Journal of Chromatography, 166 (1978) 587–592

CHROM. 11,282

QUICK ANALYTICAL SEPARATION OF GLUCOSE AND FRUCTOSE WITH IMPREGNATED WOVEN GLASS FIBER

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(First received February 21st, 1978; revised manuscript received June 26th, 1978)

SUMMARY

(1) A wide separation of glucose and fructose was obtained in 30 min with simple, inexpensive equipment using polysilicic impregnated woven glass fiber and the solvent acetone–*n*-butanol–1 *M* H_3BO_3 (50:40:10).

(2) A calibration of a radiochromatogram scanner was performed for ^{11}C.

(3) Three R_F values could be compared to provide positive chemical identification of [^{11}C]glucose and [^{11}C]fructose.

(4) Radiochemical composition and approximate specific activity were determined from a small aliquot (1–4 μl).

INTRODUCTION

This work was undertaken so that a separation of glucose and fructose could be accomplished in a time comparable to the half-life of ^{11}C (20.4 min). ^{11}C-labeled fructose and glucose were biosynthetically produced using Swiss chard leaves with a modification[1] of the technique described by Lifton and Welch[2]. The analytical technique presented here was directed at the separation of glucose from fructose because they account for approximately 90% of the ^{11}C activity in hydrolyzed leaf extract after biosynthesis with $^{11}CO_2$. The labeled products were used for research in nuclear medicine[1]. The positive chemical identification of the labeled compounds, and the determination of radiochemical composition and specific activity and screening for the presence of impurities in the purified leaf extract were necessities in this work. This method could be applied in situations where an inexpensive and quick separation of glucose and fructose is needed. Because glucose and fructose are the hydrolysis products of sucrose, they frequently are found together.

Typical analytical separations[3–7] of the monosaccharides take 3–24 h, and have been used for the analysis of ^{14}C-labeled or non-radioactive sugars. The most rapid separations of glucose and fructose have been obtained with gas–liquid chromatography of the trimethylsilyl ether derivatives of the carbohydrates[2,8–11]. The reten-

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tion times of glucose and fructose are below 20 min, but additional time is required for preparation of the derivatives. Recently liquid chromatography has been used to separate [^{11}C]glucose and [^{11}C]fructose in about 25 min[12].

Published techniques for separating glucose and fructose with thin-layer chromatography (TLC) have several disadvantages for the separation required in this work. The separations take over 60 min or three ^{11}C half-lives and glucose and fructose typically have R_F values not differing by more than 0.1. Most TLC of carbohydrates is performed with impregnates[13–15] in the silica gel layer.

Woven glass fiber impregnated with polysilicic acid which is similar in separating ability to thin layers of silica gel was used in this work as a chromatographic support and sorbent because solvents migrate about twice as fast as on 250 μm silica gel plates. Glass fiber impregnated with silica gel has been used to separate sugars in urine with a solvent system that runs 10 cm in 90 min[16]. Simple sugars have been separated on impregnated woven glass fiber with several solvent systems[17]; however, glucose and fructose were not resolved clearly.

EXPERIMENTAL

All solvents and reagents were analytical grade. Uniformly labeled [^{14}C]-glucose and [^{14}C]fructose (300 mCi/mmole) were obtained from Amersham-Searle (Arlington Heights, Ill., U.S.A.). D-[1-^{14}C]glucose-6-phosphate (^{14}C-G6P) (525 mCi/mmole) was obtained from New England Nuclear (Boston, Mass., U.S.A.). Woven glass fiber impregnated with polysilicic acid (ITLC-SA; Gelman, Ann Arbor, Mich., U.S.A.) was used. A Packard radiochromatogram scanner (Model No. 7201 RGS, No. 385 recording ratemeter, Honeywell Electronic 18 recorder–integrator) adapted for TLC was used to record the activity along the chromatogram. The collimating aperture was 2 mm, the time constant 1 sec, the scanning speed 5 cm/min, and the glass fiber sheet was placed on 3.2 mm thick glass plates on the TLC tray. A digital readout ion chamber was used to assay ^{11}C activity. The solvent system used was acetone–*n*-butanol–1.0 *M* boric acid (50:40:10). ^{11}C-Labeled leaf extract (1–4 μl) and approximately 0.5 μCi (in 1 μl) of [^{14}C]fructose, [^{14}C]glucose, and/or ^{14}C-G6P were applied at the origin. The sheet was placed in a tightly covered developing tank lined with filter paper. The development took 28–30 min to 10 cm.

