METABOLISM OF GALACTOSE IN *CANNA* LEAVES AND WHEAT SEEDLINGS*

by

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Although free galactose is not found in plants, it is of widespread occurrence in combined form as polysaccharides[1,2]. Preliminary experiments showed that when ^{14}C-labeled glucose was introduced into *Canna* leaves or wheat seedlings, the galactose isolated from the hemi-cellulose of these plants was radioactive, indicating a conversion of glucose to galactose. In view of this observation, it was of interest to study the reverse transformation, the conversion of galactose to glucose, and to other compounds. For this purpose *Canna* leaf tissue and wheat seedlings were used as experimental material. ^{14}C-labeled galactose was introduced into the plant tissues, and after the plants had been allowed to respire for various periods of time, the metabolic products were isolated, identified and assayed for radioactivity.

It was previously shown that when either glucose or fructose randomly ^{14}C-labeled is introduced into respiring plant leaf tissues, the radioactivity is rapidly incorporated into sugar phosphates, sucrose, organic acids, amino acids and the polysaccharide constituents[3,4,5,6]. Simultaneously, the respired CO_2 becomes labeled.

In the present investigation similar results have been obtained when ^{14}C-labeled galactose has been introduced into *Canna* leaf tissue and wheat seedlings.

MATERIALS AND METHODS

Plant material

In most of the experiments 18 mm diameter *Canna* leaf disks were used. The disks were infiltrated with radioactive sugars and allowed to metabolize in the dark for time periods up to 3.5 hours during which the CO_2 was collected. The disks were then extracted with boiling 80% ethanol and the sugars and other compounds analyzed chiefly by paper chromatography and radioautography[4].

In the experiments with wheat seedlings, the radioactive sugar was absorbed by the plants while the solution was aerated[3]. The rate of absorption by the seedlings was approximately 3 mg of hexose per g of dry weight per hour.

Radioactive sugars

Uniformly ^{14}C-labeled glucose used in the experiments with *Canna* disks was prepared photosynthetically from *Iridophycus flaccidum* in this laboratory as previously described[7]. Its specific activity was 52 μc per mg.

Galactose-1-^{14}C used in the experiments with wheat seedlings was obtained from Dr. H. S. ISBELL of the National Bureau of Standards.

Fructose-1-^{14}C was prepared[8] by enolization of glucose-1-^{14}C.

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Estimation of radioactivity

Samples were counted directly on Whatman No. 1 filter paper with a Tracerlab rate meter (SU-3A) supplied with a TGC-2 Geiger tube[4]. This instrument can be read with an accuracy of ± 5% of the full scale deflection in ranges of 0 to 200, 0 to 2000, and 0 to 20,000 c.p.m. When more accurate estimations were required in the degradation studies, the samples were mounted on aluminium disks and counted by means of a scaling unit. The radioactivity measurements represent mean values of duplicate samples corrected for self absorption, daily counter variation, and background.

Degradation of ^{14}C-labeled glucose

The method used for degradation of the glucose was essentially that of GUNSALUS AND GIBBS[9]. The radioactive glucose was subjected to heterolactic fermentation by *Leuconostoc mesenteroides*, which degrades glucose to CO_2, ethanol and lactic acid. The latter two compounds were then further degraded by chemical methods to their individual carbon atoms as CO_2[10,3].

Sugar analysis

Total reducing sugar was determined by the method of SOMOGYI[11] as modified by NELSON[12]. Fructose was determined by the method of ROE[13]. The sucrose was estimated by determination of the reducing sugars after hydrolysis of the non-reducing fraction with invertase[4].

Extraction and separation of ethanol-soluble components

A group of seven of a total of 49 *Canna* leaf disks were extracted with boiling 80% ethanol and the remaining 42 infiltrated with 6.0 ml of a solution containing 8.6 mg randomly ^{14}C-labeled galactose with a specific activity of 52 μc per mg. After infiltration and washing procedures which required approximately 10 minutes, a second set of 7 disks was placed in boiling 80% alcohol for subsequent extraction, and 35 disks were placed in a respirometer in the dark[4]. At intervals of 15, 30, 60, 110 and 200 minutes, samples of 7 disks were removed from the apparatus for extraction with alcohol. Simultaneously, the carbon dioxide respired by the disks throughout these time periods was collected, precipitated as $BaCO_3$ and assayed for ^{14}C activity.

