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A NOTE OF THE OCCURRENCE OF NUCLEOTIDES IN STRAWBERRY LEAVES

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Abstract—The individual nucleotides in a trichloroacetic acid extract of mature strawberry leaves were isolated by chromatography on Dowex 1 (formate form) columns using aqueous formate and formic acid as the eluting agents, and by TLC. CMP, UMP, 3'UMP, UDP-xylose, UDP-arabinose, UDP-*D*-fructose, UDP-*D*-glucose, UDP-galactose (UDP sugars in ratio of 1:1·3:2·9:10·8:2·5 respectively), CDP, UDP, CTP, UTP, ADP, GDP, GDP-*D*-mannose, GDP-*D*-xylose, GDP-*D*-glucose, GDP-*D*-galactose (ratio of GDP sugars 40:10:1:1 respectively) UDP-glucuronic acid, ATP and GTP were identified. The amount of each present was estimated from the analytical data.

INTRODUCTION

THE PRESENT paper describes the identification of a range of nucleotides from mature strawberry leaves using the same general methods as those described in a previous paper.[1]

RESULTS

Identification of Nucleotides and Phosphate Esters

To assist the systematic identification a standard method of nomenclature was adopted as follows: each peak on the first Dowex 1 (formate form) column using the ammonium formate system was numbered from I to X and the peaks derived from each of these on the second Dowex 1 (formate form) column using the formic acid system, an appropriate additional number. Thus peak I-2 would be from Peak I on the first column and would be the second peak on the second column. If these peaks were split into several individuals by TLC the same nomenclature was employed.

The nucleotides finally isolated were characterized from their position on the first and second ion-exchange columns, pentose and phosphorus (total and labile) analyses, TLC, u.v. spectra at pH 1, 7 and 12 and in some cases by enzymic methods.

A summary of the results is given in Table 1.

Enzymic estimates of the ATP present in peak IX indicated that only 86% of the total triphosphate (calculated on the basis that the extinction was entirely due to adenosine) was ATP. In order to identify the other triphosphates the ATP was selectively converted to ADP by treatment with hexokinase and excess glucose. ITP reacts slowly with yeast hexokinase under the same conditions but GTP reacts to a negligible extent. The final reaction mixture was passed through a column of Dowex AG50 (H^+ form) to remove Mg^{2+} and triethanolamine and then concentrated. Chromatography on a column of Dowex AG1 (formate form) using the ammonium formate system resolved the products into three fractions of which ADP and GTP were identified. The approximate composition of peak IX was ATP: GTP:IX-3, 17:2:1 respectively.

[1] R. R. SELVENDRAN and F. A. ISHERWOOD, *Biochem. J.* **105**, 723 (1967).

Table 1. Nucleotides isolated from strawberry leaf extract

Peak	TLC R_f (solvent)	Composition (mole/mole base) Total P	Acid labile P	Pentose	Reducing sugar (as glucose)	Compounds identified	Amount (μmole/100 g leaves)
I-1	0·44 (A)	1·01	0·12	0·58		CMP	0·3
II-2	0·47 (A)	·98	0	0·61		UMP	0·84
III-1	0·49 (A)	1·01	0·08	0·5		U-3′-MP	1·2
IV-1-1	0·34 (A)	1·85	0·92	0·66		CDP	0·6
IV-1-2	0·47 (A)	1·05	0	0·59		UMP	*
IV-2	0·31	1·88	0·95	0·75	1·1	†UDP-xylose UDP-arabinose UDP-galactose UDP-glucose UDP-fructose	15·1 (9·8)
IV-3	0·37 (A)	1·82	0·92	0·6		UDP	2·8
V-3	0·37 (A)	2·0	1·21	0·62		UDP	*
VI-1	0·29 (A)	2·78	1·9	0·67		CTP	1·8
VII-3	—	1·9	0·92	—		UDP	*
VII-4	0·47 (B) 0·88 (C)	2·8	1·78	0·59		UTP	4·0
VIII-1	0·33 (B) 0·34 (C)	2·04	0·98	1·02		ADP	5·9 (6·5)
VIII-2	0·25 (A)	2·12	1·22	1·81	1·14	‡GDP-xylose GDP-mannose GDP-glucose GDP-galactose	0·15
VIII-3	0·28 (A) 0·30 (B)	2·06	1·1	1·05		GDP	1·1
VIII-4	0·25 (A)	1·91	0·88	0·65	1·3	UDP-glucuronic	0·42
IX	—	2·2	1·6	—		ATP	9·8 (9·2)
	0·29 (B) 0·62 (C)	2·9	1·76	1·02		GTP	1·44

* These are given as a combined figure under the appropriate heading even though it is realized that some may be due to decomposition of other nucleotides. Figures in parenthesis were enzymically determined.

