CARBOHYDRATE METABOLISM IN THE SHOOTS OF BAMBOO, *PHYLLOSTACHYS EDULIS*

I. Separation and Identification of Nucleotides[1,2]

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The shoot of bamboo *Phyllostachys edulis* (winter bamboo shoots) is one of the important winter vegetables in Taiwan. It's harvest season is far different from those of the shoots of other bamboo plants cultivated in this island, such as *Leleba oldhami* and *Dendrocalamus latiflorus*. Whereas the carbohydrate metabolism in the shoots of *Leleba oldhami* has been investigated extensively by one of the authers, so far no work of the type has been done on winter bamboo shoots. From the comparative biochemical standpoint, it must be interesting to compare the carbohydrate metabolic pattern of winter bamboo shoots with that of the shoots of *Leleba oldhami*. Since sugar nucleotides have been known to be the key compound in monosaccharide transformation and transglycosylation reactions, a study of their occurrence in the shoots of *Phyllostachys edulis* may provide information useful in furthering the studies on carbohydrate metabolism of the plant.

Materials and Methods

Separation of Nucleotides:

A sample of fresh shoots of bamboo *Phyllostachys edulis*, weighing 5.7 kg, was purchased from a local market near this institute on Feb. 1, 1966, and was desheathed to obtain 2.5 kg of the edible portion. From the edible tissue, nucleotides were extracted with dilute ethanol, precipitated with mercuric acetate reagent, fractionated on a Dowex-1 column and finally separated by paper chromatography in 95% ethanol-1 *M* ammonium acetate pH 7.5, 7:3,

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which is 0.001 *M* with respect to ammonium versenate, as described previously (Su, 1965). In some cases, the paper chromatograms were developed twice in the same solvent system for complete separation. The nucleotides on the paper were located by the ultraviolet contact printing technique (Markham and Smith 1949), using a germicidal lamp as the light source. The separated nucleotides were then eluted from the chromatogram with water.

Analysis of Nucleotides:

The isolated nucleotides were characterized by ultraviolet absorption spectra, total phosphorus and labile phosphorus (1 *N* H_2SO_4, 100°, 10 min.) analyses, estimation of reducing sugar after mild acid hydrolysis (0.01 *N* HCl, 100°, 10 min.), and coelectrophoresis and cochromatography with authentic specimen (Su and Hassid 1962).

Ultraviolet absorbtion spectra were obtained with a Perkin-Elmer 202 recording spectrophotometer. Phosphorus was determined by the method of Fiske–SubbaRaw (1925). Reducing sugar analysis was performed according to the micromethod of Park and Johnson (1949).

The terminal sugar moieties of the sugar nucleotides were further characterized as following. The sugar nucleotide samples were hydrolyzed with 0.05 *N* HCl at 100° for 10 minuts and the hydrolysate was deionized with Dowex-50 (H^+) and Amberlite IR-45 (OH^-). The deionized solution was concentrated in a vacuum desiccator over KOH pellets and analyzed by descending paper chromatography. The following solvent systems were used: n-butanol-acetic acid-water (4:1:1 V/V), ethylacetate-pyridine-water (8:2:1 V/V) and n-butanol-pyridine-water (3:2:1.5 V/V).

For the detection of saccharides on paper, aniline hydrogenphthalate spray, periodate-benzidine dipping reagent (Gordon *et al* 1956), silver nitrate dipping method (Block *et al* 1958) and modified Morgan-Elson reagent (Partridge 1948) were employed.

Further identification of sugar nucleotides by the use of specific methods is described in the next section.

Results and Discussion

Fig 1. is the ion exchange chromatographic pattern of nucleotides from 2.5 kg of bamboo shoots by a Dowex-1 column. The analytical data of the nucleotides are shown in Table 1. The components of each peak are named A. B. C. etc. in the order of migration in the chromatographic separation in the ethanol-ammonium acetate solvent system.

Five ultraviolet absorbing spots remained uncharacterized because their spectral types could not be matched with known nucleotides.

Compound IXA was found to be a mixture of UDPG* and UDP-D-galactose according to the following criteria: The deionized hydrolysate of the nucleotide fraction was reduced by sodium borohydride, and the reduction products were paper electrophoresed in 0.05 *M* borax buffer pH 9.2. Two spots were found on paper by periodate-benzidine dipping method. The mobilities of the spots were identical with those of D-sorbitol and dulcitol. UDPG was the major component.

