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SEPARATION AND IDENTIFICATION OF SOLUBLE NUCLEOTIDES IN CHINESE CELERY (*OENANTHE JAVANICA* DC)[1,2]

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Abstract

The soluble nucleotides present in the Chenese celery (*Oenanthe javanica* DC) have been isolated and identified by using paper chromatography, paper electrophoresis, spectral analysis and chemical analysis. The characterized nucleotides are: 5′-AMP, ADP, ATP, CDP, GDP, 5′-UMP, UDP, UDP-D-glucose, UDP-D-galactose, UDP-D-galacturonic acid, UDP-D-xylose and UDP-D-arabinose. AMP, ADP and UDP-sugars are the major nucleotides in the Chinese celery.

Introduction

It is well known that nucleotides participate in and regulate all phases of metabolisms, such as carbohydrate, lipid, protein and nucleic acid. Since the discovery of UDP-D-glucose as a cofactor in the interconversion of D-glucose and D-galactose in yeast (Caputto *et al.*, 1950), it has become increasingly evident that nucleotide diphosphate glycosyl compounds are of primary importance in carbohydrate metabolism. They play two distinct, but related, roles in the anabolism of carbohydrates; as substrates for enzymes that transform monosaccharides and as glycosyl donors in the biosynthesis of oligo- and poly-saccharides (Feingold *et al.*, 1964; Hassid, 1967).

Some investigators have studied the presence of soluble nucleotides in bamboo shoots (Su and Hassid, 1962; Su, 1965; Chen and Su, 1968) and European larch (Cumming, 1970). The Chinses celery (*Oenanthe javanica* DC) is one of the important vegetables in Taiwan; however, the information concerning the biochemical studies of sugar nucleotide of this plant has not been documented. Thus, a study of soluble nucleotides in the Chinese celery may provide some information useful in furthering the studies on carbohydrate metabolism of this plant. This paper is to report the results of separation and identification

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of some sugar nucleotides and soluble nucleotides in the edible portion of the Chinese celery.

Materials and Methods

Plant materials

Fresh Chinese celery (*Oenanthe javanica* DC) was perchased from local market near by Taipei. After removing its leaves and roots, the edible portion (stem) was washed with deionized water and blotted with tissue paper, then chopped into small pieces and froze at −30°C for 24 hr.

Extraction of soluble nucleotides

A 6 kg of the frozen Chinese celery was homogenized with 6 liters of 95% ethanol for 10 min with a Warring blendor in cold room (4°C). The homogenate was heated in boiling water bath until the ethanolic solution started to boil, and kept at that temperature with occasional stirring for 5 min. After cooling down to 4°C in a ice bath, the ethanolic solution was squeezed through four layers of cheesecloth, and the greenish turbid solution was further clarified by filtering through Whatman No. 1 filter paper. The clear filtrate was adjusted to pH 2.5 with concentrated nitric acid and then one liter of mercuric acetate reagent was added to precipitate nucleotides as the mercuric salts (Caputto *et al.*, 1950). The mercuric salts were collected by centrifugation and washed with water.

Ion-exchange sepatation of nucleotides

The mercuric salts of nucleotides were suspended in ice bath, gassed with hydrogen sulfide for 4 hr while the suspension was chilled in an ice bath and filtered. The filtrate was aerated to remove excess hydrogen sulfide and neutralized to pH 7.0 with concentrated ammonium hydroxide. The following ion-exchange chromatographic procedures were carried out in a cold room at 4°C.

The nucleotide solution thus prepared was put in a well washed Dowex-1 column (chloride form, 4.6×35 cm, 200–400 mesh, ×8). The material which was not adsorbed on the column was washed through with water and the nucleotides were eluted with 0.01 N HCl, containing increased concentration of NaCl according to Volkin and Cohn (1953) at a rate about 5 ml per minute, and the eluate was collected in 250 ml fractions by means of an automatic fraction collector (Ginsburg, 1956). Elution of the mucleotides was followed by changes in optical density of the eluate at 260 mμ. The fractions falling under the same peak were pooled and concentrated by adsorption on charcoal

(Norit A, acid washed; 1 gm charcoal was enough for 1 liter of eluate to obtain the O. D. equals to 1) and subsequent elution with ammonium-ethanol-water (Cabid *et al.*, 1953). The alkaline solution was neutralized with acetic acid to pH 6.8 and concentrated under diminished pressure below 40°C.

