REMOTE SYSTEM FOR PRODUCTION OF CARBON-11 LABELED GLUCOSE VIA PHOTOSYNTHESIS

Carmen S. Dence, Keith A. Lechner, Michael J. Welch, and Michael R. Kilbourn, The Edward Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO 63110

SUMMARY

A remote system has been constructed for the preparation of ^{11}C-glucose via photosynthesis. The system is totally contained in a shielded hood, with all manipulations done from the outside. This system is capable of routine delivery of 25-50 mCi of ^{11}C-glucose which has a radiochemical purity >95% and is sterile and pyrogen free. The design, construction and operation of this system are fully described.

Key words: Carbon-11, glucose, positron tomography

INTRODUCTION

Although [1-^{11}C]-2-deoxyglucose (1) and 2-[^{18}F]fluoro-2-deoxyglucose (2) are widely used for studying glucose uptake and metabolism in vivo using positron emission tomography (PET), the use of the natural substrate, glucose (labeled with carbon-11) has been proposed (3,4): use of glucose itself may obviate possible problems which have arisen in determining the differences in metabolic rate constants for glucose and glucose analogs (5).

[1-^{11}C] Glucose has been prepared chemically using the classical Kiliani-Fischer synthesis (6), and [U-^{11}C] glucose has been prepared biosynthetically using photosynthesis by Swiss chard leaves (7,8), Broad bean leaves (9), or algae (10). In order to produce sufficient amounts of ^{11}C-glucose for in vivo studies (20-30 mCi), large amounts of carbon-11 are needed at the beginning of the synthesis: such high levels of radioactivity require the construction of remote, possibly automated apparatus. A remote automated apparatus for preparing ^{11}C-glucose via photosynthesis was recently described by Ishiwata et al (11), but the product obtained was a mixture of ^{11}C-glucose and ^{11}C-fructose in only 90% radiochemical purity. A synthesis of [U-^{11}C] glucose, of high radiochemical purity, using photosynthesis was recently reported by Ehrin et al (12), but details of the remote apparatus are sketchy. We describe here a totally remote apparatus for the preparation of

0362-4803/84/080743-08$01.00

Received March 12, 1984
Revised April 10, 1984

[^{11}C]glucose via photosynthesis, including purification by HPLC. This system is capable of routinely delivering more than 25 mCi of radiochemically pure ^{11}C-glucose suitable for human use. The synthetic apparatus is fully described and its operation outlined in detail.

EXPERIMENTAL

SYSTEM CONSTRUCTION

A schematic of the remote synthesis system for ^{11}C-glucose is shown in Figure 1. Except for the HPLC pump, injector and detector, and a colortan tungsten iodide lamp (670 W), all components are mounted on a 76 x 46 cm metal sheet. The system consists of the following components: glass spiral trap (A), liquid nitrogen dewar (B), small rotary evaporator (C:Buchi Inst.), pear shaped reaction vessel (D), radiant heater (E), fluorescent light (F: Micolight II cold daylight lamp, 3200 lumens), peristaltic pump (G), pressure gauge (H), six solenoid valves (V1-V6), two C18 sep-pak cartridges (I:Waters Assoc) and product collection tube with side arm (J). The rotary evaporator has been modified, with the condenser portion replaced with a

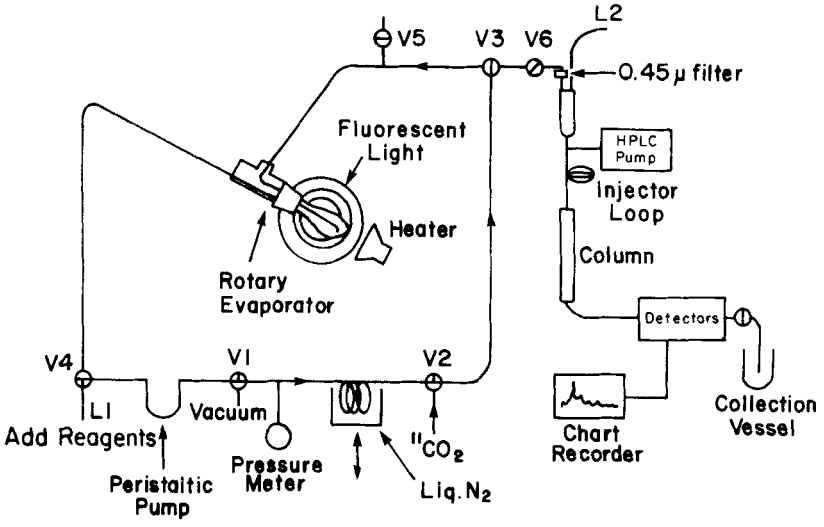

Figure 1. Schematic of the remote synthesis system for ^{11}C-glucose.