† Approximate ratio of xylose, arabinose, galactose, glucose and fructose was 1:1·3:2·5:10·8:2·9 respectively.

‡ Approximate ratio of xylose, mannose, glucose and galactose was 10:40:1:1 respectively.

Enzymic methods also showed that glucose-6-phosphate and fructose-6-phosphate were present in peak I, and with glucose-1-phosphate in peak II. 3-Phosphoglyceric acid was present in peak V and fructose-1,6-diphosphate in peak V.

The concentration of sugars in the strawberry leaves was glucose 2·1, fructose 1·7 and sucrose 6·4 mmole/100 g fr. wt.

The concentrations of the various phosphate esters estimated in the original extract were as follows: glucose-6-phosphate 24·7, fructose-6-phosphate 4·6, glucose-1-phosphate 2·8, fructose-1,6-diphosphate 0·43, glucose-1,6-diphosphate 0·83, sucrose-6-phosphate 0·36 and 3-phosphoglyceric acid 22·9 μmole/100 g leaves.

DISCUSSION

In the course of the present study, a wide range of nucleotides were isolated and identified. The guanosine diphosphate derivatives of *D*-xylose, *D*-mannose, *D*-glucose and *D*-galactose have already been described in a previous paper[1] but no mention was made about the other nucleotides. Of these, the uridine diphosphate sugars occupy a prominent place and will be discussed in detail. The difficulty experienced in the present study in attempts to separate the group suggests that they possess marked structural similarities which is in agreement with the fact that all of them give UDP and free sugars under identical conditions of hydrolysis. The identification by an enzymic method of UDP-glucose as a major component suggests by analogy that all the sugars may be attached to the uridine through a 5′ pyrophosphate group. Since the rate of liberation of reducing sugars during acid hydrolysis is comparable to the rate of hydrolysis of UDP-glucose and sucrose[2] the pyrophosphate group is probably joined to position C_2 in fructose and C_1 in all other sugars. However, the information is inadequate in the case of fructose to decide between positions C_2 or C_1. The identification of the UDP derivatives of *D*-glucose, xylose, galactose, arabinose and glucuronic acid in strawberry leaves confirms previous work[3,4] on mung bean seedlings. The identification of UDP-*D*-fructose in appreciable amounts suggests that this nucleotide sugar may be important in the metabolism of fructose. Recently the isolation and identification of UDP-fructose from Jerusalem artichoke tubers has been reported[2] without, however, establishing the configuration of the sugar moiety. The amount isolated was rather small (1·3 μmoles from 10 kg of the tubers of Jerusalem artichoke) whereas in the present study the amount was much larger (265 μmoles/10 kg of strawberry leaves). This is surprising because the identification of UDP-fructose suggests the presence of appreciable amounts of fructosans which are known to be present in certain tubers (*Dahlia* and Jerusalem artichoke) and grasses (monocotyledons) but not in strawberry plants (dicotyledon). The smaller amount isolated from a typical fructosan-containing plant may be due to the plant tissue used but could be due to the fact that 12% perchloric acid was used for extracting the phosphate compounds and this could have resulted in the hydrolysis of the UDP-sugars. In addition the nucleotides were isolated by adsorption on charcoal followed by elution with ethanol–ammonia, a process which often results in considerable losses. Of the other uridine diphosphate sugars, it is interesting to note that the concentration of UDP-glucuronic acid is of the same order as UDP-xylose. UDP-glucuronic acid is an intermediate in the formation of UDP-xylose from UDP-glucose.

[2] Y. Umemura, M. Nakamura and S. Funahashi, *Arch. Biochem. Biophys.* **119**, 240 (1967).
[3] V. Ginsburg, P. K. Stumpf and W. Z. Hassid, *J. Biol. Chem.* **223**, 977 (1956).
[4] J. Solms and W. Z. Hassid, *J. Biol. Chem.* **228**, 357 (1957).

The phosphate esters were estimated by enzymic methods. Relatively few data are available from the literature concerning the average concentration of the main phosphate esters in higher plant tissues but it does appear that the hexose-6-phosphates predominate among the sugar phosphates, their concentration ranging from 8 to 30 μmol/100 g fr. wt. In general, the figures obtained in the present study are of the same order as those obtained in recent studies on other plant materials.[5,6] The very low concentration of fructose-1,6-diphosphate in most tissues, and the important role played by this compound in intermediary metabolism, reinforces the arguments given earlier on enzyme inactivation[7] and the care that should be taken in isolating these compounds quantitatively from plant tissues. Glucose-1,6-diphosphate is usually assigned a catalytic role in carbohydrate metabolism, namely the conversion of glucose-1-phosphate to glucose-6-phosphate. However, in the present study its concentration was found to be appreciable and greater than that of fructose-1,6-diphosphate, which suggests it may have some other role. The concentration of sucrose-6-phosphate in strawberry leaves is very small. Its presence, however, gives further evidence supporting the important role assigned to this compound in sucrose synthesis. The concentration of 3-phosphoglyceric acid is relatively higher in leaves than in the other plant tissues examined, except peas.

