Bot. Bull. Academia Sinica 22: 83-92 (1981)

INCORPORATION OF $^{14}CO_2$ INTO CARBOHYDRATES OF THE PTERIDOPHYTES *ADIANTUM CUNEATUM* L., *ASPLENIUM BULBIFERUM* L. AND *PTERIS CRETICA*

A. A. A. SULEIMAN*

Department of Botany, The University, Shefield S10 2TN, England

(Received January 3, 1981; Accepted March 2, 1981)

Abstract

The soluble carbohydrates of the Pteridophytes *Adiantum cuneatum*, *Asplenium bulbiferum* and *Pteris cretica* consist of sucrose, glucose and fructose. In addition, trehalose is present in both *Asplenium* and *Pteris*. After photosynthesis in [^{14}C]bicarbonate for 1 hour, sucrose was detected as the major product of photosynthesis in all three ferns. In *Adiantum* and *Asplenium*, glucose and fructose as well as some unidentified compounds of high molecular weight were also labelled. In *Pteris*, trehalose became labelled after 1 hour photosynthesis in $^{14}CO_2$ in addition to sucrose, glucose and unidentified compounds of high molecular weight. After incubation of *Pteris* in the dark for 24 hours, the label in trehalose and glucose disappeared while that in sucrose remained. In *Adiantum* and *Asplenium*, the label in sucrose, glucose and fructose was detected on incubation in the dark for up to 48 hours. Incorporation of ^{14}C into starch declined progressively with time of incubation of all three ferns in the dark. Results are discussed in relation to presence of carbohydrates and their metabolism in Pteridophytes and other plants generally.

Introduction

A few studies on the soluble carbohydrates of Pteridophytes have been carried out. Towers and Maass (1965) found sucrose to be the major soluble sugar in 21 species and varieties of *Lycopodium*. Investigating 12 species of Pteridophytes, Ludlow *et al.* (1966) also found sucrose to be the major soluble sugar present. In some species, glucose, fructose, galactose, erythrose and xylose were also recorded. Yamashita and Sato (1929) recorded the presence of trehalose in 21 species of *Selaginella*.

In a study of the photosynthetic products of some Pteridophytes, White and Towers (1967) found trehalose to be the major sugar formed in photosynthesis in *Selaginella* while sucrose was the major product in *Lycopodium*.

* Present address: Department of Botany, Faculty of Science, University of Riyadh, Riyadh, Saudi Arabia.

* Permanent address: Department of Botany, Faculty of Science, University of Khartoum, Sudan.

Both sugars were photosynthetic products of *Isoetes*.

This paper further examines the soluble carbohydrates of three Pteridophytes, *Adiantum cuneatum* L., *Asplenium bulbiferum* L. and *Pteris cretica* (Polypodiacae). They were primarily chosen because of their availability. The incorporation of ^{14}C into soluble sugars and starch during photosynthetic exposure of the three Pteridophytes to [^{14}C]bicarbonate in the light has been examined as well as the fate of ^{14}C on dark incubation.

Materials and Methods

Plant material. *Adiantum cuneatum* L., *Asplenium bulbiferum* L. and *Pteris cretica* were supplied by the Green House of the Department of Botany of Sheffield University. For *Adiantum*, 10 leaves/sample were used; for *Asplenium*, 5 apical segments (0.5–1.0 cm)/sample were used and for *Pteris*, 10 discs (0.5 cm diameter)/sample were used. Leaves, apical segments or discs were excised, washed, kept for 24 hours in the dark to destarch the tissue and then sampled.

Exposure of tissue to $^{14}CO_2$ in the light and dark

A rectangular feeding perpex box (20×12×10 cm) was used. Samples were placed in glass dishes (2.5 cm diameter) containing 2 ml distilled water. $^{14}CO_2$ was generated in the box by addition of 1 ml of 10% lactic acid to 100 μl of $NaH^{14}CO_3$ containing 100 μCi together with 13 μl of cold $NaHCO_3$ ($\equiv$ approximately 600 ppm CO_2 at the start of the experiment) in a glass dish, and the feeding box then covered and sealed with paraffin. Samples were then allowed to photosynthesize for 1 hr. in fluorescent lights (intensity 20 Wm^{-2}) at a constant temperature of 15°C, and then 1 ml of 1 M KOH was added to absorb the remaining $^{14}CO_2$. For samples to be incubated further in the dark, the feeding box was wrapped with aluminium foil.

