THE SYNTHESIS OF HIGH RADIOCHEMICAL PURITY OF 2-[^{18}F]-FLUORO-2-DEOXY-D-GLUCOSE WITHOUT THE USE OF PREPARATIVE HPLC

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SUMMARY

A process is described for preparing routinely sterile and pyrogen-free, multimillicurie amounts of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-2FDG) with and without carrier-added fluoride and with a radiochemical purity of greater than 98% without the use of preparative HPLC. For 13 consecutive runs, the radiochemical yield with carrier-added fluoride was 35.5 ± 2.2%. The [^{18}F]fluoride used was produced by the reaction ^{16}O(^{3}He,p)^{18}F or by the reaction ^{18}O(p,n)^{18}F. Analytical HPLC, ^{1}H and ^{19}F NMR spectral analysis were used to establish the identity and purity of ^{18}F-2FDG.

KEY WORDS: 2-[^{18}F]-fluoro-2-deoxy-D-glucose, fluorine-19 nuclear magnetic resonance, high performance liquid chromatography

INTRODUCTION

A recent article (1) that describes the scale-up preparation of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-2FDG) by a modified nucleophilic displacement reaction of [^{18}F]fluoride anion with methyl-4,6-O-benzylidene-2,3-O-cyclic sulfato-β-D-mannopyranoside (MBCM) (2) reports in detail chromatographic and TLC analytical techniques used to establish the identity and purity of ^{18}F-2FDG. In addition, it reports that what appears to be one radioactive peak (^{18}F-2FDG) under a given set of HPLC conditions is partially resolved into two peaks when two HPLC analytical columns are used in series.

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The first eluted peak is shown to be ^{18}F-2FDG, followed by a second partially resolved peak suspected to be a fluorinated altrose.

We wish to report here that by using the same nucleophilic displacement reaction, but with altered reaction conditions, we have been able to prepare a sterile and pyrogen-free ^{18}F-2FDG in 40-80 mCi with a radiochemical purity of greater than 98% without resorting to prepartive HPLC separation. For the past two years we have successfully used ^{18}F-2FDG prepared routinely by the Tewson method (2), as modified by us, for positron emission tomographic (PET) measurements of regional cerebral glucose metabolism in humans (3,4). More recently, Tewson et al (5) have replaced the O-methyl group of MBCM with propenyl in a preparation requiring a ten-step synthesis. This facilitates the complete removal of all the protective groups by treatment with 2N HCl instead of with 1.0 M boron tris (trifluoroacetate) in trifluoroacetic acid (BTTF). Moreover, it should be noted that Hamacher et al (6) have reported a rapid stereospecific synthesis of no-carrier-added ^{18}F-2FDG with uncorrected radiochemical yield of a maximum of 50%.

EXPERIMENTAL

Materials. MBCM was synthesized as described in the literature (2,12) with few modifications. In its final purification step, MBCM was dissolved in a minimum amount of methylene chloride followed by the addition of heptane. The colorless needle-like crystals obtained had a m.p. of 164-166°C (decomp.) (Lit. 157-159°C with decomp. (12); Mara Specialty Chem.: 156-158.5°C with decomp.). TLC in several solvent systems as well as HPLC analysis showed our MBCM to be pure. The MBCM purchased from Mara Specialty Chem. was used as a reference compound and was shown by HPLC as well as TLC to contain a minor impurity that depressed the m.p. of the compound. Otherwise, its major component coeluted with our MBCM. Tetramethylammonium hydroxide pentahydrate (97% pure) (TMAOH), tetramethylammonium fluoride tetrahydrate (98% pure) (TMAF), anhydrous acetonitrile (99+% pure) in 100 ml Sure/Seal™ bottles, BTTF, and activated neutral aluminum oxide (150 mesh, 58A) were purchased from Aldrich Chemical Company. Ion retardation resins, AG 11A8 (50-100 mesh) and Dowex (50-100 mesh), were purchased from Bio-

Rad and Sigma Chemical Co., respectively. 2-FDG, used as a reference compound, was purchased from Sigma Chemical Co. All HPLC solvents were purchased from J.T. Baker and Fisher Scientific. BTTF was removed from the bottle as needed by means of a glass syringe equipped with a 20-gauge needle. When not in use, it was stored over a desiccant and under refrigeration with a teflon liner between the seal and the cap to minimize corrosion of the metallic cap.