The collimating aperture of the scanner was covered with a sheet of Parafilm (13 mg/cm^2) to absorb ^{14}C β^- emissions ($E_{max.} = 0.155$ MeV) so that the radiochromatogram produced was only from ^{11}C (β^+ $E_{max.} = 0.980$ MeV). The ability of the Parafilm to absorb the ^{14}C β^- was checked independently. Twelve hours later, after ^{11}C had decayed, another radiochromatogram, this time of ^{14}C, was produced without the Parafilm covering on the collimating aperture. The sheets were then sprayed with a solution of 4 g diphenylaniline, 4 ml aniline, and 20 ml of 85% phosphoric acid dissolved in 200 ml of acetone[18,19] and heated at 95° for 15 min. This reagent yielded characteristic colors for the hexoses; glucose gave blue and fructose, scarlet. The chemical quantity of the sugars in the hydrolyzed leaf extract was approximated from the visual comparison of size and color density of spots obtained after chromatographing known amounts of glucose and fructose.

Glucose was determined in the leaf extract using either the glucose oxidase or hexokinase methods.

The total counts in a peak of a radiochromatogram were estimated from the area of the peak as determined by the disk integrator on the chart recorder of the radiochromatogram scanner. The activity in counts per minute is then equal to total counts times the scanning velocity divided by the collimating aperture width. Efficiency was then determined by dividing the activity determined from the peak area in counts per minute by the absolute activity in the peak. The absolute activity of the total volume of labeled lead extract was determined in a digital readout ion chamber. The absolute activity in a peak was determined from the volume spotted and the fraction of that peak area to the total area of the chromatogram and was corrected for the decay of ^{11}C during the time between the assay and the peak.

RESULTS

Fig. 1 shows tracings of the chromatograms from two separate ^{11}C preparations (run Nos. 1 and 2). Each panel of this figure shows the spots in the chemically