Extraction of tissue and analysis of extracts

After exposure of tissue to $^{14}CO_2$ for the appropriate period in the light or dark (for details see "Results"), samples were thoroughly washed in ice-cold distilled water, gently blotted and soluble sugars extracted 3 times under reflux with 80% ethanol. No loss of carbohydrates was found in the washing water. The residue left after ethanol extraction was ground with amyloglucosidase (1 mg/ml) in citrate buffer (0.05 M, pH 4.5), incubated at 45°C for 2 hr., boiled for 5 minutes to denature the enzyme and then samples centrifuged and the supernatant kept for subsequent analysis (Holligan, McGee and Lewis, 1974).

Identification of the soluble carbohydrates

Paper chromatography was used for separation and detection of soluble

carbohydrates. Ethanolic extracts were chromatographed on paper using ethyl acetate/pyridine/water (8:2:1 v/v—Hatch, 1964) and ethyl acetate/acetic acid/water (14:3:3—Smith, 1960). Soluble carbohydrates were detected on chromatograms using silver nitrate-sodium ethoxide (Travelyan *et al.*, 1950). No attempt was made for the quantitative determination of carbohydrates.

Autoradiography, scanning and counting

For autoradiograph, chromatograms were covered with "Melinex" and exposed to "Kodak" X-ray films for a suitable period. For determination of percentage incorporation of C^{14} in individual compounds, chromatogram strips were scanned using a "Berthhold" paper chromatogram scanner in conjunction with a "Berthhold" integrator. Extracts (0.1-0.2 ml) were dried on aluminium planchets and counted using a "Nuclear Chicago" gas flow counter.

Results

Characterization of soluble carbohydrates

In *Adiantum*, three silver nitrate positive spots, denoted by A to C in Table 1 were detected on paper chromatograms. From their paper chromatographic mobilities, spots A to C were identified as sucrose, glucose and fructose, respectively. In both *Asplenium* and *Pteris*, four silver nitrate positive

Table 1. *Paper chromatographic mobilities of the soluble sugars of* ***Adiantum cuneatum, Asplenium bulbiferum*** *and* ***Pteris cretica*** *using two solvents and detected with silver nitrate-sodium ethoxide*

Pteridophyte	Spot in chromatogram	PC mobility relative to glucose		Authentic sugar with identical PC mobility
		(1)	(2)	
Adiantum	A	44.3	51.7	Sucrose
	B	100.0	100.0	Glucose
	C	136.2	142.8	Fructose
Asplenium	A	31.2	38.8	Trehalose
	B	44.3	51.7	Sucrose
	C	100.0	100.0	Glucose
	D	136.2	142.8	Fructose
Pteris	A	31.2	38.8	Trehalose
	B	44.3	51.7	Sucrose
	C	100.0	100.0	Glucose
	D	136.2	142.8	Fructose

(1) Ethyl acetate/pyridine/water (8:2:1—Hatch, 1964)
(2) Ethyl acetate/acetic acid/water (14:3:3—Smith, 1960)

spots were detected, denoted by A to D in Table 1. Their paper chromatographic mobilities suggest that the spots are trehalose, sucrose, glucose and fructose, respectively.

Thus the three Pteridophytes have sucrose, glucose and fructose as common soluble sugars, and in *Asplenium* and *Pteris*, the disaccharide trehalose is present in addition.

Incorporation of C^{14} into photosynthetic products and its fate on incubation in the dark

Between 60.1–68.3% of C^{14} was incorporated into the ethanolic fraction in all three ferns on exposure to $^{14}CO_2$ for 1 hr. in the light (Table 2), while the amyloglucosidase fraction, ascribed mainly to starch, incorporated between 31.7–39.9% of the label. Total and percentage incorporation of ^{14}C into the amyloglucosidase fraction declined progressively with time of incubation in the dark (Table 2) suggesting rapid turnover of starch in all three ferns.