The alcohol extracts of the infiltrated disks obtained after each time period were concentrated to 1.00 ml and the total amount of ^{14}C activity in each extract was estimated by counting a 4% aliquot. The extracted leaf disk residues were dried by pressing between filter paper sheets, and the ^{14}C activity which was fixed in the alcohol insoluble residue of the plant tissue was determined by counting the activity with the Geiger counter in each disk individually.

Two-dimensional paper chromatographic analyses, using butanol-acetic acid-water mixture and water-saturated phenol as solvents[14,4] were employed for the separation of the compounds present in the 80% ethanol extracts and the preparation of the chromatographic patterns.

Estimation of the ^{14}C activity present in the various compounds on the chromatograms was made by direct count of the areas containing the sugars after their positions had been located by radioautography.

The ^{14}C activities of the glucose and fructose moieties of sucrose derived from the incorporated D-galactose were determined as follows: a 0.25 ml aliquot of each of the alcohol extracts was treated with ion exchange resins and the sucrose isolated by paper chromatography. It was then hydrolyzed by treatment with yeast invertase and the monosaccharides produced were subsequently separated chromatographically.

The phosphorylated sugars were identified as follows: the area on the chromatogram containing the organic phosphate compounds was eluted, the solution concentrated and hydrolyzed with alkaline intestinal phosphatase. The free sugars were then rechromatographed two-dimensionally and identified by their R_F values. The amount of radioactivity in the individual sugars was determined by locating the areas they occupied on the chromatogram by the aid of radioautography and subsequent counting of the spots on the paper.

RESULTS

The results of the distribution of the ^{14}C activity of the various fractions obtained from the *Canna* leaf disks after incorporation of randomly ^{14}C-labeled galactose is presented in Table I. The table shows that the total activity in the *Canna* leaf samples is approximately the same at various time intervals. The ^{14}C activity of the respiratory CO_2 progressively increases, while that in the alcoholic extract decreases. The increase of the activity in the insoluble leaf residue could only be explained by the assumption that as time progresses the ^{14}C label of the soluble constituents present in the alcohol

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TABLE I

DISTRIBUTION OF ^{14}C-ACTIVITY IN FRACTIONS DERIVED FROM RESPIRING *Canna* LEAF DISKS AFTER INFILTRATION WITH A 0.13% SOLUTION OF UNIFORMLY LABELED ^{14}C-GALACTOSE

Forty-two *Canna* leaf disks, having a fresh weight of 3.85 g were infiltrated with 6 ml of a solution containing 8.6 mg of ^{14}C-galactose with a specific activity of 52 μc/mg.

Time, minutes	*0*	*15*	*30*	*60*	*110*	*200*
Total activity c.p.m.	294.000	242.000	201.000	214.000	232.000	271.000
% Activity Resp. CO_2	0	0.7	2.0	4.9	4.8	20.8
% Activity Alcohol ext.	95.8	93.1	87.2	84.8	75.5	69.5
% Activity Leaf residues	4.4	6.4	10.7	10.6	9.7	9.9

fraction are gradually incorporated into the insoluble compounds, chiefly carbohydrate in nature.

Examination of the chromatograms showed that immediately after infiltration of the *Canna* tissue with randomly ^{14}C-labeled glactose, the dominant compounds containing in excess of 10% of the total activity were sucrose, galactose, alanine, and compounds which migrated to positions normally occupied by hexose mono- and diphosphate esters. The changes in ^{14}C activity of several of the more active compounds is shown in Fig. 1.

A marked decrease in the activity in galactose is observed after 15 minutes, falling to 4% of the activity in the alcohol extract after 60 minutes. The diminution of activity in the galactose corresponds to an increase of activity in sucrose, lactic acid polysaccharide and respired CO_2. The radioactivity of the sugar phosphates (Fig. 1) which became radioactive simultaneously with the sucrose, had fallen after 60 minutes to 1% of the total activity of the sample. At the end of 60 minutes the only compounds on the chromatogram showing more than 4% of total radioactivity were sucrose (40%), and lactic acid (11%). Other compounds which appeared on the chromatogram were identified as triose phosphates, phosphoglyceric acid, citric, malic, succinic, glyceric and glycolic acids, and the amino acids, aspartic, glutamic, and γ-aminobutyric acid. At no time did ^{14}C-labeled free glucose or free fructose appear on the chromatogram.

