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ESTIMATION OF SUCROSE-6-PHOSPHATE AND GLUCOSE-1,6-DIPHOSPHATE IN TEA LEAVES AND STRAWBERRY LEAVES

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Abstract—A method for the estimation of sucrose-6-phosphate and glucose-1,6-diphosphate in a highly purified extract of strawberry and tea leaves is described. This is based on hydrolysis to the corresponding hexose-6-phosphates and estimation of these by specific enzymes using a fluorimetric technique.

INTRODUCTION

SUCROSE-6-PHOSPHATE is an intermediate in one proposed route for the biosynthesis of

$$\left.\begin{array}{l}\text{UDP-glucose + fructose-6-phosphate} \underset{\text{Sucrose phosphate synthetase}}{\rightleftharpoons} \text{UDP + sucrose-6-phosphate} \\ \text{Sucrose-6-phosphate} \xrightarrow[\text{Specific phosphatase}]{} \text{Sucrose + } P_i\end{array}\right\} \text{Equation 1}$$

sucrose (Equation 1) while glucose-1,6-diphosphate is involved in the formation of glucose-1-phosphate since it is known to be a co-enzyme for the enzyme phosphoglucomutase which catalyses the interconversion of glucose-1-phosphate and glucose-6-phosphate. UDP-glucose involved in the proposed route for the synthesis of sucrose is formed from UTP and glucose-1-phosphate.[1] Both sucrose-6-phosphate and glucose-1,6-diphosphate are assumed to be present in plant tissues because the related enzymes have been isolated from plant tissues (sucrose phosphate synthetase,[2-4] sucrose phosphatase,[5] and phosphoglucomutase[6,7]), and when $^{14}CO_2$ was fed to plant tissues, spots corresponding in position to sucrose phosphate and glucose-1,6-diphosphate were shown to be present on radio-autographs of paper chromatograms of extracts[8-10] of the plant tissues.

Since both compounds are involved in aspects of the photosynthetic carbon reduction cycle, it was of interest to measure the amounts present during a study of the effect of light–dark transition on the concentration of the phosphate compounds in strawberry leaves.

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The method, which is described as follows, was originally designed to estimate the small amounts of sucrose-6-phosphate present in plant tissues but was later found suitable for the estimation of glucose-1,6-diphosphate; by analogy it appears likely that mannose-1,6-diphosphate and ribose-1,5-diphosphate could also be estimated in a similar way. The method depends (1) upon the preparation of an extract from which all interfering substances have been removed, and (2) a mild acid hydrolysis of this to give a hexose-6-phosphate (with ribose 1,5-diphosphate it would be ribose-5-phosphate) which is then estimated by a specific enzymic procedure using a fluorimetric technique.

RESULTS AND DISCUSSION

Preparation of Extract

In analysing plant extracts it was found that even after most of the solutes, including P_i and phytin, had been removed by the procedure described by Isherwood and Barrett[11] and polyphenols by adsorption on poly *N*-vinylpyrrolidone,[12] there was still present material which fluoresced when excited with light at 340 nm and interfered with fluorimetric estimations. In addition, large amounts of glucose-6-phosphate and fructose-6-phosphate were present as well as smaller amounts of fructose-1,6-diphosphate, and since these would interfere with the estimations the extracts had to be given an extra treatment before analysis. The extract was first treated with the minimum amount of charcoal to remove fluorescent material and then the reducing sugar phosphates were completely destroyed by heating with dilute alkali.[13] The solution was passed through a cation-exchange column and again treated with a small amount of charcoal. Preliminary experiments were carried out to determine the minimum amounts of charcoal required to reduce the interference from the fluorescent material and from the caramelized sugars to a negligible value and these amounts were used in subsequent experiments. Nucleotides were adsorbed by the charcoal but not the sugar phosphates. Glucose-1-phosphate, glucose-1,6-diphosphate and sucrose-6-phosphate and 6-phosphogluconate[14] were not affected by the treatment and could be estimated in the purified extract.

Conditions of Hydrolysis

The minimum conditions necessary to hydrolyse the sucrose-6-phosphate and the glucose-1,6-diphosphate were determined by studies on purified strawberry leaf extract which was assumed to contain both compounds, and by comparison with the rate of hydrolysis of glucose-1-phosphate, glucose-1,6-diphosphate and sucrose. The procedure was as follows: To purified strawberry leaf extract[11] from which the reducing sugar phosphates had been removed (1 ml),[13] HCl was added to give in separate experiments a normality of 1, 0·1 and 0·01. The solutions were heated to 100°, cooled, neutralized and the amount of fructose-6-phosphate and glucose-6-phosphate measured fluorimetrically[15, 16] (Table 1).