Table 2. *Incorporation of ^{14}C into ethanolic and amyloglucosidase fractions of* ***Adiantum cuneatum, Asplenium bulbiferum*** *and* ***Pteris cretica*** *which had been exposed to $^{14}CO_2$ for 1 hr. in the light followed by periods in the dark. Values are means of triplicate determinations. Percentage incorporation of ^{14}C is given in brackets*

Sample and treatment	Incorporation of ^{14}C as counts/minute/sample		
	Ethanolic fraction	Amyloglucosidase fraction	Total counts
Adiantum, 1 hr light in $^{14}CO_2$	429,660 (60.1%)	284,892 (39.9%)	714,552
Adiantum, 1 hr light in $^{14}CO_2$+24 hr dark	480,780 (88.4%)	62,608 (11.6%)	543,388
Adiantum, 1 hr light in $^{14}CO_2$+48 hr dark	384,180 (92.8%)	29,472 (7.2%)	413,652
Asplenium, 1 hr light in $^{14}CO_2$	259,880 (68.3%)	120,596 (31.7%)	380,476
Asplenium, 1 hr light in $^{14}CO_2$+24 hr dark	470,580 (94.1%)	29,328 (5.9%)	499,908
Asplenium, 1 hr light in $^{14}CO_2$+48 hr dark	243,380 (94.0%)	15,528 (6.0%)	258,908
Pteris, 1 hr light in $^{14}CO_2$	139,068 (61.4%)	87,540 (38.6%)	226,608
Pteris, 1 hr light in $^{14}CO_2$+24 hr dark	72,040 (77.4%)	20,928 (22.6%)	92,968
Pteris, 1 hr light in $^{14}CO_2$+48 hr dark	92,280 (84.0%)	17,576 (16.0%)	109,856

The incorporation of C^{14} into the ethanolic fraction increased in all three Pteridophytes with time of incubation in the dark suggesting synthesis of some unidentified compounds of high molecular weight (Table 2).

Plate 1 is an autoradiograph of a chromatogram of ethanolic extracts of leaves of *Adiantum* and apical segments of *Asplenium* which had been exposed to $^{14}CO_2$ for 1 hr. in the light and 24 hr. and 48 hr., respectively in the dark. Photosynthetic products after 1 hr. in both ferns are some unidentified compounds of high molecular weight, sucrose (major product) and the hexoses glucose and fructose, which incorporated less actively (Plate 1). After 24 and 48 hr. in the dark, activity was still mainly in sucrose, unidentified compounds of high molecular weight and less so in glucose and fructose (Plate 1).

Plate 2 is an autoradiograph of a chromatogram of ethanolic extracts of discs of *Pteris* which has been exposed to $^{14}CO_2$ for 1 hr. in the light and 24 and 48 hr., respectively in the dark. Incorporation of C^{14} after 1 hr. was mainly into sucrose and less so into trehalose, glucose and some unidentified compounds of high molecular weight. After 24 and 48 hr. in the dark, the label was only detectable in sucrose (Plate 2).

Table 3 gives the percentage incorporation of C^{14} into individual compounds in the three ferns after exposure to $^{14}CO_2$ in the light for 1 hr. and 24 and 48 hr., respectively in the dark. Sucrose is shown in all cases to have the major percentage incorporation of C^{14} after 1 hr. in $^{14}CO_2$ in the light (see Table 3). This is followed by a rise after 24 hr. in the dark (*Adiantum* and *Pteris*) and then a decline after 48 hr., or alternatively by a progressive decline (*Asplenium*). The unidentified compounds of high molecular weight behaved in the opposite manner. Glucose in *Adiantum* behaved similar to sucrose, but percentage incorporation into fructose increased progressively with the time of incubation in the dark (Table 3).

Discussion

Soluble carbohydrates

The soluble carbohydrates of the three Pteridophytes investigated are sucrose, glucose and fructose in all three, but in addition, the presence of trehalose in both *Asplenium bulbiferum* and *Pteris cretica* reported here is of interest.

Trehalose is abundant in fungi (Cochrane, 1958; Lewis, 1975). Trehalose has also been reported in the moss, *Polytrichum juniperinum* (Holligan and Drew, 1971), the Pteridophytes, *Selaginella spp.* (Quillet and Soulet, 1964), *Isoetes spp.* (White and Towers, 1967), *Botrychium lunaria* (Kandler and Senser, 1965) and *Ophioglossum vulgatum* (Lohr, 1968). In higher plants, Hopf and