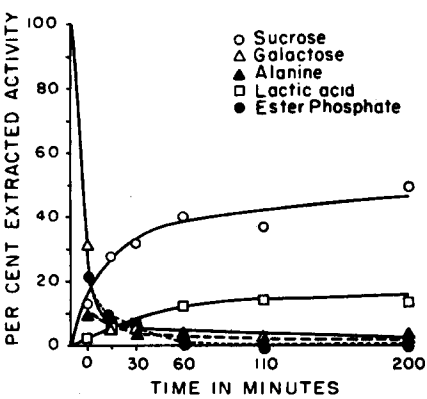

Fig. 1. Distribution of ^{14}C activity in the 80% ethanol extracts of *Canna* leaf disks after infiltration with ^{14}C-galactose.

Hydrolysis of the sucrose with invertase and determination of the ^{14}C activity of the resulting glucose and fructose showed that the two moieties were radioactive to the same extent.

Quantitative analysis of a 0.5 ml aliquot of each of the alcoholic extracts for glucose, fructose and sucrose revealed that there was essentially no change in the levels of these naturally occurring sugars in the *Canna*

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disks during the 3.5 hour period of the experiment. Each set of seven disks contained approximately 11 mg total sugars (6 mg sucrose, 2 mg glucose, and 3 mg fructose). The 0.06 mg of ^{14}C-labeled galactose, infiltrated into each set of 7 disks, could not have been detected by the analytical methods used.

The sugar phosphate fraction was isolated chromatographically from the remaining 0.21 ml alcohol extracts of the 0 and 15 minute experiments, treated with intestinal phosphatase, the hydrolysis products rechromatographed, and the radioactive spots radioautographed. At least seven free sugars could be detected on the chromatogram in addition to some unhydrolyzed material that remained at the origin. The most prominent of these was galactose. About 38% of the total activity originally present in the phosphate fraction was found in this hexose. When the sugar, identified by its R_F value as galactose, was oxidized with bromine, compounds were obtained that corresponded chromatographically to galactonic acid and its lactone. When subjected to a Ruff degradation both these compounds yielded lyxose. The amount of activity present in most of the other sugars was too low for positive identification. However, the radioautograms suggested the presence of either sedoheptulose of mannose, pentose and dihydroxy acetone, in addition to glucose and fructose.

In order to identify the phosphorylated galactose derivative, another lot of *Canna* leaf disks was infiltrated with a more concentrated solution of ^{14}C-labeled galactose of a lower specific activity. For this purpose, forty 9 mm disks were infiltrated with 2 ml of 0.5% ^{14}C-labeled galactose containing 3 μc per mg. After the external solution was decanted the disks were allowed to respire for 15 minutes and then extracted with boiling 80% ethanol. The extract was concentrated and the sirup chromatographed unidimensionally as a band with phenol as a solvent. The area containing the phosphorylated sugars was eluted with water, rechromatographed as a band in butanol-acetic acid-water, the product located by means of a radioautogram, eluted again and concentrated to dryness. The residue was then subjected to hydrolysis with 1 *N* HCl for 10 minutes at 100°. The products of hydrolysis were frozen and lyophylized in a desiccator over solid KOH. On rechromatographing of the products, a new area

TABLE II

PER CENT DISTRIBUTION OF ^{14}C IN GLUCOSE DERIVED FROM SUCROSE AFTER INCUBATION OF WHEAT SEEDLINGS WITH GALACTOSE-1-^{14}C AND FRUCTOSE-1-^{14}C

Two lots, 70 seedlings each, were incubated for 2 hours in 10 ml of media, one containing 5.3 mg of galactose-1-^{14}C (sp. activity, 1.22 μc/mg), and the other 8 mg of fructose-1-^{14}C (sp. activity 0.8 μc/mg). The values in the table are obtained by *L. mesenteroides* degradation, and expressed as percentages of total activity recovered as $BaCO_3$.