y-shaped fitting to allow two tubes to pass through and into the reaction vessel (D): one tube extends to the bottom of the flask whereas the second ends at the opening of the flask. Valve V5 serves as a vent for the system; reagents can be added into the system from outside the shielded hood via line L1 which enters at valve V4: and the system is connected to the cyclotron $^{11}CO_2$ target through valve V2. Throughout, the system is constructed using 2 mm I.D. teflon tubing to interconnect the solenoid valves.

SYSTEM OPERATION

Swiss chard plants are grown (in our laboratory) under controlled light, temperature, and humidity conditions (Klima-Gro^R). Twenty-four hours in advance, a leaf is cut off, moistened, and wrapped in aluminum foil. At synthesis time a portion of leaf, about 4 cm in diameter, is cut out of the upper end of the leaf. This portion of the leaf is placed in the flask and against the flask wall. To the flask is added 0.70g of a strong cation exchange resin (Rexyn 101(H+)16-50 mesh) and 0.5 ml of water, and the flask attached to the rotary evaporator (C) and rotated such that the leaf is facing the fluorescent light (F). The entire synthetic apparatus is then thoroughly flushed with nitrogen (in through line L1 and vented through valve 5). The $^{11}CO_2$ is produced by proton irradiation of nitrogen gas containing a trace of oxygen. At the beginning of the synthesis all valves in the system are closed. The cyclotron target gas ($^{11}CO_2$ + N_2) is pulled into the cooled spiral trap (A) by opening of valves V1 and V2. After trapping of all the activity, valves V1 and V2 are closed, and the trap warmed by lowering the nitrogen dewar and heating the trap with a hot air gun (heating is optional). The $^{11}CO_2$ is then circulated through the system by turning on the peristaltic pump, and photosynthesis initiated by turning on the fluorescent light. Irradiation and circulation are continued for 10 minutes. At the end of the photosynthetic step, the peristaltic pump and lights are turned off. Through line L1 is added a mixture of 0.3 ml of 1N HCl and 12 ml of 80:20 acetonitrile:water (valves V3 and V4 opened for addition, then closed). Valve V5 (vent) is opened and the radiant heater

turned on, and the mixture gently refluxed for 10 minutes. At this point the rotary evaporator is switched on and the mixture is evaporated to a volume of less than 1 ml. Four ml of water are added, and the mixture cooled by passing through a stream of nitrogen.

The next step consists of transfer of the crude reaction mixture onto the HPLC system. Using a large syringe, air pressure is supplied (from outside hood) through line L1 (V5 closed) and valves V3, V4, and V6 are opened; the contents of the reaction flask are pushed out and through the two C18 Sep-paks and into the test tube collection vessel (vented through the side arm). Valve V6 is now closed. The clear, colorless product solution is transferred to the automatic injection loop (5 ml volume) by supplying air pressure through line L2. The injector (Altex 210) is equipped with a pneumatic actuator (operated at 40 psi). The HPLC columns include a short pre-column (C18 porasil B) and three 60 x 0.9 cm stainless steel columns packed with cation exchange resin (Bio-Rad AG50W-4X, 200-400 mesh, calcium form). The columns are eluted with 0.005M $Ca(OH)_2$, Ph 3.5, at flow rate of 2.5 ml/min. The column effluent flows past a radioactivity detector (K) and through a three-way solenoid valve (L) which is used to divert the flow for collection of the desired peaks.

The ^{11}C-glucose is prepared for injection by addition of 0.5 ml of 0.5M phosphate buffer (pH 7.0) to precipitate calcium ions, addition of 3N NaCl to adjust the ionic strength to that of isotonic saline, and filtration through a 0.22 Millipore filter.

