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UPTAKE OF LABELED GLUCOSE BY ROOT TIPS FROM ETIOLATED WHEAT SEEDLINGS*

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Abstract—Wheat root tips from germinated seedlings were incubated in labeled D-glucose-U-L-^{14}C for periods up to 30 min. Uptake of activity into glucose-1-phosphate, glucose-6-phosphate, sucrose and UDP-glucose was monitored Extremely low, but significant, activities were found in ADP-glucose and GDP-glucose. A Golgi fraction was isolated. Activity was found in a particulate form but it could be solubilized in mild base or acid. Sephadex chromatography of the base solubilized material demonstrated that it had high mol wt Acid hydrolysis yielded mainly radioactive xylose, arabinose, glucose, galactose, with lesser quantities of mannose and uronic acids

INTRODUCTION

FROM classical metabolism, labeled glucose incorporated into plant tissue is phosphorylated by glucokinase to glucose-6-phosphate. After that it may be converted to glucose-1-phosphate, UDP-glucose, sucrose and polysaccharides. Many early studies indicated that cell wall polysaccharides contained most of the activity after incubation in labeled sugars for a few hours.[1,2] That implies rapid uptake of activity into UDP-glucose, GDP-glucose and other sugar nucleotides, necessary for biosynthesis of complex polysaccharides.

While many studies on uptake for periods of several hours have been reported, short periods have not been extensively studied. Northcote and Pickett-Heaps[3] reported that Golgi bodies were active after wheat root tips were incubated in radioglucose for 15–20 min. Their studies indicated that these organelles were involved in the biosynthesis of hemicellulose, that was subsequently incorporated into the root cap and cell walls. Presumably the appropriate sugar nucleotides containing xylose, arabinose and uronic acids, as well as glucose and galactose were labeled also. UDP-glucose is the most abundant sugar nucleotide in wheat seedlings.[4–6] ADP-glucose is present in the endosperm,[7] and the coleoptiles incorporate ^{32}P into ADP-glucose and GDP-glucose but at low levels compared to UDP-glucose.[8] The roots seem to contain only UDP-glucose in quantities sufficient to be identified.

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A quantitative study therefore was conducted on the uptake of radioglucose into the sugar nucleotides containing adenosine, uridine and guanosine bases to determine if uptake of activity is consistent with their reported roles in carbohydrate biosynthesis. Uptake of activity into the Golgi fraction, sucrose, glucose-1-phosphate and glucose-6-phosphate was monitored also.

RESULTS AND DISCUSSION

^{14}C *Metabolites.* The uptake of labeled glucose into sucrose, glucose-6-phosphate, glucose-1-phosphate, UDP-glucose and three unidentified compounds is shown in Table 1. Specific activities, i e. activity divided by the amount of carbohydrate extracted, are reported because irregular sized samples were taken for analyses. At least nine radioactive compounds could be observed on the X-ray film after the 30 min incubation. Fructose was identified by its R_f but was not counted because it contained such low levels of activity. The three closely running compounds, X-1, X-2 and X-3 (Table 1), were produced in fairly high concentration

TABLE 1. RADIOACTIVITY* IN METABOLITES FROM WHEAT ROOT TIPS AFTER INCUBATION IN 20 μC D-GLUCOSE-U-L-^{14}C

Metabolite	Incubation time, min								
	2	4	6	8	10	15	20	25	30
Sucrose	3	9	13	18	19	33	37	52	
Glucose-1-phosphate	2	6	7	8	7	8	7	8	10
Glucose-6-phosphate	3	8	12	10	14	14	14	19	22
UDP-glucose	1	5	6	6	6	12	9	13	15
GDP-glucose	—	—	—	—	0 3	—	—	—	0 5
ADP-glucose	—	—	—	—	0 15	—	—	—	0 2
X-1	2	20	—	24	2	3	3	4	5
X-2	5	26	—	24	2	3	3	5	7
X-3	4	30	—	22	3	0	0	0	0

* Activity shown in counts/min/μg carbohydrate

in the first 5–8 min. After 10 min they declined to nearly zero in activity. They were similar in R_f to a compound described as G-X by Peaud-Lenoel and de Gournay-Margerie,[9] and exhibited the same type of rapid uptake of activity. Unfortunately, further work showed that, unlike the *G–X* compound, none of our three compounds could be hydrolyzed to glucose. Chromatography in a variety of solvents demonstrated further minor differences in R_f (Table 2). Other compounds identified by these workers,[9] namely, alanine, glutamine, glutamic acid, aspartic acid and glycine, were ruled out because they had different R_f values in one or more chromatographic systems. Our compounds failed to react with ninhydrin or with silver nitrate.