Carbon atom	*Glucose moiety of sucrose**	
	Derived from galactose-1-^{14}C	*Derived from fructose-1-^{14}C*
1	72	73
2 }	5	3
3 }		
4	2	1
5	0	0
6	21	17

* The methods used for extraction and hydrolysis of the sucrose from the plants are described in a previous publication[3].

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appeared on the chromatogram corresponding to galactose (71%). The remaining 29%, which occupied the original position, probably representing a more acid resistant hexose monophosphate, was not identified. The galactose spot was eluted and the identity of this sugar confirmed by two-dimensional co-chromatography when mixed with authentic galactose, glucose, mannose and fructose. The radioactivity was confined to the area occupied by galactose when the position of the sugars was revealed by treating the chromatogram with *p*-anisidine hydrochloride. The easily hydrolyzable galactose is probably galactose-1-phosphate.

The results of degradation of the glucose moiety of the sucrose isolated from wheat seedlings after incubation with either galactose-1-^{14}C or fructose-1-^{14}C are shown in Table II. There was practically no difference between the distributions of ^{14}C activity among the individual carbon atoms in the glucose chain, whether galactose or fructose was used as substrate for the wheat seedlings.

DISCUSSION

When randomly ^{14}C-labeled galactose is infiltrated into *Canna* leaf tissue the metabolic products immediately formed are organic phosphates, chiefly hexose monophosphates (mainly galactosephosphate, with smaller amounts of sedoheptulose phosphate, pentose phosphate and phosphates of glucose, fructose and mannose) and sucrose. The results are similar to those obtained when glucose or fructose is introduced into this tissue[4]. The fact that the glucose and fructose constituents isolated from the synthesized sucrose are labeled after infiltration of ^{14}C-labeled galactose indicates that the galactose is readily transformed into these monosaccharides. Evidence that its transformation to glucose is direct, and that it occurs without prior degradation of the chain to smaller fragments may be adduced from the observation that when galactose-1-^{14}C is introduced into wheat seedlings, the glucose isolated from the synthesized sucrose is predominantly labeled in C-1. The smaller proportion of ^{14}C activity in C-6 appears to be derived through randomization with C-1, possibly by a reversal of the glycolytic system, involving a recombination of two triose phosphates[3].

Inasmuch as an easily hydrolysable galactose phosphate, probably galactose-1-phosphate, is present among the phosphorylated products after incorporation of galactose into the *Canna* leaf tissue, it may be postulated that the galactose $\rightleftharpoons$ glucose interconversion occurs through a mechanism involving the enzyme galactowaldenase[15,16].

Previous experiments showed[4] that when radioactive glucose was incorporated into *Canna* leaf tissue, the labeled sucrose contained about 50% more activity in the glucose than in the fructose moiety; infiltration of fructose gave rise to sucrose labeled with about 50% more ^{14}C in the fructose than in the glucose moiety. In the present work incorporation of galactose into the tissue resulted in sucrose with equal activity in both monosaccharide constituents. This difference cannot be explained at the present time.

SUMMARY

The transformation of randomly ^{14}C-labeled galactose was studied in *Canna* leaf disks during the course of a 3.5 hour respiration period in the dark.

Introduction of this radioactive sugar into *Canna* leaf tissue caused a rapid appearance of ^{14}C-labeled sucrose and ^{14}C-labeled hexose monophosphates. When the radioactive sucrose was

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hydrolyzed to its monosaccharide constituents, the activity of the glucose was equal to that of fructose.

The major proportion of the radioactive phosphorylated hexoses isolated from *Canna* leaf tissue, after infiltration of randomly ^{14}C-labeled galactose was an easily hydrolyzable galactose phosphate, which is probably galactose-1-phosphate.

When galactose-1-^{14}C was introduced into wheat seedlings and the glucose isolated from the sucrose after 2 hours was degraded to its individual carbon atoms, C-1 contained the major proportion of the activity (72%). C-6 contained practically all the remainder of the label (21%) which may have been produced through randomization with C-1 by a reversal of the glycolytic system in the respiring plant. The other four carbon atoms in the glucose chain possessed very little activity.

The data indicate that galactose is converted directly to glucose without prior degradation of the chain, probably through a mechanism involving the enzyme, galactowaldenase.

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