ENZYMATIC HYDROLYSIS OF SOYBEAN POLYSACCHARIDES

II. INTERMEDIATE PRODUCTS IN THE HYDROLYSIS OF THE HEMICELLULOSE B_1 OF SOYBEAN COTYLEDONS BY TAKA-DIASTASE

Teiiti NARASAKI

(Laboratory of Agricultural Products Technology)

In the first paper of this series,[1] hydrolysis of the hemicellulose B_1 of soybean cotyledons by Taka-diastase was reported. Taka-diastase liberated xylose, arabinose, galactose, and galacturonic acid in the hydrolyzate and gave an unhydrolyzable polysaccharide residue rich in rhamnose, fucose, xylose, glucose, and galacturonic acid. Further, five intermediate hydrolysis products were formed in an early stage of the incubation and two of them remained unchanged in the hydrolyzate after 48 hours' incubation. These results seemed to indicate that some informations on the components and the structures of the hemicellulose B_1 would be obtained by examination of the intermediate hydrolysis products.

The present investigation was undertaken to see if examination of the intermediate hydrolysis products could give any informations for the components and the structures of the hemicellulose B_1.

Experimental

1. Materials and Methods

The hemicellulose B_1 and the enzyme solution were prepared according to the procedures reported in the preceding paper.

Enzymatic hydrolysis was performed under the standard conditions described in the preceding paper.

2. Separation of the Intermediate Hydrolysis Products

To 100 ml each of the reaction mixtures at 4 hr and 48 hr incubation 200 ml of absolute ethanol was added to stop the reaction and to precipitate the residual polysaccharides. After centrifugation, The supernatant clear solutions were concentrated *in vacuo* below 40° C to about 0.5 ml. The obtained sugar solutions were used for the separation of the intermediate hydrolysis products by ascending paper chromatography on Toyo Roshi No.50 filter paper sheets of 40×40 cm. The solvent mixtures were *n*-butanol-acetic acid-water (4:1:2, double development) and *n*-butanol-pyridine-water (6:2:3, single development). The separated areas were detected by consulting the guid strips colored by *p*-anisidine-HCl. The zones containing each intermediate hydrolysis products were cut and extracted with 10 ml of water. The obtained sugar solutions were hydrolyzed with $2N$ H_2SO_4 (final concentration) in boiling water for 4 hr. The hydrolyzates were neutralized with $BaCO_3$ and the formed

precipitate was removed by centrifugation and filtration through Toyo Roshi No. 5-C filter paper. The component sugars of these hydrolyzates were determined by paper chromatography.

3. The Intermediate Products at 4 Hours' Incubation

Paper chromatograms of the acid hydrolyzates of the intermediate hydrolysis products are given in Fig. 1.

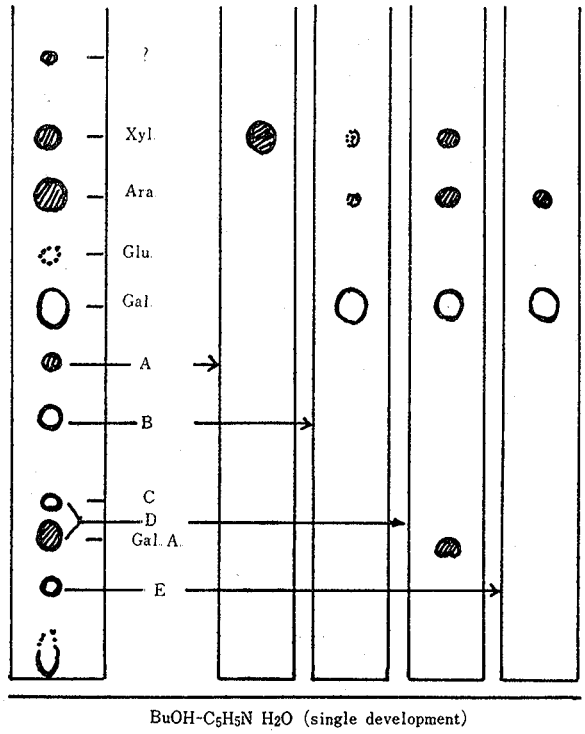

Fig. 1. Component sugars of the oligosaccharides in 4 hours' enzymatic hydrolyzate

This enzymatic hydrolyzate contained 5 intermediate products, A, B, C, D,and E, as reported in the preceding paper.

The spot A gave only xylose by hydrolysis with H_2SO_4. The spot B consisted of galactose and trace amounts of xylose and arabinose. The spot E gave arabinose and galactose in the ratio of 1 : 2.

4. The Intermediate Products at 48 Hours' Incubation

The reaction mixture incubated for 48 hr contained only 2 intermediate products, C and D, as shown in Fig. 2.