SYSTEM PERFORMANCE

For production runs, 1-1.5 curies of $^{11}CO_2$ are produced by cyclotron irradiation. The entire synthetic procedure-photosynthesis, hydrolysis, and purification via HPLC-requires 60 minutes. A typical chromatogram of C-11 labeled products obtained from the HPLC system is shown in Figure 2. The columns can separate the products, which are ^{11}C-sucrose, ^{11}C-glucose, and ^{11}C-fructose; ^{11}C-labeled five carbon sugars (xylose and ribose: see below) co-elute with the ^{11}C-glucose. Using this system, we have consistently obtained 25-54 mCi of 11C-glucose with radiochemical purities of 95-99% (see below).

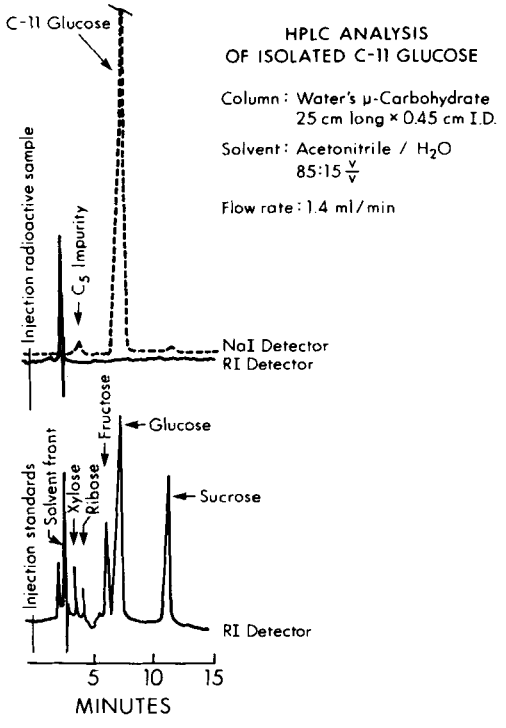

Figure 2.

QUALITY CONTROL

The ^{11}C-glucose obtained using this apparatus has been carefully checked for chemical and radiochemical purity. The radiochemical purity has been determined by both analytical HPLC and thin-layer chromatography (TLC). The analytical HPLC was done using a Waters carbohydrate column (25 x .45 cm) eluted with 85:15 acetonitrile:water (flow rate 1.4 ml/min), and peaks detected using a refractive index detector and NaI(Tl) radioactivity detector. This analytical HPLC column is capable of resolving glucose, fructose, sucrose, and 5-carbon-sugars ribose and xylose; A chromatograph of a typical preparation of ^{11}C-glucose is shown in figure 3. In most preparations, small amounts (total 5%) of sucrose and a five-carbon sugar are usually observed. The ^{11}C-glucose has also been analyzed by TLC using silica gel plates devel-

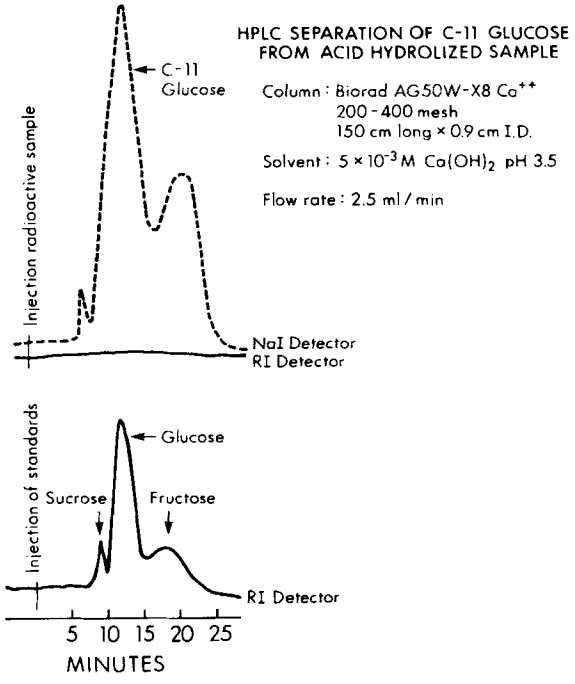

Figure 3.

oped with 15/5/5 (v/v/v) ethyl acetate/acetone/pyridine and visualized with anisaldehyde-sulfuric acid. In this system glucose can be cleanly separated from sucrose and the five carbon sugars. Purity by radio-TLC agreed with that determined by HPLC, with radiochemical impurities sucrose and xylose (or ribose, a non-separable stereoisomer). The source of the ^{11}C-labeled five carbon sugars is either oxidative degradation of the hexoses during the hydrolysis step, or because these pentoses arise during the normal course of photosynthesis. The amount of this impurity varies (0-5%) and is not removed by the preparative HPLC.