^{19}F and ^{1}H NMR Analysis of ^{18}F-2FDG. Fluorine-19 NMR spectra were obtained on a Bruker WM-250 operating at 235.4 MHz (Fig. 2 and 3). Hexafluorobenzene was used as an internal reference (δ_F = -163.0 ppm). ^{19}F chemical shifts were obtained from the hydrogen decoupled spectrum using two-level broadband decoupling (Fig. 3). In order to prepare a sufficient quantity of 2-FDG for NMR studies, we doubled the amounts of reagents and maintained the same reaction conditions as described in part B, below. The following changes were introduced, made possible by the use of a 15 ml reaction flask: MBCM was dissolved in 5.0 ml of anhydrous acetonitrile instead of 8.0 ml, the azeotroping of water was carried out with 3.0 ml of anhydrous acetonitrile instead of 10.0 ml, and the extraction of ^{18}F-2FDG was effected with 2.3 ml of $NaHCO_3$ solution. After analysis by HPLC, fractions Nos. 13 to 18, containing about 95% of the ^{18}F-2FDG produced with a mean radiochemical purity of greater than 98%, were combined with those of a second, identically repeated run and lyophilized overnight. A light white fluffy solid was obtained. This was dissolved in D_2O and used for the NMR spectra.

HPLC Analysis. This was performed with a Laboratory Data Control (LDC) modular liquid chromatograph consisting of a Refractomonitor III refractive index detector, a Constametric Model III solvent pumping system, a Rheodyne sample injection valve, Model 7110, equipped with a 20 µl loop. An Alltech analytical column (25 cm x 4.6 mm) with RSiL packing (10 µ) was used singly or connected in tandem with an analytical Alltech NH_2 column (25 cm x 4.6 mm). The mobile phase used was acetonitrile:water (80:20) with flow rates of 1.5 ml/min or 2.0 ml/min. In addition, an LDC 308 Computing Integrator was used connected to a

two-channel recorder, LDC 3401/3402. As a radioactivity detector, a Victoreen "Vamp" Area Monitor, model 808D--MOD, to which a Victoreen w/808-520 probe was attached, was connected to the recorder. The teflon tube leading from the refractive index detector to the waste reservoir was coiled once around the probe and the probe shielded from background radiation in a lead container. Readings were displayed on a logarithmic scale and expressed as mR/hr. The ratio of each reading corresponding to each radioactivity peak, minus the background radioactivity, to the sum of the readings corresponding to the total number of radioactivity peaks, minus the background radioactivity, expressed the percent radioactivity associated with the eluted band.

In order to validate the method, eluted fractions corresponding to each eluted radioactivity band were collected and measured individually in a Capintec Isotope Calibrator CRC-10R. The results of the two methods were in very good agreement. Nevertheless, as an alternative, we also used the Berthold HPLC Radiactive Monitor LB 503 connected with a Hewlett Packard HP 3392A Integrator to monitor the radiochemical purity of product and compare the results obtained with the "Victoreen" detector. The radiochemical purity obtained by the Berthold detector was usually higher than that obtained by the other two methods.

Synthesis of ^{18}F-2FDG

A. Use of [^{18}F]fluoride produced from $^{16}O(^{3}He,p)^{18}F$

^{18}F-2FDG was synthesized according to a previously reported procedure (2) which we modified in order to augment the radiochemical purity and adapt its preparation to our "hot cell" conditions. [^{18}F]Fluoride is produced routinely in a retractable, recirculating-water target (7) from the reaction $^{16}O(^{3}He,p)^{18}F$. The volume of the recirculating water irradiated is 45 ml, of which 37.5 ml containing the [^{18}F]fluoride is transferred to a 100 ml round-bottom reaction flask containing 1.0 ml (41 μmol) of TMAOH and 0.8 ml (33 μmol) of TMAF. The reaction flask is attached through a vapor trap to a modified Buchi rotary evaporator, and the water is evaporated under high vacuum and with heating. To insure complete removal of moisture, 10 ml of anhydrous acetonitrile is added and evaporated. This is repeated twice before