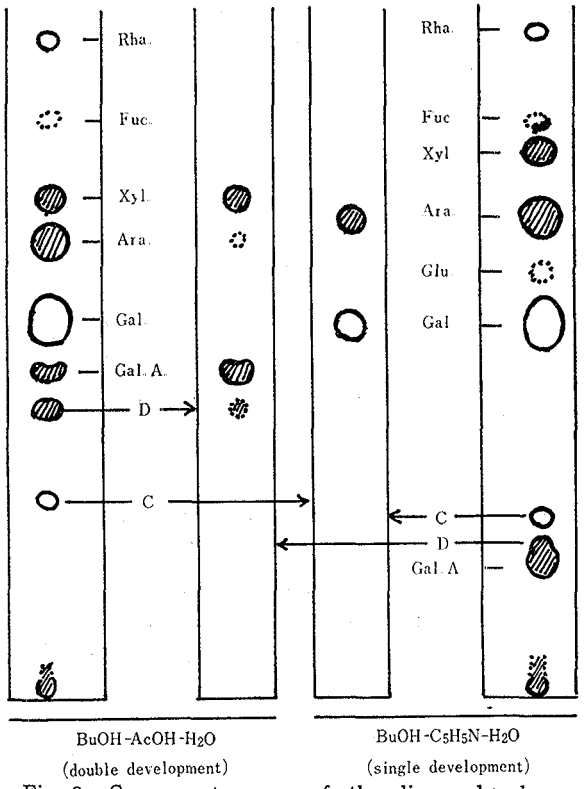

Fig. 2. Component sugars of the oligosaccharides in 48 hours' enzymatic hydrolyzate

The spot C consisted of arabinose and galactose in the ratio of 1:1. The spot D gave xylose and galacturonic acid in the ratio of 1 : 1.

Discussion

The spot A indicates the presence of xylose-xylose linkage and the linkage can be hydrolyzed by Take-diastase, since the spot disappeared after 12 hr incubation as has been shown in Fig.6 in the preceding report. The spot B shows the presence of galactose-galactose linkage and this linkage can also be hydrolyzed by Taka-diastase, because this spot appeared in an early stage of the reaction and was not detected after 8 hr incubation. The spot E seemed to be arabinose-galactose and hydrolyzed to the spot C, which appeared to be arabinose-galactose and resistant to Taka-diastase, because the spot E disappeared after 8 hr incubation while the spot C increased with the progress of incubation and was detected from the reaction mixture incubated 48 hr. The behaviors of the spots B, C, and E seemed to confirm the presence of an arabogalactan in the hemicellulose B_1. The spot D gradually increased with the progress of incubation as the spot C and was detectable in the reaction mixture incubated for 48 hr. This spot may be a xylosyl-galacturonic acid resistant to the activity of Taka-diastase. The increase and decrease of the spots A and D during the incubation seemed to establish the presence of a galacturonic acid-containing xylan in the hemicellulose B_1. It is well known that plant xylans contain galacturonic acid. The above results seemed to show that no essential difference exists between wood xylans and a xylan of soybean cotyledons.

Acknowledgment

The author is grateful to prof. Sin'itiro KAWAMURA, Laboratory of Biological Chemistry. this University, for his interest in this work. Valuable suggestions of prof. Akira KAJI, Laboratory of Fermentation Chemistry, this University, are greatefully acknowledged.

A part of expenditure was defrayed by the research fund donated by the Ministry of Education to T. NARASAKI (Enzymatic hydrolysis of soybean hemicelluloses, 1962) and to

A. KAJI (Improvement of the utilization of agricultural and horticultural products by the use of enzymes, 1962-1963).

Reference

(1) NARASAKI, T.: *Kagawa Daigaku Nogakubu Gakuzyutu Hokoku*, 18,16(1966).

ダイズ多糖類の酵素分解

II. ダイズ子葉ヘミセルロースB$_1$のタカジアスターゼ分解により生ずる中間生成物

楢崎丁市

要旨 前報においてタカジアスターゼによるヘミセルロース B$_1$の加水分解が，ヘミセルロースB$_1$の構造を解明するのに有益な知見をあたえる事を報告した．

本報では酵素分解によって生ずる中間分解生成物を分離し，それらの構成糖を調べることによってその構造を推定した．又前報に示した中間生成物の反応経過中の変動とその推定構造とからヘミセルロース B$_1$ 構成多糖類中にはアラボガラタンとガラクツロン酸を含むキシランが存在することを確認した．又タカジアスターゼはアラビノース：ガラクトースおよびキシロース：ガラクツロン酸の結合を分解することができないことがわかった．

本研究を行なうにあたり有益な助言を賜わった川村信一郎教授および梶明教授に深く感謝します．

この研究の経費の一部分は文部省科学研究費（昭和37年度各個研究費，楢崎丁市，ダイズヘミセルロースの酵素分解；昭和37，38年度機関研究費，梶明，酵素による農園芸産物の高度利用に関する研究）により支払われた．

(Received May 16, 1966)