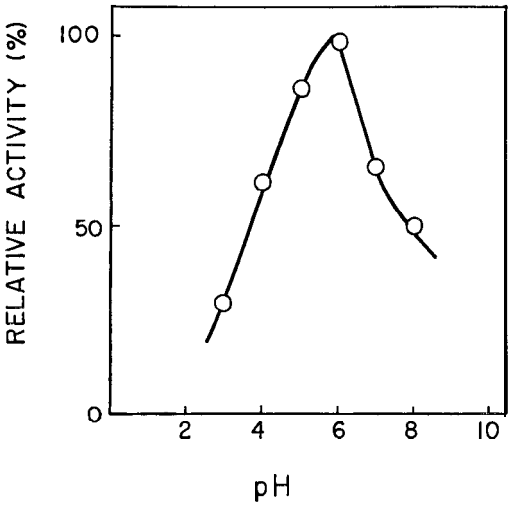

Fig. 1. The pH-dependence of the activity of the crude enzyme preparation toward glycol hemicellulose.

Temperature-dependence of activity toward glycol hemicellulose

The activity was measured at pH 6.0 by incubating the reaction mixture for 60 minutes at various temperatures. Contrary to the case of unaltered hemicellulose as a substrate, the crude enzyme preparation exhibited considerably sharp temperature-activity profile with maximum at 50°C as shown in Fig. 2. In the former case, the crude enzyme preparation showed about half activity of the maximum even at 80°C.

Comparison of both substrates, hemicellulose and glycol hemicellulose

1. Effect of substrate concentration : One milliliter of 0.5 % crude enzyme solution from ***Neurospora*** sp. was added to 1 ml of the substrate solution at a given concentration. As shown in Fig. 3, both substrates show typical curve usually observable in enzyme kinetics.

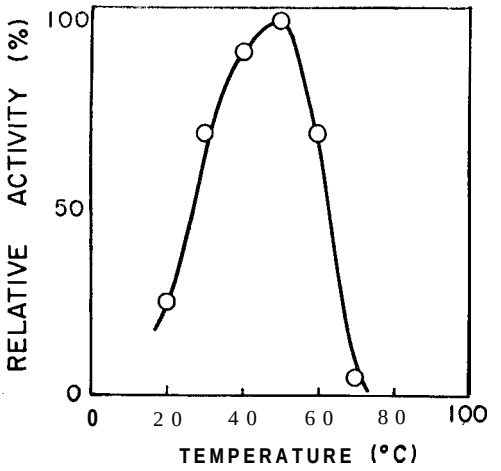

Fig. 2. Temperature-dependence of the activity of the crude enzyme preparation toward glycol hemicellulose.

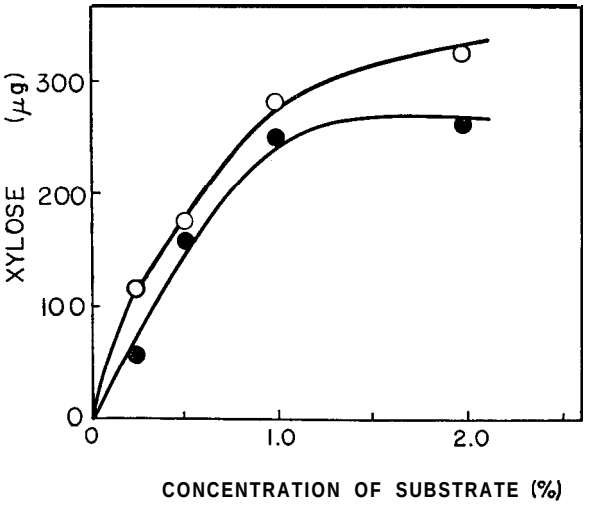

Fig. 3. Effect of substrate concentation on the activity of the crude enzyme preparation.
○ : glycol hemicellulose,
● : hemicellulose.

2. Effect of enzyme amount : The formation of reducing power from glycol hemicellulose was proportional to the amount of the enzyme added, while that from hemicellulose showed a maximum value and then gradually decreased with increase in the enzyme amout, as shown in Fig. 4.

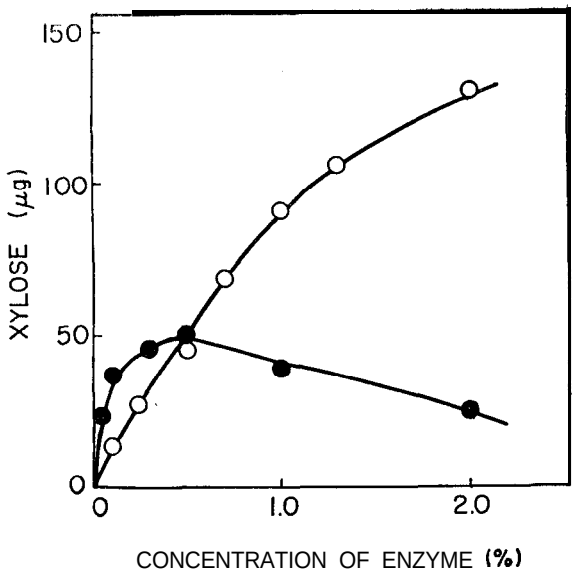

Fig. 4. Effect of enzyme concentration.
○: glycol hemicellulose,
●: hemicellulose.