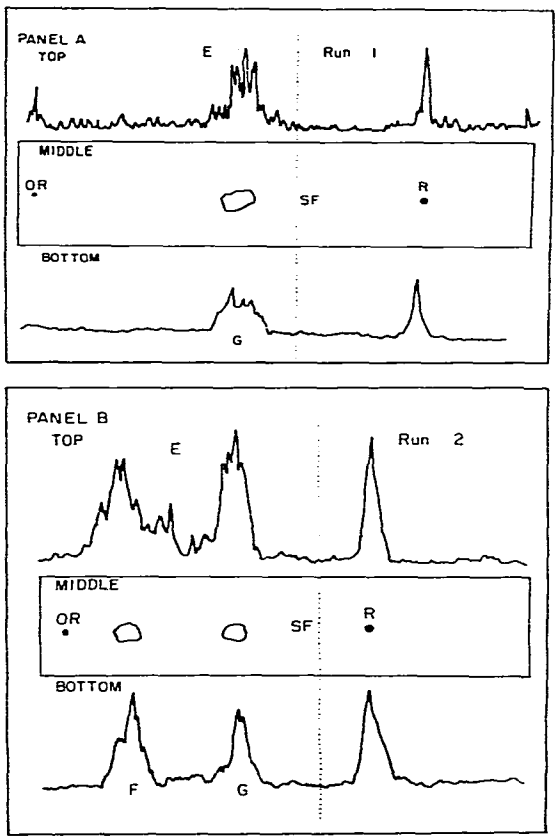

Fig. 1. Panel A; top: E, ^{11}C radiochromatogram showing a peak at $R_F = 0.78$; middle: the chromatogram with the scarlet spot at $R_F = 0.75$ circled; bottom: the radiochromatogram of [^{14}C]-glucose (G, $R_F = 0.78$). The circled reference spot (R) containing ^{11}C and ^{14}C is 14.6 cm from the origin (OR). Panel B; top: E, ^{11}C radiochromatogram, with peaks at R_F values of 0.23, 0.40, and 0.68; middle: the chromatogram with a blue spot of $R_F = 0.25$ and a scarlet spot at $R_F = 0.68$; bottom: radiochromatogram of [^{14}C]glucose (G, $R_F = 0.68$) and [^{14}C]fructose (F, $R_F = 0.24$). The circled reference spot (R) is 12 cm from the origin (OR). SF refers to the solvent front.

stained glass fiber sheets, the radiochromatogram of the ^{11}C taken immediately after removal of the sheet from the developing tank with an absorber over the collimating aperture of the radiochromatogram scanner and the radiochromatogram of ^{14}C-sugars taken the next day without the absorber. In both Fig. 1A and 1B, one of the ^{11}C activity peaks has the same R_F as the blue spot characteristic of glucose and as the [^{14}C]glucose peak. These two facts provide positive identification of [^{11}C]glucose. In Fig. 1B, there is a ^{11}C peak at an R_F of about 0.25 that is fructose, as confirmed by the occurrence of the [^{14}C]fructose peak plus the light red spot at the same R_F. In Fig. 1B there is an unknown peak at R_F 0.40 in the ^{11}C radiochromatogram.

The efficiency of the radiochromatogram scanner was determined to be 0.37 $\pm$ 0.04 (S.D.) for 14 determinations.

The R_F values obtained from the ^{14}C-sugars, from the colored spots and from the ^{11}C solution are compared in Table I for eight chromatograms. The similarity of these three R_F values are documented. The specific activity of glucose and fructose at the end of the photosynthesis with $^{11}CO_2$ is presented. The specific activity is calculated using the efficiency of the radiochromatogram scanner. The fructose concentration in run Nos. 1, 3, 5, 8 and 9 was too low to be detected with the spray reagent, so the specific activity could not be calculated. The fraction of ^{11}C associated with glucose, fructose, and the unknown peak is also presented in Table I. Typically, glucose contained 55% of the ^{11}C. The results for fructose showed greater variability than the results for glucose.

TABLE I

RADIOCHEMICAL COMPOSITION, SPECIFIC ACTIVITY AND COMPARISON OF R_F VALUES OF [^{11}C]GLUCOSE AND [^{11}C]FRUCTOSE

—, None present or not detected.

Run No.	*Glucose*					*Fructose*					*Unknown*	
	R_F			*Fraction ^{11}C in glucose*	*Spec. act.* (μCi/μg)*	R_F			*Fraction ^{11}C in fructose*	*Spec. act.* (μCi/μg)*	R_F	*Fraction ^{11}C in unknown*
	*Chem.***	^{14}C	^{11}C			*Chem.***	^{14}C	^{11}C			^{11}C	
1	0.75	0.78	0.78	0.64	0.14	—	—	—	<0.12	—	—	—
2	0.68	0.68	0.68	0.40	0.17	0.25	0.24	0.23	0.42	0.51	0.40	0.11
3	0.80	0.79	0.80	0.50	0.54	—	0.30	0.30	0.50	—	—	—
5	0.79	0.78	0.78	0.55	0.080	—	—	—	<0.18	—	0.37	0.32
6	0.73	0.73	—	0.12	—	0.25	0.25	—	—	—	0.40	0.51
7	0.73	0.71	0.69	0.34	9.65	0.19	0.20	0.17	0.29	8.11	0.37	0.26
8	0.75	0.77	0.79	0.54	16.7	—	—	—	<0.14	—	—	—
9	0.75	0.75	0.75	0.59	2.58	—	—	—	<0.05	—	0.40	0.20

* Specific activity at end of photosynthesis.