From the results in Table 1 it was clear that hydrolysis in 0·1 N HCl at 100° for 7 min was sufficient to split completely the glycosidic link in both compounds. This was confirmed

[11] F. A. ISHERWOOD and F. C. BARRETT, *Biochem. J.* **104**, 922 (1967).
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TABLE 1. ACID HYDROLYSIS OF NATURAL SUCROSE-6-PHOSPHATE AND GLUCOSE-1,6-DIPHOSPHATE IN STRAWBERRY LEAF EXTRACT

Hydrolysis conditions		Yield of sugar phosphates (μmoles/100 g leaves)	
Normality	Time (min)	Sucrose-6-phosphate (as fructose-6-phosphate)	Glucose-1,6-diphosphate (as glucose-6-phosphate)
0·01	7	0·34	0·66
0·1	7	0·33	0·82
1·0	10	0·23	0·84

by control experiments using sucrose, glucose-1,6-diphosphate and glucose-1-phosphate under the same conditions. The lower value for sucrose-6-phosphate in N acid is possibly due to destruction of the fructose moiety in the hot acid (furfural formation).

Analysis of Plant Material

In a typical experiment on strawberry leaves, the procedure was as follows: the purified extract[11, 12] (containing about 4 μmoles of glucose-6-phosphate from 10 g leaves, 4 ml), acidified to pH 3 with formic acid, was thoroughly stirred with activated charcoal (Norite, 100 mg) and centrifuged. The supernatant was filtered to remove fine particles of charcoal and then freeze-dried. The concentrate was taken up in water (4 ml), 3 N potassium hydroxide (0·2 ml) added with stirring and the mixture heated for 20 min in a boiling water bath. It was then cooled and passed through a cation exchange column (Dowex AG 50, H^+ form, 8–10 per cent cross-linked, 100–200 mesh, A.R. grade; 2·5 × 1 cm dia.) to remove cations. The effluent was treated with activated charcoal (100 mg) and filtered. The amount of charcoal was critical. Just enough to remove the caramelized sugar was used but not an excess. The filtrate was freeze-dried and taken up in water (3·9 ml). To this 4 N HCl (0·1 ml) was added and the mixture heated in a boiling water bath for 7 min. The solution was cooled, neutralized with potassium hydroxide, made up to volume (6 ml) and an aliquot was used for the estimation of glucose-6-phosphate and fructose-6-phosphate. This procedure will include any combined glucose-6-phosphate or fructose-6-phosphate which survives the alkali treatment and is decomposed in the hot dilute acid to the hexose phosphate. This means that the figures given in Table 2 must be regarded as an estimate of the maximum amount likely to be present.

TABLE 2. SUGAR PHOSPHATES IN STRAWBERRY AND TEA LEAVES

Plant material	Sugar phosphates (μmoles/100 g fresh wt.)		
	Glucose-1-phosphate*[17]	Glucose-1, 6-diphosphate	Sucrose-6-phosphate
Strawberry leaves (*Fragaria vesca* var. Royal Sovereign)	2·6	0·83	0·36
Tea leaves (from Botanic Gardens, Cambridge; *Camellia sinensis* var. *assemica*)	1·25	0·2	0·05
Tea leaves (from Tea Research Institute, Ceylon. Clone 777 flown to England and analysed within 3 days of plucking. Temp. of storage 3–5°)	0·63	0·07	0·01

* This was measured on the extract immediately before the acid hydrolysis.

[17] H. U. BERGMEYER and H. KLOTZSCH, in *Methods of Enzymatic Analysis* (edited by H. U. BERGMEYER), p. 131, Academic Press, New York (1963).

A control experiment in which glucose-1,6-diphosphate (0·42 μmole) was added to a strawberry leaf extract and analysed by the above procedure gave a recovery figure of 0·39 μmole.

EXPERIMENTAL

The phosphoglucomutase, glucose-6-phosphate dehydrogenase, phosphoglucoisomerase enzymes were purchased from Boehringer Corp. (London) Ltd., Bilton House, Ealing, London. The fluorimeter used was Model 27A, Electronic Instruments, Ltd., Richmond, Surrey. The chemicals used were purchased from the Sigma Chemical Co., St. Louis, Mo., U.S.A.