an 8 ml solution of 13.9 mg (40 μmol) of MBCM in dry acetonitrile is added. The solution is refluxed for 12 min and then evaporated to dryness under high vacuum and with heating. Five milliliters of BTTF is added and the solution is heated at 55°C with slow rotation for 15 min. At the end of the reaction, 2.5 ml of water is added and the solution heated for an additional 5 min. Finally, the volatiles are removed under high vacuum and with heating. The dark amber-colored residue is dissolved in 4.5 ml of 30% $NaHCO_3$ aqueous solution and transferred to a column (50 cm x 9 mm) consisting of two segments, an upper segment of neutral alumina (10 cm) and a lower segment (40 cm) of ion retardation resin. The alumina/resin column is eluted with at least 500 ml of sterile, pyrogen-free water prior to its use. The crude extract is applied on the column and eluted with sterile water and a flow rate of 1.5 ml/min maintained by a Pharmacia P-3 peristaltic pump. A series of 2.0 ml clear colorless fractions are collected with a Pharmacia Frac 100 fraction collector. The radioactivity and pH of each fraction is measured, the ^{18}F-2FDG-containing fractions are analyzed by HPLC, and the fraction with highest radiochemical purity is routinely selected and used to prepare an isotonic solution rendered sterile through a Millipore Millex-GS 0.22 μm filter.

B. Use of [^{18}F]fluoride produced from $^{18}O(p,n)^{18}F$.

In a recently acquired target (8), [^{18}F]fluoride was also produced from the $^{18}O(p,n)^{18}F$ reaction with ^{18}O-enriched water. The use of 1.80 ml volume of water containing the [^{18}F]fluoride allowed us to use a 15 ml pear-shaped reaction flask instead of the 100 ml round-bottom flask and to scale down the amounts of reagents and volumes of solutions by a factor of two or more. Thus, the amounts of MBCM, TMAOH and TMAF used are, respectively, 6.90 mg (20 μmol) in 4.0 ml anhydrous acetonitrile, 200 μl (20 μmol) and 200 μl (16.5 μmol). To remove the protective groups, 2.5 ml of BTTF was used followed by 1.0 ml of water, and, to extract the crude ^{18}F-2FDG, 2.5 ml of 30% $NaHCO_3$ solution was added. Reaction, purification and HPLC conditions are the same as those described in part A. With the same target and the same reaction conditions and reagent quantities, ^{18}F-2FDG was prepared without added carrier (TMAF). All

operations involved in the synthesis and purification of ^{18}F-2FDG are carried out by remote control.

RESULTS AND DISCUSSION

Our attempts to prepare ^{18}F-2FDG using reaction conditions previously reported (2) gave in our hands ^{18}F-2FDG of only 85-95% purity as determined by HPLC analysis of fractions eluted through the alumina/resin column. Using a single HPLC column, HPLC analysis showed four distinct radioactivity peaks with retention times 4.0 min, 4.38 min, 6.25 min and 7.65 min. The peak with retention time of 6.25 min was identified as the ^{18}F-2FDG after comparing it with an authentic compound detected with a refractive index monitor. No attempt was made to identify the other three peaks, but one of the first two peaks is thought to be the partially deprotected 2-[^{18}F]-fluoro-2-deoxymethylglucoside, in agreement with the findings of others (2). The radioactivity peak with retention time of 7.25 min is suspected of being a borate ester of ^{18}F-2FDG, as suggested in a private communication (9). No attempt was made to identify the suspected borate ester. The addition of water to the BTTF solution upon completion of the deprotection step followed by a five-minute heating at 55°C served in effectively eliminating the undesirable fourth radioactivity peak. Nevertheless, even in its absence, the radiochemical purity of ^{18}F-2FDG rarely exceeds 95% and often ranges between 90-95%. However, by heating the BTTF solution at 55°C for 15 min, one can obtain ^{18}F-2FDG with a reproducible radiochemical purity equal to or greater than 98%, but at the expense of radiochemical yields as the result of the extensive defluorination, as evidenced by the higher fluorine-18 activity trapped in the dry-ice/acetone trap.

Based on 13 consecutive synthetic preparations, the radiochemical yield was found to be 35.6 ± 2.2% and as much as 80 mCi of ^{18}F-2FDG has been prepared. The time of synthesis from EOB to the end of elution through the alumina/resin column is approximately 2 hours. This includes the time required (20-25 min) to evaporate the water containing the [^{18}F]fluoride. The use of the enriched ^{18}O-labeled water target with a volume of only 1.8 ml and the use of smaller volumes of acetonitrile reduced the time of synthesis by a half hour. In addition,

whereas the ^{18}O-labeled water target requires a bombardment of only 45 min with a proton beam current of 17 μA to yield about 550 mCi of the [^{18}F]fluoride, the continuously circulating water target under the same conditions with a helium-3 beam yields approximately 50-60 mCi. To obtain 555 mCi would require a 45 μA helium-3 beam current with an irradiation time of a little over 4 hours.