** R_F from colored spots.

For four chromatograms of the ^{11}C material, the glucose concentration estimated from the volume spotted and from the spot size was compared with the glucose concentration obtained from enzyme analysis. Table II shows these results. The agreement between the two determinations is satisfactory.

The R_F of ^{14}C-G6P is zero. An estimate of the maximum fraction of ^{11}C activity at the origin indicated not more than 3% of the ^{11}C activity was associated with G6P.

TABLE II

COMPARISON OF GLUCOSE CONCENTRATIONS OBTAINED FROM CHROMATOGRAPHY AND FROM ENZYME ANALYSIS

Runs No.	*Volume spotted (μl)*	*Quantity estimated from spot size (μg)*	*Glucose concentrations (mg/ml)*	
			Chromatography	*Enzyme analysis*
3	2	8	4.0	4.6
4	4	5	1.2	1.5
7	2	5	2.5	2.7
8	6	5	0.83	0.83

DISCUSSION

Previously, glass fiber impregnated with silica gel (ITLC-SG) had been used to separate carbohydrates in urine with a solvent system that developed the chromatogram in 90 min[16]. ITLC-SA, which acts more like a standard silica gel plate than ITLC-SG, allowed the use of a solvent system similar to that used by Prey *et al.*[14] for the separation of carbohydrates with 250-μm silica gel glass plates in 60 min. Prey *et al.* used water–acetone–*n*-butanol (10:50:40) with boric acid present as an impregnate in the silica gel layer. In this work boric acid was used in the solvent system instead of being impregnated on the chromatographic support. The solvent contained *n*-butanol–acetone–1.0 *M* boric acid (40:50:10). The final system combined the speed of polysilicic acid impregnated glass fiber sheets and a 50% acetone solvent which, with the additional separating ability of boric acid, allowed a suitable separation of glucose and fructose in less than 30 min so that the radiochemical composition of the lead extract could be determined before the ^{11}C decayed.

The maximum efficiency for the 2π solid angle counter used in this work is 0.50 (ref. 20). With self-absorption of the ^{11}C β^+ in the glass fiber sheet and in the Parafilm absorber this theoretical maximum would be reduced. An estimate of the self-absorption in the Parafilm using a mass absorption coefficient of 18 cm^2/g (ref. 21) for the 0.98-MeV positron of ^{11}C, gives a maximum efficiency of $0.5\, e^{-(18)(0.013)} = 0.40$ which agrees well with the measured efficiency of 0.37, especially because the relatively small absorption in the glass fiber sheet was neglected in the calculation.

The radiochromatogram scanner was calibrated so that the specific activity could be determined from the area of the activity peak, the time interval between the end of the biosynthesis and the radiochromatogram, and visual comparison of size and color intensity of the spot with known standards after chemical spraying. The calibration for this radiochromatogram scanner applies to ^{11}C activity under specific conditions of a Parafilm covered, 2 mm collimating aperture with a scanning speed of 5 cm/min.

The R_F values presented in Table I are not consistent from chromatogram to chromatogram. One possibility that could account for this is that the other components in leaf extract (including *ca.* 0.15 *N* sodium chloride), which could vary from preparation to preparation, would change the R_F. Also, the R_F values of glucose and fructose have been shown to vary with the amount of material spotted in a system similar to the one presented here[22]. Neither of these factors affect the identification of

the peaks in Table I because the three R_F values compared were from the same chromatogram.

The specific activities of glucose and fructose show wide variation (see Table I). This is due to the variability in the ability of the leaf to incorporate $^{11}CO_2$ and to the variability in the amount of $^{11}CO_2$ presented to the leaf.

ACKNOWLEDGEMENTS

Financial support from the Health Sciences Fund of the Massachusetts Institute of Technology is gratefully acknowledged. The radiochromatogram scanner was loaned by Dr. Maloof.

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