Analysis of nucleotides

Paper chromatographic analysis of the concentrated fractions from Dowex-1 column showed that each peak contained more than one UV absorbing compound, thus the nucleotides were further separated by descending paper chromatography with a solvent of ethanol—1 M ammonium acetate, (7:3, v/v, pH 7.5) (Paladini *et al.*, 1952). The nucleotides on the paper chromatogram were located by the ultraviolet contact printing technique (Markham and Smith, 1949) using a germicidal lamp as the light source. The nucleotides on the chromatogram were then eluted with water.

The isolated nucleotides were characterized by ultraviolet absorption spectra and co-chromatography and co-electrophoresis with authentic specimen (Su and Hassid, 1962). All the reference nucleotides were perchased from National Biochemical Corp. U. S. A. Paper electrophoresis was performed on Whatman No. 1 filter paper, using 0.1 M ammonium acetate, pH 5.8, or 0.1 M ammonium formate, pH 3.6 of for characterization of the nucleotides.

Ultraviolet absorption spectra were obtained with a Perkin-Elmer 202 recording spectrophotometer. The optical density ratios of 250 mμ/260 mμ, 280 mμ/260 mμ, and 290 mμ/260 mμ were calculated.

Total phosphorus content and phosphorus liberated after hydrolysis with 1 N sulfuric acid at 100°C for 10 min were determined by Fisk-SubbaRow method (1925). Reducing values of the sugars, which were liberated from sugar nucleotides after hydrolysis with 0.01 N HCl for 10 min at 100°C were estimated according to the method of Park and Johnson (1949).

The terminal sugar moieties of the sugar nucleotides were further characterized according to the method of Su (1965). The identification of sugars was performed by descending paper chromatography using Whatmen No. 1 filter paper developed separately with *n*-butanol-acetic acid-water (4:1:1, v/v/v) and ethyl acetate-pyriding-water (8:2:1, v/v/v). Simultaneously, the authentic sugars were chromatographed under the same condition. In addition, sugars on the chromatograms were detected by using silver nitrate dipping method (Block *et al.*, 1958) or by spraying with aniline hydrogen phthalate (Partridge, 1949).