Because others have reported (1) that, under a given set of HPLC conditions, what appeared to be the radioactive peak, identified as ^{18}F-2FDG, was actually partially resolved into two peaks when two HPLC columns were used in

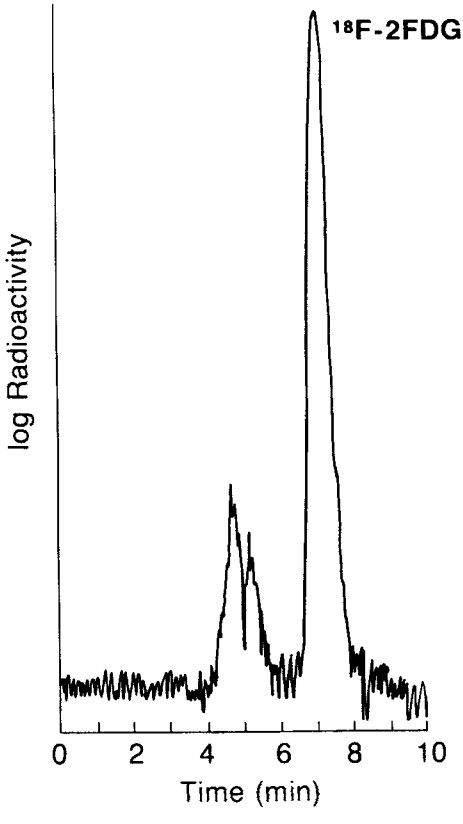

Figure 1: HPLC elution radiochromatograms of ^{18}F-2FDG prepared using modified procedure as described in text. HPLC conditions: eluant: acetonitrile/water (90:10); flow rate: 2.0 ml/min; HPLC column: Alltech analytical column (RSiL packing; 10 μ) connected in series with an analytical Alltech NH_2 column.

series, we applied the same analytical procedure in analyzing the ^{18}F-2FDG obtained by our modified procedure. Only one major peak was obtained with retention times of 7.30 min (Fig. 1). The other two minor radioactive peaks with retention times of 4.75 and 5.25 min, respectively, were not identified. One of them is presumably the partially deprotected 2-[^{18}F]-fluoro-2-deoxymethyl-glucoside (2). Furthermore, fluorine-19 NMR spectra (Figs. 2 and 3) confirmed that there was essentially one fluorinated compound aside of the two radioactive peaks detected only by HPLC and which constituted less than 2% of the total radioactive mixture. Figure 2 shows that the fully coupled hydrogen-fluorine-19

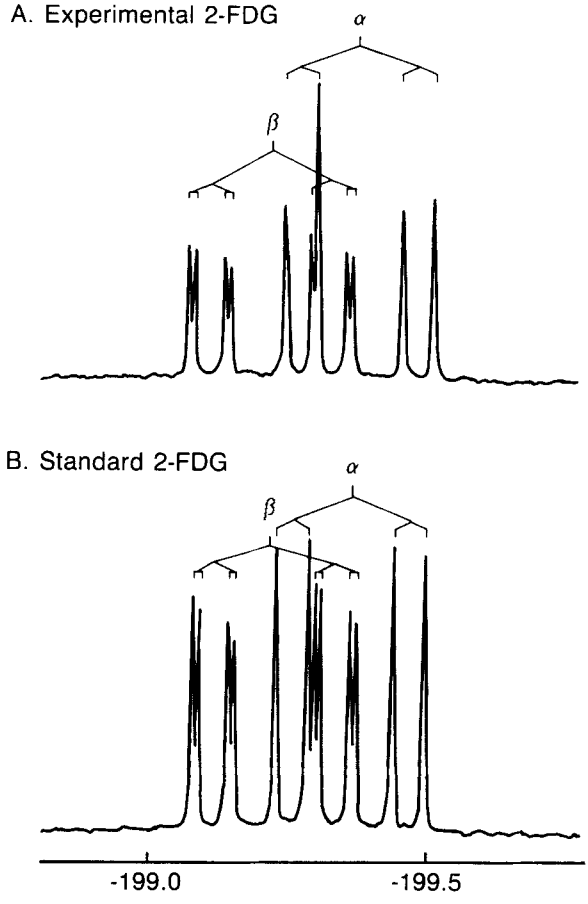

Figure 2: Fully coupled hydrogen-fluorine-19 ^{19}F-NMR spectra of experimental 2-FDG, obtained as described in section: ^{18}F-^{1}H NMR analysis of ^{18}F-2FDG.

