CARBOHYDRATE METABOLISM IN THE SHOOTS OF BAMBOO, *LELEBA OLDHAMI.*

I. Preliminary Survey of Soluble Saccharides and Sucrose-degrading Enzymes[1]

JONG-CHING SU[2][3]

(Received April 5, 1965)

Bamboo shoot is a popular vegetable in Taiwan during the summer season. It is harvested for marketing while the whole shoot is still underground. As soon as the tip of the shoot breaks the soil, the light brown sheath starts turning into green, and accompanying the development of photosynthetic activity, cavities are formed inside the shoot, the taste of the shoot becomes bitter, the texture of the tissue gets more fibrous, and the value of the shoot as vegetable diminishes.

We have chosen young bamboo shoots as a model system for the systematic study of some aspects of carbohydrate metabolism in higher plants from the following standpoints:

1. The growth rate of bamboo shoots is very high, and the size of the shoot is large enough to permit us to use a single shoot for various purposes of studies.

2. The young tissue is devoid of photosynthetic activity and is completely parasitic to the mother plant. It receives carbohydrate translocates, probably

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(1) A contribution from the Department of Agricultural Chemistry, National Taiwan University.

(2) Professor of Biochemistry, Department of Agricultural Chemistry, National Taiwan University, and Research Fellow, Institute of Botany, Academia Sinica.

(3) This investigation was supported by U.S. Public Health Service Research Grant GM-10577 from the Institute of General Medical Sciences, and also by a subsidy from the National Council on Science Development, Republic of China.

The author wishes to thank Dr. H. W. Li for the encouragement.

The following abbreviations are used: Tris, tris(2-amino-2-(hydroxymethyl)-1, 3-propanediol); UDP, uridine diphosphate; UDPG, uridine diphosphate D-glucose; ADPG, adenosine diphosphate D-glucose.

in the form of sucrose (Zimmermann, 1960), from the mother plant through rhizome to sustain its rapid growth. It is so completely isolated from the site of carbohydrate reserve that the possibility of complicating carbohydrate metabolism pattern within the tissue by the transformation of the reserve carbohydrate, or the photosynthetic products, into the translocate form of the compound can be avoided.

3. As it is reported in this paper, no trace of starch can be detected in young bamboo shoots. This further simplifies the picture of carbohydrate metabolism of bamboo shoots by eliminating the starch synthesizing system.

4. Our unpublished results indicate that no uronosyl residue is present in the cell wall polysaccharides of bamboo shoots. This does imply that the pectin synthesis activity, which is so common to young plant tissues, may be absent in bamboo shoots.

Therefore, bamboo shoot is regarded as an ideal system for the study of carbohydrate metabolism, especially the biosyntheses of cell wall polysaccharides, starting from a simple substrate, such as sucrose.

This paper reports the results of the survey of soluble saccharides and the activities of enzymes which act directly on sucrose. This study was done for the purpose of obtaining the information concerning the starting point from which commence all the carbohydrate metabolism pathways of bamboo shoots.

Material and Methods

For the extraction of carbohydrates, bamboo shoots purchased from a local grocer were used. For the preparation of enzymes, the shoots were cut from a bamboo bush located about 5 km south of the main campus of National Taiwan University, and brought into the laboratory as soon as possible.

The shoots were de-sheathed to obtain the edible portion. From 6 kg of bamboo shoots, 4 kg of edible portion was obtained. After slicing with a knife, the tissue was blended with 4 liters of 95% ethanol in a Waring blendor. The homogenate was heated in a boiling water bath until the ethanolic solution started boiling, and kept at that temperature, with occasional stirring, for 5 minutes. The ethanolic extract, after cooling, was squeezed through two layers of cheesecloth, and the yellowish turbid solution was further clarified by filtering through filter paper. On standing the extract in a deep freeze overnight, a white precipitate formed. The precipitate, which was shown to contain a ninhydrin-negative peptide, was removed by filtration in a cold room. The clear filtrate was adjusted to pH 2.5 with concentrated nitric acid, and 250 ml of mercuric acetate reagent (Caputto *et al*, 1950) was added to precipitate nucleotides as the mecuric salts. The precipitate was collected by centrifugation, washed with water, and saved for the study of the nucleotides. The

supernatant liquid was treated with H_2S, while chilled in an ice bath, until the excess mercuric ions were precipitated as HgS. After removing the precipitate by filtration, the excess H_2S was aerated off, the pH of the solution adjusted to 5 with ammonia, and the solution was evaporated under vacuum to remove ethanol. After diluting the concentrate with water, it was deionized, while cold, by passing though columns of ion-exchange resins IR-120(H^+) and IR-4B (OH^-). On concentrating the almost colorless deionized solution, first in a vacuum evaporator at 40° and then in a vacuum desiccator over KOH pellets, a light brown-colored immobile sirup resulted. From 4 kg of the edible tissue, 62 g of the sirup, which was shown to contain 92% of sugar solid, was obtained.