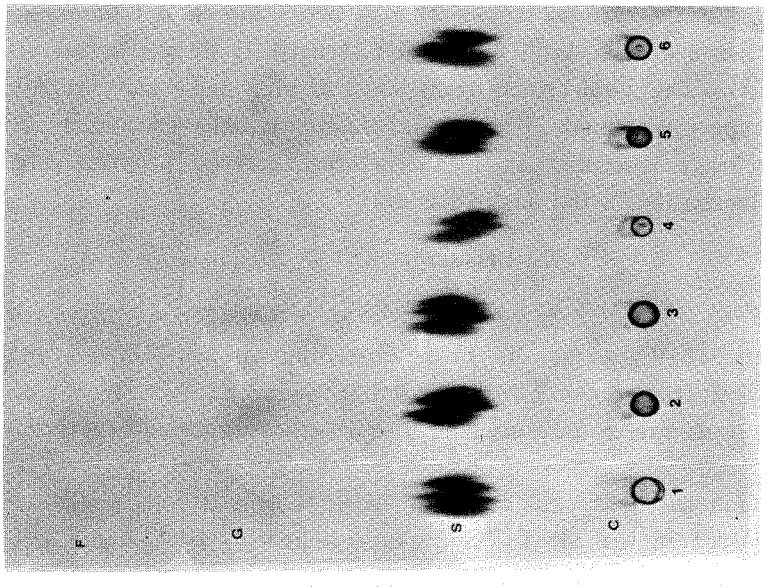

Plate 1. Autoradiograph of chromatogram of ethanolic extracts of leaves of ***Adiantum cuneatum*** (1, 2, 3) and apical segments of ***Asplenium bulbiferum*** (4, 5, 6) which had been exposed to $^{14}CO_2$ for 1 hr. in the light (1 and 4), and 24 hr. (2 and 5) and 48 hr. (3 and 6) in the dark after removal of $^{14}CO_2$. (C=unidentified compounds of high molecular weight, S=sucrose, G=glucose and F=fructose. Solvent=ethyl acetate/pridine/water (8:2:1 v/v).).

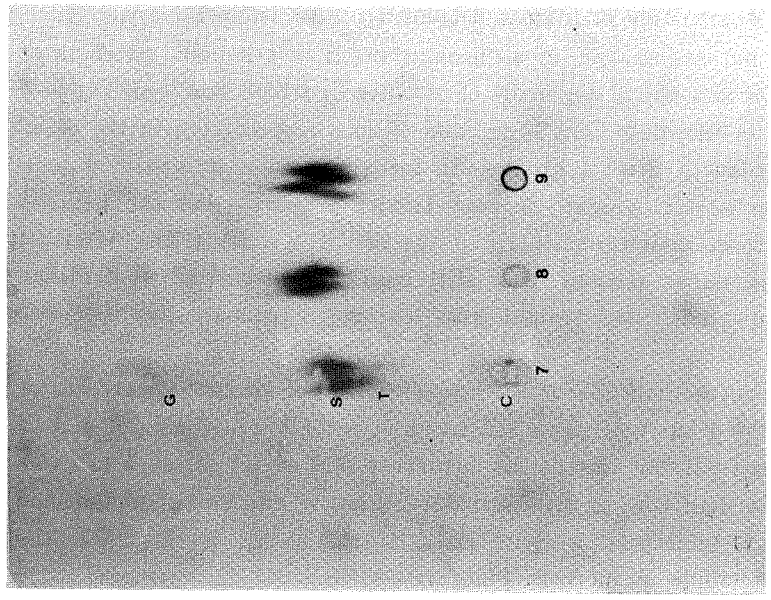

Plate 2. Autoradiograph of chromatogram of ethanolic extracts of discs of ***Pteris cretica*** which had been exposed to $^{14}CO_2$ for 1 hr. in the light (7), and 24 hr. (8) and 48 hr. (9) in the dark after removal of $^{14}CO_2$ (Notation additional to plate 1: T=trehalose. Solvent system as in plate 1).

Table 3. *Percentage incorporation of ^{14}C into individual soluble sugars of the Pteridophytes,* ***Adiantum cuneatum, Asplenium bulbiferum*** *and* ***Pteris cretica*** *on exposure to $^{14}CO_2$ for 1 hr. in the light followed by periods in the dark, calculated from integrated results of scanned paper chromatograms*

Sample and treatment	Percentage incorporation of ^{14}C			
	Unidentified baseline compounds	Sucrose	Glucose	Fructose
Adiantum, light 1 hr in $^{14}CO_2$	19.5	75.2	3.5	1.8
Adiantum, light 1 hr in $^{14}CO_2$+24 hr dark	10.8	80.2	6.2	2.8
Adiantum, light 1 hr in $^{14}CO_2$+48 hr dark	18.7	72.7	4.9	3.7
Asplenium, light 1 hr in $^{14}CO_2$	14.8	85.2	ud*	ud*
Asplenium, light 1 hr in $^{14}CO_2$+24 hr dark	14.5	84.7	0.8	ud*
Asplenium, light 1 hr in $^{14}CO_2$+48 hr dark	18.6	81.4	ud*	ud*
Pteris, light 1 hr in $^{14}CO_2$	17.9	74.8+T	7.3	ud*
Pteris, light 1 hr in $^{14}CO_2$+24 hr dark	8.3	91.7	ud*	ud*
Pteris, light 1 hr in $^{14}CO_2$+48 hr dark	13.9	86.1	ud*	ud*