An examination of the exudates by paper chromatography demonstrated that radioactive X-1, X-2, X-3, fructose and other compounds had been excreted into the medium at an early stage. However, no significant amount of sucrose or any of the phosphorylated compounds (Table 1) was detected. Uptake of activity into X-1, X-2 and X-3 therefore is

[9] C PEAUD-LENOEL and C DE GOURNAY-MARGERIE, *Bull Soc Franc Physiol Vegetale* **10**, 162 (1964)

even greater than shown in Table 1. However since they could not be hydrolyzed, presumably they contain no phosphate or glucose. Therefore a possible role in polysaccharide biosynthesis seems remote.

Uptake of activity in the Golgi fraction. The dialyzed Golgi fraction remained insoluble during dialysis and the radioactive material could not be solubilized in hot water or by sonication. It was readily solubilized with mild base or acid (0 05 N KOH or 0·01 N HCl at 100° for 15 min). Elution of the material on Sephadex G-15 is illustrated in Fig. 1. Most of the activity was in high mol. wt. compounds. The high mol. wt. components on acid

TABLE 2 PAPER CHROMATOGRAPHY OF X-1, X-2 AND X-3 COMPOUNDS EXTRACTED BY 50% ETHANOL FROM WHEAT ROOT TIPS USING GLUCOSE AND ANALINE AS REFERENCES

Compound	Solvent			
	I		II	III
	R_G	R_{Ala}	R_G	R_{Ala}
X-1	0 34	0 71	0 74	0·52
X-2	0 37	0 97	0 84	0 77
X-3	0 41	0 87	1 20	—

Solvent I: EtOAc–pyridine–water (10·4.3, v/v)
Solvent II: *n*-BuOH–propionic acid–water (EDTA) 3·6 mM (10 5 7, v/v)
Solvent III. 80% *iso*PrOH.

hydrolysis produced the radioactive sugars reported in Table 3 (isolated by paper chromatography). The analysis is typical of a 'hemicellulose'. Results from the 'chase' experiment (Table 3) are consistent with the theory advanced by Northcote,[3] i.e. the Golgi produce hemicellulose that is subsequently deposited via vesicles in the slime layer of the root cap and cell wall.

Some puzzling inconsistencies appear with regard to other aspects of cell wall biosynthesis. Although root tips from wheat seedlings were shown to have a full complement of enzymes necessary for biosynthesis of the guanosine and adenosine containing sugar nucleotides, extremely low levels of activity were incorporated compared to the amount incorporated by UDP-glucose. Significant levels of activity were not incorporated into the pentose or uronic acid moieties of these nucleotides, necessary for the biosynthesis of the hemicellulose found in the Golgi fraction. Apparently there is an extremely rapid turnover of these nucleotides but not of UDP-glucose. Furthermore, the hemicellulose was particulate, bound perhaps to a lipid component, which was readily freed with mild basic or acidic hydrolysis. Lipid involvement in bacterial cell wall,[10,11] *O*-antigen[12,13] and cellulose[14] biosynthesis is well documented. The bacterial systems produce polysaccharides that are excreted. The hemicellulose produced in plants is excreted also, and some type of lipopolysaccharide may be involved.

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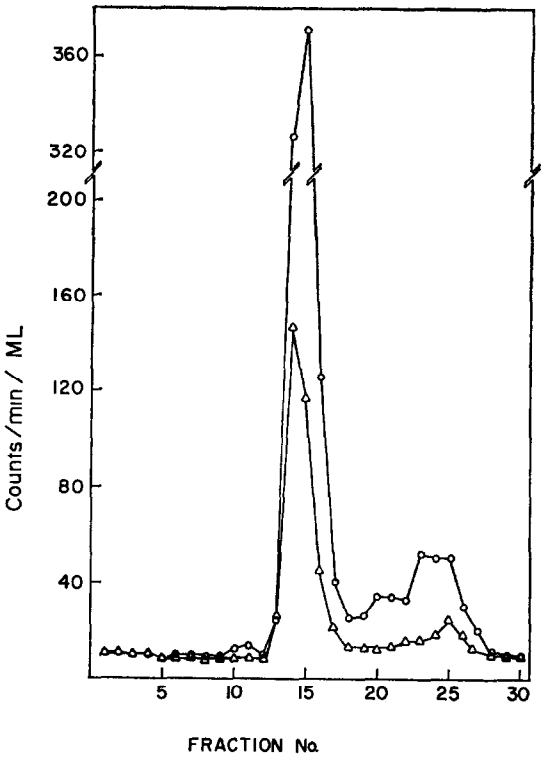

FIG. 1. FRACTIONATION OF RADIOACTIVE POLYSACCHARIDE SAMPLES OF EQUAL CARBOHYDRATE CONTENT OBTAINED FROM GOLGI FRACTION SOLUBILIZED IN 0·5 N KOH AT 100° FOR 15 min. THE SEPHADEX G-15 COLUMN (1 × 41 cm) WAS ELUTED WITH 6·5 mM KH_2PO_4 –K_2HPO_4 BUFFER pH 8, FLOW RATE 7 ml/hr, RECOVERY—95% OF ACTIVITY APPLIED.