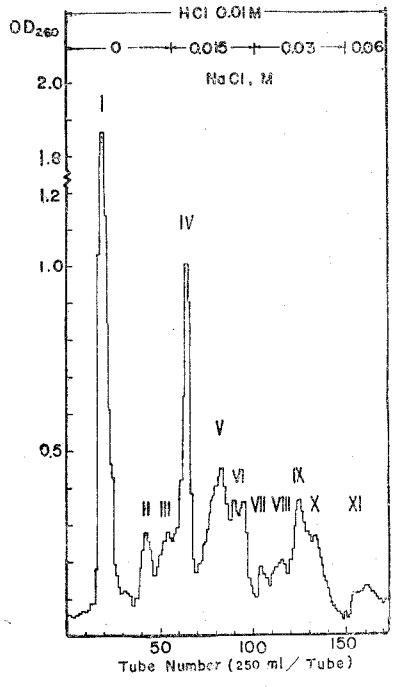

Fig 1. Ion Exchange Chromatographic Pattern of *Phyllostachys edulis* Nucleotides. Resin used: Dowex-1, 200-400 mesh, x8, chloride form.

The deionized hydrolysate of compound XA was separated by descending paper chromatography in ethylacetate-pyridine-water (8:2:1 V/V). In addition to the not well separated spots of D-glucose and D-galactose, a faint spot cochromatographed with D-xylose was detected.

For the characterization of N-acetylhexosamine in VIIIA, a sample containing 0.25 μmoles of uridine compound as calculated from extinction coefficient was hydrolyzed in 1 *N* HCl at 100° for 1 hr. After neutralization with NaOH,

* The following abbreviations are used: UDPG, uridine diphosphate-D-glucose; UDP-Gal, uridine diphosphate-D-galactose; UDP-Xyl, uridine diphosphate-D-xylose; AMP, adenosine 5'-monophosphate; UMP, uridine 5'-monophosphate; GMP, guanosine 5'-monophosphate; IMP, inosine 5'-monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; TPN, triphosphopyridine nucleotide; UDPAG, uridine diphosphate N-acetylglucosamine; UDPAGal, uridine diphosphate N-acetylgalactosamine.

Table 1. *Chemical Analysis of Nucleotides Isolated from* ***Phyllostachys edulis***

Peak No.	μmole	Spectral Type	Compound Identified	Chemical Composition (moles per mole base)		
				Total P	Labile P	Reducing Sugar* (after Hydrolysis)
I	141	Adenosine	AMP	0.94	0	
IIA		(Unknown)				
B		(Unknown)				
C	29	Uridine	UMP	0.90	0	
IIIA		(Unknown)				
B	31	Guanosine	GMP	1.13	0	
IV		(Unknown)				
V	34	Adenosine	ADP	2.01	0.99	
VIA	10	Uridine	UMP	1.05	0	
B	5	Adenosine	AMP	0.92	0	
VII		(Unknown)				
VIIIA	9	Uridine	UDPAG UDPAGal	1.54	0.90	0.98
B	6	Uridine	(UDP?)	1.64	0.66	0.43
IXA	60	Uridine	UDPG UDP-Gal UDP-Xyl	1.68	0.87	0.92
B	6	Guanosine	GDP	1.34	0.69	0.21
XA	19	Uridine	UDP-Xyl	1.70	0.81	0.89
B	7	Uridine	UDP	2.26	0.75	0
XIA	25	Uridine	UDP	1.95	0.41	0.07
B	12	Adenosine	ATP	2.65	1.86	

*Glucose is used as standard.

the hexosamine content was analyzed by Dische's indole method (1950) with glucosamine as standard. A hexosamine/uridine ratio of 0.8 was obtained.

The absorption spectrum in the visible region of the color reaction product was also determined. The maximum was found to be at 492 mμ, corresponding to that of authentic glucosamine.

When compound VIIIA was hydrolyzed in 0.05 *N* HCl at 100° for 10 minutes, the hydrolysate gave negative indole reaction, although nearly an equimolar amount of reducing sugar was liberated (see Table 1). Since the indole reaction gives negative response toward N-acetylhexosamine, it is considered that the hexosamine residues of VIIIA are N-acetylated.