ud* = undetectable
+T = plus trehalose

Kandler (1976) reported trehalose in species of Umbelliferae. Oesch and Meier (1967) reported trehalose in cambial sap of *Fagus silvatica.* Suleiman *et al.,* (1979) reported traces of trehalose in the leafy liverwort *Plagiochila asplenioides.* So presence of trehalose in two Pteridophytes in this study adds to the species in which trehalose has been detected.

Photosynthetic products and their fate in the dark

Sucrose is reported in this study as the major product of photosynthesis in all three Pteridophytes investigated on exposure to $^{14}CO_2$ for 1 hr. in the light. Trehalose was also slightly labelled after 1 hr. photosynthesis in *Pteris cretica.* White and Towers (1967) found sucrose to be the major product of photosynthesis in the Pteridophyte, *Lycopodium,* but they found both sucrose and trehalose incorporating C^{14} from $^{14}CO_2$ after 2 hr. in the light in *Isoetes.* In the present study, and after 24 hr. in the dark, the slight label in trehalose was shown to disappear, but that in sucrose was not. Thus it seems that the

suggestion of White and Towers (1967) that trehalose is utilized for the synthesis of sucrose in Pteridophytes holds in case of *Pteris cretica*. They suggested that such synthesis follows one of two ways:

(i) Trehalose $\xrightarrow{H_2O}$ Glucose $\xrightarrow{ATP}$ $\xrightarrow{UTP}$ UDPG $\xrightarrow{Fructose}$ Sucrose

or

(ii) Trehalose $\xrightarrow{UDP}$ UDPG $\xrightarrow{Fructose}$ Sucrose

Alternatively, trehalose could be utilized during respiration of *Pteris* in the dark.

In this study in *Asplenium*, although trehalose is present, no C^{14} was detected in it after 1 hr. photosynthesis in $^{14}CO_2$. It would seem that possibly C^{14} is incorporated into trehalose in *Asplenium* in shorter periods (less than an hour) and then used for synthesis of sucrose as suggested by White and Towers (1967). This is supported by the fact that other ferns incorporate C^{14} from $^{14}CO_2$ in the light after short periods. Kandler and Senser (1965) found 20% of the radioactivity after 30 minutes photosynthesis of the fern, *Botrychium lunaria* was into trehalose.

There was some turnover of ^{14}C-sucrose and more in C^{14}-starch in the three ferns investigated here. It appears that Pteridophytes in this respect seem to resemble other higher plants but not bryophytes, particularly leafy liverworts in which the turnover of C^{14} metabolites in the dark is very slow (Suleiman and Lewis, 1980).

Acknowledgement

I am grateful to Professor A. J. Willis and Dr. D. H. Lewis for use of the facilities of the Department of Botany of the University of Sheffield, U. K.

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放射性二氧化碳介入蕨類植物 *Adiantum cuneatum* L., *Asplenium bulbiferum* L., 及 *Pteris cretica* 的醣類之研究

A. A. A. SULEIMAN

Department of Botany, The University, Shefield S10 2TN, England

蕨類植物 *Adiantum cuneatum, Asplenium bulbiferum* 及 *Pteris cretica* 的可溶性醣類以蔗糖、葡萄糖和果糖爲主。 *Asplenium* 和 *Pteris* 尙含有海藻糖。這三種羊齒植物以碳十四標示的二氧化碳進行光合作用一小時後，得到的主要光合作用產物是蔗糖。除蔗糖外 *Adiantum* 和 *Asplenium* 也合成放射性葡萄糖、果糖及一些帶電性化合物。在上述條件下 *Pteris* 除了合成放射性蔗糖、葡萄糖及帶電性化合物之外也合成放射性海藻糖。將 *Pteris* 置於暗處二十四小時後，海藻糖及葡萄糖的放射性消失，但是蔗糖的放射性仍然存在。將 *Adiantum* 和 *Asplenium* 置於暗處四十八小時後，其蔗糖、葡萄糖及果糖仍然具有放射性。在暗處此三種羊齒植物的澱粉之碳十四含量隨着時間減少。這些實驗結果與蕨類植物及其他植物的醣類代謝的關係有加以討論。