Results

The ion-exchange chromatographic pattern of nucleotides from Dowex-1

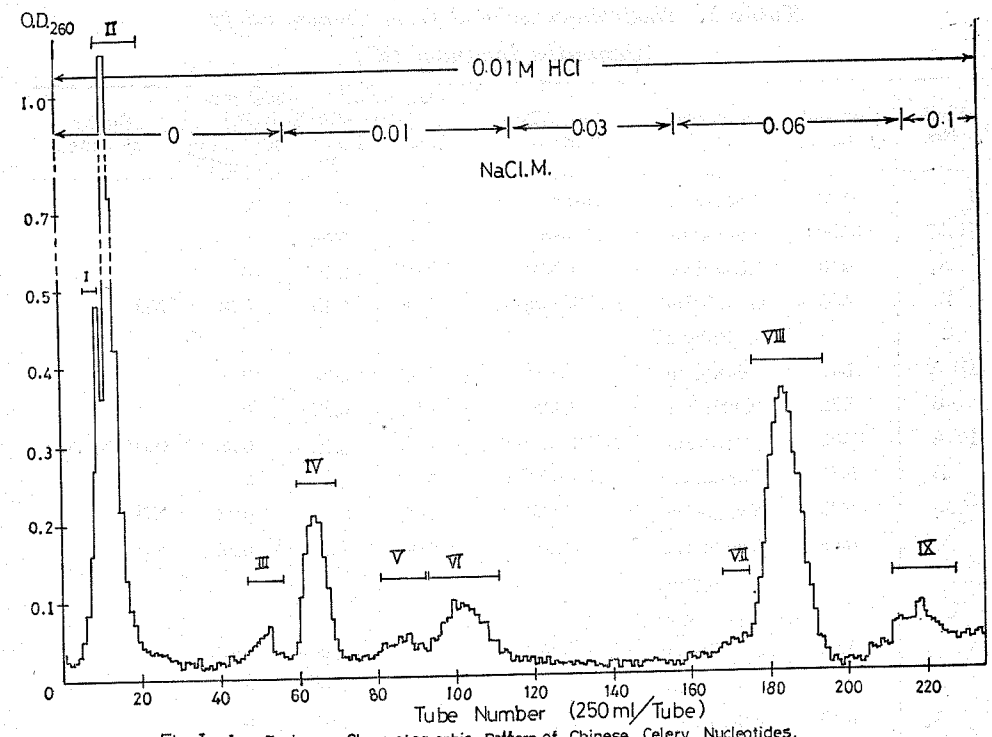

Fig I. Ion Exchange Chromatographic Pattern of Chinese Celery Nucleotides.
Resin used: Dowex-1, 200–400 mesh, X8, Chloride form.

column was shown in Fig. 1. There were nine separated nucleotide fractions in the chromatogram as indicated by the Roman numerals. The nucleotides identified in the various peaks were given in Table 1. The numbers I to IX in this table refer to the nine peaks from the Dowex-1 column.

Paper chromatographic analysis showed that each fraction contained more than one UV absorbing components. The components of each fraction were named as A, B, C, etc. in the order of migration on the chromatographic separation in ethanol-ammonium acetate (7:3, v/v, pH 7.5) (Paladini *et al.*, 1952). The eluates of components from the chromatogram were further separated by paper electrophoresis in 0.1 M ammonium formate, pH 3.6. The results showed that each component had more than one UV absorbing compounds. These compounds were named A_1, A_2, B_1, B_2, etc. in the order of their migration on the paper. The total quantities of each identified compound in μ mole per 6 kg fresh Chinese celery showed in Table 1.

From Fig. 1 the major fractions of the Dowex-1 elution-chromatogram were II, IV and VIII. Fraction II contained mainly the AMP and UMP as well as some nucleotide in gaunosine type. Fraction IV contains a large amount of UDP-sugar. The sugar moiety in IV A was identified as mainly the D-

Table 1. *Nucleotides isolated from Chinese celery (**Oenanthe javanica** DC)*

Peak No.	Content (μ mole)	Spectral type	Compound indentified	Chemical composition (moles/mole base)			Sugar identified
				Total P	Labile P	Reducing sugar(1)	
I	0.73	Adenosine	Nucleoside	0	0	0	
II A_1	12.06	Adenosine	AMP	0.86	0.06	0	
A_2	3.18	Uridine	UMP	0.89	0.15	0	
B	2.55	Gaunosine	(GDP-sugar?)	0.46	0.19	0.31	ND
C		(Unknown)					
III A	1.13	Adenosine	ADP	2.31	1.02	0	
B	1.22	Cytidine	CDP	1.93	0.79	0	
IV A	12.05	Uridine	(UDP-sugar?)	1.26	0.16	0.38	GalUA, Ara
B	0.47	Adenosine	Nucleoside	0	0	0	
V A_1	0.25	Adenosine	(AMP)	1.29	0.26	0.34	ND
A_2	0.59	Adenosine	ADP	2.06	1.31	0.12	ND
B		(Unknown)					
C		(Unknown)					
VI A_1	1.60	Adenosine	AMP	1.24	0.26	0	
A_2	7.34	Adenosine	ADP	2.31	1.11	0	
A_3	0.23	Adenosine	ATP	3.06	1.91	0	
VII A	0.41	Gaunosine	GDP	1.92	1.11	0	
B	0.37	Uridine	UMP	1.10	0.27	0	
VIII A_1	9.49	Uridine	(UDP-sugar?)	1.31	0.08	1.27	Glu, Gal, Xyl
A_2	5 91	Uridine	UDP-sugar	2.22	1.13	0.94	Glu, Xyl
B	29.68	Uridine	UDP-sugar	2.13	0.86	0.83	Glu, Gal
IX A_1	0.25	Adenosine	ADP	2.20	1.01	0	
A_2	0.64	Gaunosine	GDP	2.19	1.04	0	
B	1.09	Adenosine	AMP	1.12	0	0	

(1) Glucose is used as the standard.