EXPERIMENTAL

Chemicals. The nucleotides and sugar phosphates were purchased from the Sigma Chemical Co., St. Louis, Mo., U.S.A., and enzymes from Boehringer Corp. (London) Ltd., Bilton House, Ealing, London.

Isolation and separation of the nucleotides. Mature strawberry leaves (variety Royal Sovereign) were extracted with trichloroacetic acid (TCA) and the extract treated exactly as described in a previous paper.[1]

It was found that the use of an ion-exchange resin to remove all cations from the original TCA extract in order that the TCA could be more readily removed from the solution also adsorbed some of the phosphorus in organic combination (10%) as well. The same was observed in experiments on soaked peas except that the amount adsorbed was higher (30%). This phosphorus was eluted from the resin with 2 N ammonia and the major component in the eluate identified as phosphoryl choline[8,9] (at least 90%). NAD was also shown to be present by an enzymic method.[10] The adsorption of NAD by this column explains the notable absence of this compound from the final nucleotide fractions. Control experiments showed that, in addition to these compounds, pyridoxal phosphate and thiamine phosphate were also readily adsorbed.

It was also found that during the removal of Pi and organic acids on the second cellulose column[7] the presence of oxalic acid (Pk_1 1·23), which may make up 50% of the dry weight of some leaves, improved the separation between 3-phosphoglyceric acid and Pi; 3-phosphoglyceric acid and Pi have similar chromatographic properties in this system in the absence of oxalic acid.

Chemical analysis of the nucleotides. The methods for the estimation of total phosphorus, acid labile phosphorus, and reducing sugars liberated by mild acid hydrolysis of the nucleotides have been described in a previous paper.[1] Pentose sugars present in purine nucleotides[11] and in pyrimidine nucleotides[12] were measured separately (recovery of pentose from pyrimidine nucleotides is usually about 60–65% of the theoretical).

Chromatography. TLC of the nucleotides was carried out on cellulose powder as described previously.[1] In addition to the isobutyric acid–*N*-ammonium hydroxide–0·1 M-EDTA solvent (solvent A), EtOH-M-ammonium acetate (3:7, v/v, pH 7·5)[13] (solvent B) and saturated aq. $(NH_4)_2SO_4$-M-sodium acetate–Isopropanol (79:19:2, v/v/v) (solvent C)[14] was used.

[5] J. BARKER, F. A. ISHERWOOD, R. JAKES, T. SOLOMOS and M. E. YOUNIS, *Nature* **196**, 1115 (1962).
[6] J. BARKER, R. JAKES, T. SOLOMOS, M. E. YOUNIS and F. A. ISHERWOOD, *J. Exptl. Botany* **15**, 284 (1964).
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For the paper chromatography of the sugars the following solvents were used: ethyl acetate–pyridine–water (2:1:1, v/v/v top layer),[15] ethyl acetate–acetic acid–water (3:1:3, v/v/v top layer),[15] and phenol saturated with water.[16] For the identification of phosphoryl choline the last solvent and *n*-propanol–water–aq. NH_3 (d-0·88) (60:10:30, v/v/v respectively)[17] were used.

Enzymic determination of nucleotides and sugar phosphates and sugars. These were estimated[10, 5, 18] not only in the initial purified extract from strawberry leaves but also in some of the appropriate fractions from the ion-exchange columns. However, in analysing plant extracts, it was found that even after most of the solutes, including Pi and phytin, had been removed,[7] and polyphenols by adsorption on poly-*N*-vinylpyrrolidone, there was present material which fluoresced when excited with light at 340 mμ and interfered with the fluorimetric estimations of those phosphate esters which were present in low concentration. For these assays, the solution was treated with a small amount of charcoal (200 mg Norite/10 g leaves in 10 ml; pH 3·0 formic acid). Preliminary experiments were carried out to determine the minimum amount required to reduce the interference to a negligible value and this amount was used in subsequent experiments. No phosphate esters were lost through adsorption on the charcoal if the amount used was only sufficient to remove the bulk of the fluorescent polyphenols.

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