^{19}F-NMR spectra of our experimental and standard 2-FDG are essentially identical and are consistent with the literature values (10). The assignments

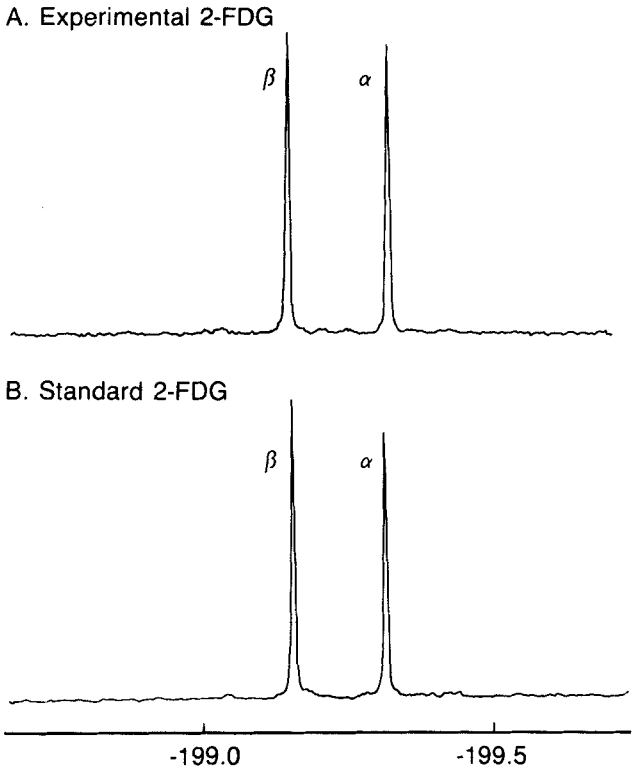

Figure 3. Hydrogen decoupled spectrum of experimental and standard 2-FDG. In both figures, chemical shifts are in ppm.

of the α- and β-anomer fluorine split bands are in accordance with those in the literature (10,11). The largest band of the experimental 2-FDG spectrum is actually the merging of the two fluorine split bands because of small changes in the chemical shifts caused by the presence of other non-fluorine-containing impurities. The fluorine-19 chemical shifts of the α- and β-anomers are -199.29 and -199.11 ppm, respectively, and were derived from the hydrogen decoupled spectrum (Fig. 3). An integration of the two bands shows that the mixture consists of 55% of the β-anomer and 45% of the α-anomer under the experimental conditions the spectra were obtained. A ^{1}H NMR spectrum of our 2-FDG showed, in addition to the 2-FDG hydrogen bands identified by comparison with those of

the standard compound, additional hydrogen bands which are presumably those of the defluorinated 2-FDG, possibly the 2,6 anhydro-β-D-glucopyranose (levoglucosan) (2,12,13). No attempt was made to identify it.

To compare the performance of the two resins, we prepared 2-FDG under simulated "hot cell" conditions using the reaction conditions and quantities described in part B and eluted through the two columns prepared as described in part A. Two milliliter fractions were collected and the pH of each recorded with a Beckman Altex pH meter. Fractions Nos. 12-16 and 17-21 were pooled and carefully lyophilized in tared flasks, and the weight of the white residue determined. Under "hot cell" conditions, about 90-95% of the ^{18}F-2FDG is collected in fractions Nos. 12 to 18 or less. Results are shown in Table I.

TABLE I

Comparison of Performance of AG 11A8 and Dowex Ion Retardation Resins

	AG 11A8		Dowex	
Fraction No.	mgs*	pH	mgs*	pH
12-16	11.8	8.41-9.53	8.4	7.90-8.88
17-21	8.2	8.7 -9.10	12.1	9.00-9.17

*Pooled eluted fractions were lyophilized in tared flasks and the solid residues weighed.

Samples collected in the manner described above and submitted to Galbraith Laboratories for elemental analysis showed B < 0.08% and N = 0.012%. It appears that the alumina/resin column is capable of separating ^{18}F-2FDG effectively from borate, tetramethylammonium, [^{18}F]fluoride and sodium salts provided one applies the conditions described above. The authors would opt for the Dowex product because it yields fractions with a lower pH and it has less of the characteristic amine odor often present in the Bio-Rad product.

As a rule, 50-60% of the ^{18}F-2FDG is contained in fractions Nos. 14-16, with the maximum amount of radioactivity in fraction No. 15, ranging from 9-17 mCi. Since the amount of ^{18}F-2FDG injected into humans is never more than 5-7 mCi, it is estimated that only a fraction of a milligram, consisting of 2-FDG, defluorinated 2-FDG and sodium salts, is injected into the subject.

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