○, 30 min incubation in D-glucose-U-L-^{14}C.

△, 30 min incubation in D-glucose-U-L-^{14}C followed by 30 min 'chase' in 0·01 M D-glucose.

TABLE 3. RADIOACTIVITY OF SUGARS, FROM THE HYDROLYZATES OF THE ISOLATED PARTICULATE FRACTION SEPARATED BY PAPER CHROMATOGRAPHY*

Sugar	Activity, % of total
Immobile fraction	18 2
Galactose	17·5
Glucose	17·2
Mannose	3·2
Arabinose	13 4
Xylose	22 9

* Root tips were incubated 30 min in D-glucose-U-L-^{14}C. Isolated particulate fraction after dialysis was hydrolyzed in 1 N HCl 6 hr at 100°.

EXPERIMENTAL

Preparation of etiolated seedlings A sample of winter wheat (Ottawa variety) was immersed in 0 1% formaldehyde 1 hr.[15] The formaldehyde was removed by washing 1 hr in distilled water. The seeds were germinated in the dark for 48–72 hr at room temp. on moist filter paper. Sterile conditions were maintained so far as possible.

Incubation studies. Following germination, root tips were excised with a razor blade and washed in cold water. Generally about 2 g of excised root tips was placed in a small beaker and covered with 3 ml of water containing 15–50 μc of D-glucose-U-L-^{14}C At various time intervals during the first 30 min of incubation, five to eight root tips were removed and dropped into boiling 50% ethanol.[9] After 5 min, the extraction mixture was cooled and the roots re-extracted for 5 min in 50% ethanol, then for 5 min in 75% ethanol. The three extracts for each sample were pooled and evaporated to dryness under vacuum. The samples were then made up to volume and analyzed for carbohydrate.[16] Aliquots were chromatographed.

Paper chromatography. Ethanolic extracts containing ^{14}C metabolites were purified by two dimensional chromatography on Whatman No. 1 paper (46 × 57 cm) It was developed in the first direction for 17 hr[17] with EtOAc–pyridine–water (19 4:3, v/v), then with *n*-BuOH–propionic acid–3 6 mM EDTA (10:5:7, v/v) in the second direction for 20 hr.[18] For separation of nucleotides a small amount of inactive ADP-glucose, UDP-glucose and GDP-glucose was added and the sample was chromatographed in 95% ethanol–1 M NH_4OAc pH 3 8 (5:2, v/v) in the first direction for 20 hr, then in 95% ethanol–1 M NH_4OAc pH 7·5 (5·2, v/v) in the second direction for 24 hr[19] Nucleotides were located by their absorption in the u v. Neutral sugars from extracts and hydrolyzates were identified by chromatography in EtOAc–pyridine–water (10:4 3, v/v) Spots were revealed with $AgNO_3$.[20]

Radioautography Kodak Blue Brand Medical X-ray film (14 × 17 5 cm) was used to locate metabolites on chromatograms.[21] Films were developed as specified by the manufacturer.

Radioactive measurements. Compounds revealed by X-ray radioautography were extracted with water and their radioactivities measured. Aq. ^{14}C samples were counted in a dioxane solvent or on Whatman No. 1 paper discs. For the latter, the sample was dried on the discs at room temp and counted in a fluor of 5 g of 2,5-diphenyloxazole/l. of toluene.

Isolation of Golgi fraction. A Golgi fraction was isolated from about 1 g of excised root tips that had been incubated in labeled glucose for 30 min. All operations were carried out at 0–4°. Root tips were washed in distilled water and ground in a loose fitting Thomas tissue grinder for 20 sec in a medium of 0 5 M sucrose, 0 1 M Na_2HPO_4–NaH_2PO_4 (pH 7·6), 0 001 M $CaCl_2$, 1% dextran and 1% bovine serum albumin[12,22] The ground tissue was filtered through cheese cloth and centrifuged in a Beckman preparative centrifuge at 4000 *g* for 30 min. The supernatant was re-centrifuged at 100,000 *g* for 30 min on a 0 25 ml pad of 1 8 M sucrose layered in the bottom of the centrifuge tube. The particulate material at the interface which contained the Golgi apparatus, was recovered and re-centrifuged to remove soluble radioactive components. The particulate material concentrated at this layer was dialyzed 2–4 days with distilled water prior to chemical studies.

The Golgi fraction so isolated could be split into three particulate bands by re-centrifuging for 3 hr at 34,000 *g* on a gradient of 0 5–1 8 M sucrose Only the heaviest band concentrated at a density corresponding to 1 25 M sucrose contained radioactivity; therefore, the gradient separation generally was omitted. Golgi apparatus from onions has been reported to be separated at a density corresponding to 1 25 M sucrose[23]

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