3. Time-course of appearance of reducing power : The difference in the rate of reducing power formation was not observed between both substrates as seen in Fig. 5.

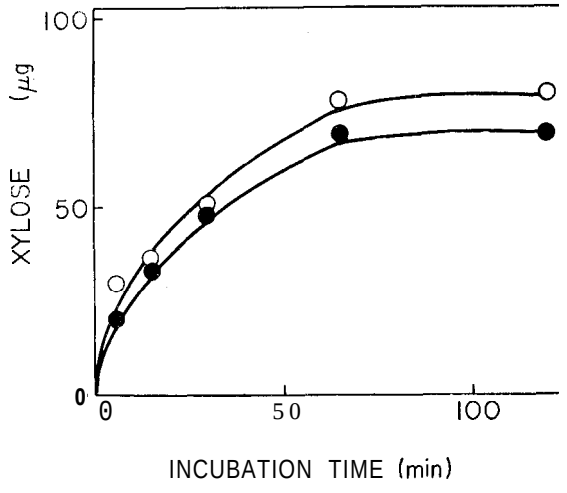

Fig. 5. Rate of hydrolysis of substrates.
○ : glycol hemicellulose,
● : hemicellulose.

Hydrolysis of glycol hemicellulose by enzymes produced by Tricoderma koningi

A ***crude*** enzyme preparation from ***Tricoderma koningi*** hydrolyzes both substrates, whereas a partially purified enzyme by column chromatography on Amberlite IRC-50 could hardly hydrolyze glycol hemicellulose. The results are summarized in Table I.

Table I. Activity of enzymes from ***Tricoderma*** *koningi*. One milliliter of 0.5 % substrate solution was hydrolyzed by 1 ml of enzyme solution at 40°C for 1 hr. OD_{280} of crude enzyme solution and partially purified enzyme solution were 2.20 and 0.95, respectively.

	Hemicellulose (μg xylose)	Glycol hemicellulose (μg xylose)
Crude enzyme	370	405
Partially purified by Amberlite IRC-50	265	55

Discussion

Corn seed hemicellulose is only soluble in a dilute alkali solution. Nevertheless, the substitution of hydroxyl group of hemicellulose by β-hydroxyethyl group did not occur in the dilute alkali solution. For the reaction of ethylene oxide with hydroxyl groups of sugar residues, higher alkali concentration than 10 % was needed, though hemicellulose was insoluble in sodium hydroxide solution at such a concentration. Only a substance with same Rf value as xylobiose was formed by acid hydrolysis of glycol hemicellulose by 0.3 N hydrochloric acid. The extent of glycolation (β-hydroxyethylation) therefore could not be estimated by acid hydrolysis and successive paper chromatography. However, it is probable that the extent of substitution of hydroxyl groups (presumably 3-OH group) by β-hydroxyethyl group is quite low, not affecting the Rf value of xylobiose unit in paper chromatography. The viscosity of glycol hemicellulose at pH 6.0 was not enough to application of viscometry for an estimation of the amount of glycoside linkag hydrolyzed.

In assay of the activity with hemicellulose unaltered, the temperatur activity profile showed a broad peak, indicating 50 % activity of th maximum even at 80°C. Contrarily, when glycol hemicellulose was us as substrate, the crude enzyme preparation exhibited a sharp temper ture-dependence of the activity, losing its activity completely at 70 These facts mean that the crude enzyme preparation from *Neurospo* sp. contains, at least, two types of HHE; one is heat-stable and has l

capability to hydrolyze glycol hemicellulose, and the other behaves just reversely. The existence of HHE, which shows low activity toward glycol hemicellulose, was also evidenced in a crude enzyme preparation from ***Tricoderma*** *koningi*. **(see** Table I).

A remarkable difference in the digestibility of both substrates, hemicellulose and glycol hemicellulose, was seen in the effect of the enzyme concentration on the production of reducing power (Fig. 4). The production of reducing power from glycol hemicellulose was proportional to the enzyme amount added, while that from hemicellulose gradually decreased with increase in the enzyme amount after it had attained a certain maximum value. The decrease in the reducing power seen in latter case may be arisen from a transglycosidation. It is, thus, evident that glycol hemicellulose is more preferable substrate for assay of some kinds of HHE.

The characterization of each HHE, which had been distinguished chromatographically from the other components, may be made by choosing a suitable substrate. The authors are now attempting to characterize HHE by its mode of action toward various substrates.

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2) M. Dirdjohadipoetro, K. Hayashi and M. Funatsu, J. Fac. Agr., Kyushu Univ., 15, 257 (1969).