The residues from the dilute ethanol extraction were combined and extracted with boiling water. The residue was separated by centrifugation and re-extracted with 0.1% NaOH at room temperature, with vigorous shaking, for 10 minutes. After centrifuging down the residue, the dilute alkali extract was neutralized with HCl to a slightly acidic pH. Both the hot water extract and the neutralized alkali extract were tested with an I_2-KI solution for the presence of starch.

For the extraction of enzymes, the freshly cut and de-sheathed shoots were chilled in a deep freeze for half an hour before slicing. Four hundred grams of the sliced tissue was homogenized with 100 ml of cold 0.5 *M* Tris buffer, pH 7.5, in a Waring blendor with a prechilled jar, until a thick slurry resulted. The extract was strained through two layers of cheesecloth, and centrifuged in a refrigerated centrifuge at 10,000 *g* for ten minutes. The cell debris was discarded. To the supernatant solution, finely ground ammonium sulfate was added until the salt concentration reached 0.5 saturation. The protein fraction precipitated contained all the required enzyme activities. The precipitate was dissolved in small amount of 0.05 *M* Tris buffer, pH 7.5, and dialyzed overnight in a cold room (2°) against 0.05 *M* Tris, pH 7.5, which is 0.1 m*M* with respect to versene.

Electrophoresis was conducted with an apparatus similar to that described by Crestfield and Allen (1955) at a field strength of 45 v/cm. The buffers used were: 0.2 *M* ammonium formate, pH 3.6, and 0.2 *M* ammonium acetate, pH 5.7. For the separation of nucleotides on paper, the solvent system, 95% ethanol–1 *M* ammonium acetate, pH 7.5, 7:3, was used (Paladini and Leloir, 1952). Paper chromatographic separation of saccharides was performed in the solvent system n-butanol-acetic acid-water, 4:1:1. Nucleotides on paper were located by contact printing under ultraviolet light using a germicidal lamp as the light source. Sugars on paper were revealed by *p*-anisidine phosphate (Feingold *et al*, 1958) and aniline hydrogenphthalate (Partridge, 1949) spraying reagents. For the detection of ketose, urea phosphate reagent was used (Wise

et al, 1955).

Total hexose was determined by a modified anthrone method (Su and Ho, 1955). Ketose was estimated with a resorcinol reagent according to Roe *et al* (1949), and reducing sugar by the method of Nelson (1944).

UDPG was isolated from the bamboo extract (Su, unpublished). Invertase and U-C-^{14}-sucrose were purchased from Nutritional Biochemicals Corporation and Volk Radiochemical Co., U. S. A., respectively. The radioactivity on paper was located, either by a conventional ratemeter equipped with an end-window GM-tube, or by the radioautographic technique using Kodak Blue Brand medical X-ray films. Whatman No. 1 filter paper was used for paper chromatographic and electrophoretic analyses.

Results

On paper chromatographic separation, three spots corresponding to glucose, fructose and sucrose were given by the sugar sirup.

Phenylosazone was prepared directly from the sirup. It melted, without recrystallization, at 211–212°. It did not give a melting point depression when mixed with an authentic specimen of D-glucose phenylosazone. When the sirup was hydrolyzed with 2 *N* HCl at 70° for 10 minutes, the spot corresponding to sucrose disappeared from the chromatogram and no monosaccharide spots other than glucose and fructose could be detected. The same result was obtained when the sirup was hydrolyzed with a yeast invertase. Phenylosazone was prepared from the acid hydrolysate, the melting point of which was found to be 212–213°. Also no melting point depression was observed on admixture with authentic D-glucose phenylosazone.

The reducing value of the sirup almost doubled after the acid inversion. When the total hexose and the ketose contents of the sirup were estmated, it was found that the sirup contained, when expressing the results as invert sugar, 92% hexose, and 47.4% of the hexose was fructose. This evidence indicates that the sirup consists of sucrose, D-glucose and D-fructose in nearly equimolar proportions.

The hot water and dilute alkali extracts of bamboo tissue gave a negative iodine test, indicating that starch was absent in the sample. When seven volumes of 95% ethanol was added to the water extract, a small amount of a white precipitate formed. The precipitate gave, after heating with 1 *N* HCl at 110° for 4 hours, besides glucose, galactose, xylose and arabinose, a spot corresponding to ribose on paper chromatographic analysis. When the precipitate was dissolved in dilute alkali, the solution showed a characteristic ultraviolet absorption peak of nucleic acid at 255 mμ. From these results, it was concluded that the hot water extract contained ribonucleic acid.

The protein fraction precipitated at 0.5 ammonium sulfate saturation was shown to contain invertase (β-fructofuranosidase) and sucrose synthetase (UDPG-fructose transglucosylase) according to the following experiment results.