The hexosamine obtained from VIIIA was oxidized with ninhydrin according to the micro-procedure of Pontis (1955). The pentoses obtained on ninhydrin oxidation cochromatographed with L-arabinose and D-lyxose in n-butanol-pyridine-water system.

For further characterization of the terminal hexosamine residues, a sample

Table 2. *Chemical Analysis of Nucleotides Isolated from* ***Leleba oldhami*** *(J. C. Su, 1965)*

Peak No.	μmole*	Compound Identified
I	270	AMP
IIA	15	UMP
B		(Unknown)
C	10	TPN
III	35	UMP
IV	30	GMP
V		(Unknown)
VIA	2	IMP
B	35	ADP
VII		(Unknown)
VIIIA	10	UDPAG, UDPAGal
B	2	(UDP?)
IXA	290	UDPG
B	50	(UMP?)
C	10	(UDP-sugar?)
XA	55	(UDP-?)
B	20	UDP
XIA		(Unknown)
B	10	(UDP-?)
C	95	ATP

* 4kg of edible portion of bamboo shoots had been used in this analysis.

of VIIIA was hydrolyzed with 0.05 *N* HCl at 100° for 15 minutes. The hydrolysate was deionized and chromatographed on a borax treated paper using n-butanol-pyridine-water, 3:2:1.5 (Pontis 1955). Two spots corresponding to N-acetylglucosamine and N-acetlgalactosamine were obtained. The same results were obtained when the deionized hydrolysate was analyzed by paper electrophoresis in 0.05 M sodium tetraborate, pH 9.2.

The evidence present above confirms that VIIIA consists of UDP-N-acetylglucosamine and UDP-N-acetylgalactosamine.

By comparing the results obtained in this work with those of *Leleba oldhami* (Su 1965), it was found that the distribution of nucleotides in these two species of bamboo shoot are very similar (Table I and Table II).

The difference of quantitative significance was found in the content of UDP-hexoses in the two species of bamboo. The more rapidly growing summer bamboo shoots (*Leleba oldhami*) contain strikingly higher amount of UDP-hexoses than the slower growing winter bamboo shoots.

Qualitatively, GDP is present but 5'-IMP and TPN are missing in the winter bamboo shoots, and for the summer bamboo shoots the reverse is true.

Both UDP-N-acetylglucosamine and UDP-N-acetylgalactosamine are found in the two species of bamboo investigated by us.

It is interesting to note that UDP-N-acetylhexosamines have been found in plant kingdom only in non-photosynthetic tissues, such as etiolated mungbean sprouts (Solms *et al* 1957, Gregoire *et al* 1963), dahlia tubers (Gonzalez 1963), and shoots of bamboo. Whether they also occur in photosynthetic tissues awaits future exploration.

Summary

From the shoots of bamboo *Phyllostachys edulis*, the following soluble nucleotides were isolated and identified: 5'-AMP, ADP, ATP, 5'-UMP, UDP, 5'-GMP, GDP, UDPG, UDP-D-galactose, UDP-D-xylose, UDP-N-acetylglucosamine and UDP-N acetylgalactosamine. The results are similar to those of shoots of bamboo *Leleba oldhami*. UDP-D-glucose is the major sugar nucleotide in both species of bamboo.

冬筍之醣類代謝

1. 核苷酸之分離與確認

陳慶三 蘇仲卿

自冬筍分離並確認下列核苷酸及核苷酸糖：一磷酸腺苷 (5'-AMP)，二磷酸腺核 (ADP)，三磷酸腺核 (ATP)，一磷酸尿苷 (5'-UMP)，二磷酸尿核 (UDP)，一磷酸鳥嘌呤苷 (5'-GMP)，二磷酸鳥嘌呤核(GDP)，二磷酸尿核葡萄糖 (UDPG)，二磷酸尿核半乳糖(UDP-D-galactose)，二磷酸尿核木糖 (UDP-D-xylose)，二磷酸尿核-N-乙醯氨基葡萄糖 (UDP-N-acetylglucosamine)，二磷酸尿核-N-乙醯氨基半乳糖 (UDP-N-acetylgalactosamine) 等，此結果與綠竹筍的核苷酸很相似，所不同者，冬筍含有 GDP 而無次黃嘌呤核苷酸 (IMP) 及三磷酸吡啶核苷酸 (TPN)，綠竹筍則反是。UDPG 是兩者的主要核苷酸糖。

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