(2) The following abbreviation are used: AMP, Adenosine 5′-monophosphate; ADP, Adenosine diphosphate; ATP, Adenosine triphosphate; UMP, Uridine 5′-monophosphate; UDP, Uridine diphosphate; GDP, Gaunosine diphosphate; CDP, Cytidine diphosphate; Glu, D-Glucose; Gal, D-Galactose; GalUA, D-Galacturonic acid; Ara, D-Arabinose; Xyl, D-xylose; ND, Not determined.

galacturonic acid and a little of D-arabinose. Fraction VIII was the major sugar nucleotides fraction. The sugar moiety of VIII A_1 contained mainly the D-glucose as well as a little of D-galactose and D-xylose; and VIII A_2 contained mainly the D-glucose and a little of D-xylose. In VIII B contained almost the equal amount of D-glucose and D-galactose.

Discussion

Among the nucleotides isolated from the Chinese celery, UDP-D-glucose,

UDP-D-galactose and UDP-D-galacturonic acid are obtained in the largest amount. This is in accord with the view that they are of prime importance in carbohydrate metabolism in Chinese celery. According to the chemical compositions of cell wall polysaccharides of Chinese celery (Yuan, 1974), it showed that the main component sugars of the cell wall polysaccharides are D-glucose, D-galactose, D-galacturonic acid and D-xylose. Obviously, the presence of UDP-D-glucose, UDP-D-galactose and UDP-D-galacturonic acid are used as cofactors in the interconversion of monosaccharides and also used as substrates for enzymes that transform monosaccharides and as glycosyl donors in the biosynthesis of oligo- and poly-saccharides.

In Table 1, it is noted that more than half of the soluble nucleotides are sugar nucleotides. And almost all of the nucleotide-bound monosaccharides are uridine derivatives. However, gaunosine derivatives may present in a small amount. D-glucose, D-galactose and D-galacturonic acid are found to be the major components of the uridine nucleotide. This raises the question of the formation of glucose-, galactose- and galacturonic acid-containing oligo- and poly-saccharides. D-xylose and D-arabinose are found to be the minor components of the uridine nucleotide. They may concern with the biosynthesis of xyloglucan and arabinogalactan.

There are large amount of AMP and UMP in fractions II A_1 and II A_2, respectively. It was probably due to the break down of ATP and UDP-sugars during the extraction and purification. There are three UV absorbing compounds II C, V B and V C which still remained unidentified because of their mobilities in electrophresis and spectral types could not match with the authentic specimen. The compound II B is very likly a GDP-sugar. Although the sugar moiety has not been identified; it is indicating that some oligo- or poly-saccharides are synthesized via GDP-sugar. We did not find any pyridine nucleotide or nucleotide in inosine type, but found a large amount of UDP-sugars. The metabolic fate of these UDP-sugars in the Chinese celery awaits further exploration.

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水芹中可溶性核苷酸之分離與鑑定

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存在於水芹 (*Oenanthe javanica* DC) 中之可溶性核苷酸，經分離後以濾紙色層分析法、電泳分析法、光譜分析法及化學分析法鑑定之。經確認之核苷酸計有：一磷酸腺苷 (5′-AMP)，二磷酸腺核 (ADP)，三磷酸腺核 (ATP)，二磷酸胞核 (CDP)，二磷酸鳥嘌呤核 (GDP)，一磷酸尿苷 (5′-UMP)，二磷酸尿核 (UDP)，二磷酸尿核葡萄糖 (UDP-D-glucose)，二磷酸尿核半乳糖 (UDP-D-galactose)，二磷酸尿核半乳糖醛酸 (UDP-D-galacturonic acid)，二磷酸尿核木糖 (UDP-D-xylose) 及二磷酸尿核阿剌伯糖 (UDP-D-arabinose)。其中以一磷酸腺苷、二磷酸尿核及二磷酸尿核糖類，爲水芹中主要的可溶性核苷酸。