Ten λ of the dialyzed enzyme solution (equivalent to about 0.8 g fresh tissue) was incubated with 0.5 μmole UDP, 0.3 μmole $MgSO_4$, 0.1 μmole sodium versenate, 1 μmole NaF and 0.025 μmole U-C^{14}-sucrose (0.18 μc) in a total volume of 25 λ at 37° for 30 minutes. The incubation mixture was directly subjected to paper electrophoresis in the formate buffer. About 90% of the radioactivity remained in the origin, while about 5% of the activity migrated to the UDPG area and the remaining activity was found in the hexose monophosphate area. The immobile spot was eluted with water and analyzed paper chromatographically. Practically all of the electrophoretically immobile activity was recovered from the glucose and fructose areas of the paper chromatogram; only a trace of unaffected sucrose was found. The spot migrated electophoretically as UDPG was eluted and sufficient amount of carrier UDPG was added to the eluate. It was then subjected to electrophoresis in the acetate buffer and chromatographic separation in the ammonium acetate-ethanol system. The complete correspondence of the spots on the UV contact prints and the radioautograms was taken as the evidence that the compound synthesized from UDP and sucrose by the bamboo shoot enzyme was UDPG. This was further confirmed by the finding that, when UDP was omitted from the incubation mixture, no electrophoretically mobile radioactive spot was formed, and the sucrose added was completely hydrolyzed to glucose and fructose.

Discussion

The water content of young bamboo tissue is high; about 93% of the edible portion of bamboo shoot consists of water. In this investigation, we recovered 58 g of sugar from the ethanolic extract of 4 kg of the edible tissue. On assuming that the recovery of the sugar was complete, the sugar content of the tissue on the dry matter basis was then estimated to be 21%. Since almost 50% of the soluble sugar consists of sucrose, and since the young tissue is non-photosynthetic, it is most probable that bamboo plants are not exceptional in using sucrose as the predominant, if not the sole, form of carbohydrate translocate.

Compared to the high content of sucrose, the presence of starch in bamboo shoots could not be demonstrated. This is to imply that: a) starch synthesizing system is absent, and b) sucrose cannot be the starch degradation product in bamboo shoots. This is in sharp contrast with the mature bamboo plants in which starches are present in significant quantities (Hsieh *et al*, 1964). The lack of starch-sucrose transforming system greatly simplifies the picture of

polysaccharide synthesis in bamboo shoots.

There have been known two types of sucrose degrading reactions in nature. One is the hydrolytic reaction catalyzed by invertase, and the other is the transglycosidic type degradation catalyzed by sucrose phosphorylase, levan sucrase, dextran sucrase, invertase, UPDG-fructose transglucosylase, ADPG-fructose transglucosylase, etc. Among the enzymes mentioned above, invertase, UDPG-fructose transglucosylase and ADPG-fructose transglucosylase have been known to occur in plants. UDPG-fructose transglucosylase was initially regarded as the enzyme responsible for sucrose synthesis in higher plants. However, it was later postulated (Neufeld and Hassid, 1963) that the *in vivo* function of this enzyme is degradative rather than synthetic. The findings by Akazawa *et al* (1964) that this enzyme was present in growing rice grains and that either sucrose or UDPG could contribute the glucosyl moiety for starch synthesis were in accord with the speculation.

The physiological nature of bamboo shoots is such that sucrose serves most probably as the starting point of all carbohydrate metabolic pathways. From this standpoint, it is considered that, the *in vivo* role of sucrose synthetase in bamboo shoots must be a degradative one, as in the case of rice grain system.

During the course of soluble saccharides isolation, care was taken to avoid hydrolysis of sucrose. In spite of the precaution, however, as much as one half of the obtained sugar sirup was found to be consisted of invert sugar. It is most probable, therefore, that the reducing sugars were formed not by acid inversion during the course of preparation, but by the invertase catalyzed hydrolysis *in situ*. The presence of an active invertase in bamboo shoots also implies that a good part of sucrose in bamboo shoots is metabolized through the invertase-catalyzed hydrolysis step.

Summary

From 4 kg of the edible portion of bamboo shoots was obtained 58 g of sugar solid, which was found to consist of sucrose, D-glucose and D-fructose in nearly equimolar proportions. No starch could be found in the hot water extract of the young tissue, however, the presence of ribonucleic acid in the extract could be demonstrated. The protein fraction of bamboo shoot extract precipitated at 0.5 ammonium sulfate saturation was found to contain invertase and sucrose synthetase activities. From the results obtained, it is postulated that both invertase and sucrose synthetase-catalyzed sucrose degradations play vital parts in the carbohydrate metabolism of bamboo shoots.

綠竹筍之醣類代謝

I. 可溶性醣類及蔗糖分解酵素之初步研究

蘇仲卿

自4公斤綠竹筍可食部分，分離得58克糖，其組成（分子比）約爲蔗糖：葡萄糖：果糖=1:1:1。

綠竹筍之熱水抽出物中，並無澱粉存在，但含有核酸（RNA）。新鮮竹筍之蛋白質抽出物中，其可以 0.5 飽和硫酸銨沉澱部分，含有蔗糖水解酵素（Invertase）及蔗糖合成酵素（UDPG-fructose transglucosylase）之活性。從所得結果，可推想此二種酵素俱在竹筍醣類代謝上有重要作用。

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