The specific activity of the ^{11}C-glucose has been estimated using two methods. Analytical HPLC (performed as described above) indicated approximately 56 micrograms of glucose per gram of Swiss chard leaves (a typical amount used in each synthesis). Analysis by enzymatic methods using a commercially available kit (Sigma Biochemicals) indicated 132 micrograms of

glucose in a typical preparation of ^{11}C-glucose. For the preparation of 20 mCi of ^{11}C-glucose, this would result in a minimum specific activity of 27 Ci/mmol.

We have also assayed the ^{11}C-glucose for proteins (a potential problem using biosynthetic methods). Protein has been determined using a commercially available kit (Bio-Rad Protein Assay kit II) which utilizes a colorimetric test. Analysis of representative batches of ^{11}-C-glucose indicated a range of zero to 25.8 micrograms of protein. Levels of calcium ions were determined by colorimetric assay (EDTA complexation) and found to be less than 0.001M. The pH has been tested and is in the range of 6.8-7.3. Finally, random batches of ^{11}C-glucose have been subjected to tests for sterility and pyrogenicity, with no positive tests reported.

SUMMARY

Using the apparatus described herein, 20-50 mCi of ^{11}C-glucose of acceptable radiochemical and chemical purity can be routinely prepared for human studies. Operator dose is minimal, as all procedures are done remotely, and the only handling of the radioactivity is in the final stages of preparation of the isolated ^{11}C-glucose for injection.

Carbon-11 labeled glucose is now a radiopharmaceutical available daily for PET studies at our institution.

ACKNOWLEDGEMENTS

This work was supported in part by National Institutes of Health grants HL13851 and NS06833. The authors thank Mr. Roland Head and Mr. William Margenau for the construction of the remote synthesis system, and Drs. Michel M. Ter-Pogossian and Marcus E. Raichle for their continued interest in this project.

REFERENCES

1. MacGregor R.R., Fowler J.S., and Wolf A.P. -- J. Nucl. Med. 22: 800 (1981).
2. Ido T., Wan C-N., Casella V., Fowler J.S., Wolf A.P., Reivich M., and Kuhl D. -- J. Label. Comp. Radiopharm. 14: 175 (1978).

3. Raichle M.E., Larson K.B., Phelps M.E., Grubb R.L., Welch M.J., and Ter-Pogossian M.M. -- Amer. J. Physiol. 228: 1936 (1975).

4. Raichle M.E., Welch M.J., Grubb R.L. Jr., Higgins C.S., Ter-Pogossian M.M., and Larson K.B. -- Science 199: 986 (1978).

5. Selikson M. -- J Nucl Med. 24, 366 (1983).

6. Shiue C-Y., and Wolf A.P. -- J. Nucl. Med. 22: 58 (1981). Abstract.

7. Lifton J.F. and Welch M.J. -- Radiat. Res. 45:35 (1971).

8. Welch M.J., and Tewson T.J. -- Radiopharmaceuticals II, Society of Nuclear Medicine, New York, 1979, pp 201-217.

9. Goulding R.W., and Palmer A.J. - Int. J. Appl. Rad. Isot. 24: 7 (1973).

10. Ehrin E., Westman E., and Nilsson S.O. -- J. Label. Comp. Radiopharm. 17: 453 (1980).

11. Ishiwata K., Monma M., Iwata R., and Ido T. -- J. Label Comp. Radiopharm 19: 1347 (1982).

12. Ehrin E., Stone-Elander S., Nilsson J.L., Bergstrom M., Blomqvist G., Brisman T., Eriksson L., Grietz T., Janssen P.E., Litton J-E., Malmborg P., Ugglas M., and Widen L. -- J. Nucl. Med. 24:326 (1983).