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Note

Syntheses, radiolabelling, and kinetic evaluation of 2-deoxy-2-fluoro-2-iodo-D-hexoses for medical imaging

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The use of 2-deoxy-2-[^{18}F]fluoro-D-glucose (2-FDG) to study glucose metabolism in the heart and brain has seen extensive clinical application. 2-FDG/positron emission tomography (PET) has become the standard against which other functional imaging techniques are judged, because of the high resolution of PET and the well-defined mechanism of 2-FDG accumulation in metabolically active tissues. However, a disadvantage of 2-FDG/PET is the relatively short half-life of ^{18}F ($t_{1/2}$ = 110 min), requiring the proximity of the imaging centre to a cyclotron. SPECT (single photon emission computed tomography) agents have the advantage of generator production (e.g., ^{99m}Tc) or longer half-lives (e.g., ^{123}I, $t_{1/2}$ = 13.1 h) which permit the use of these agents by other hospital imaging centres. However, there are currently no radioiodinated agents which provide the same quality of information about the metabolic state of tissues as does 2-FDG. Therefore, we have attempted to synthesize a structurally similar, stable ^{123}I-labelled analogue of 2-FDG.

The usefulness of 2-FDG depends on its structural similarity to glucose, permitting its transport into the cell, phosphorylation, and subsequent metabolic trapping. Following uptake by the glucose transport system, 2-FDG is phosphorylated by hexokinase; subsequent steps in the glycolytic pathway are blocked by the C-2 fluorine and the labelled sugar accumulates as 2-deoxy-2-fluoro glycosyl phosphates in tissues with high metabolic glucose demand. Hexokinase catalyzes the phosphorylation of various deoxyhalo glucose analogues as well as glucose itself [1]. The most extensive substitutions are permitted at the 2-position

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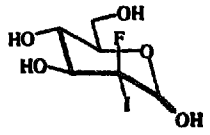
of the sugar ring, with 2-FDG ($K_M = 0.59$ mM, V_M relative to D-glucose = 0.85), 2-deoxy-2-fluoro-D-mannose (2-FDM, $K_M = 0.41$ mM, $V_M = 0.85$), 2-deoxy-2,2-difluoro-D-*arabino*-hexose ("2,2-difluoro-glucose", 2,2-DFG, $K_M = 0.13$ mM, $V_M = 0.53$), and 2-chloro-2-deoxy-D-glucose ($K_M = 2.1$ mM, $V_M = 0.54$) all being good substrates for hexokinase, while compounds modified at other positions of the sugar ring are either very poor substrates for the enzyme or are not used at all [1,2].

There have been a number of attempts by various workers to develop ^{123}I-labelled analogues of 2-FDG [3–6]. None of these compounds appear to be substrates for hexokinase and, consequently, most show little uptake into, and rapid clearance from, the heart or brain. The iodo sugar perhaps most likely to be a hexokinase substrate, 2-deoxy-2-iodo-D-glucose, is chemically unstable and could not be isolated [3,4,7], with decomposition presumably occurring through an α-iodo aldehyde in the open chain form of the sugar. We anticipated that incorporation of a geminal fluorine at C-2 of 2-deoxy-2-iodo-D-glucose or 2-deoxy-2-iodo-D-mannose might stabilize these structures relative to the nonfluorinated iodo sugars. Such sugars have no acidic proton at C-2, adjacent to the aldehyde functionality, thus are not susceptible to elimninations. Further, any displacement or elimination of the iodide at C-2 is considerably disfavoured by the presence of the second electronegative substituent at that position. Such *gem*-dihalo compounds, if stable, might be expected to be substrates for hexokinase since they retain an unmodified glucose structure except at C-2, the position where greatest substitution is apparently tolerated. Indeed, these probably represent the smallest conceivable stable analogues of 2-deoxy-2-iodo-D-glucose, and should thus be the best such candidates for a metabolic imaging agent.

1. Results and discussion

Two possible approaches to the synthesis of the desired compounds were considered: the oxidative addition of iodine to 1,5-anhydro-2-deoxy-2-fluoro-D-*arabino*-hex-1-enitol (2-fluoro-D-glucal), or the oxidative addition of fluorine to 1,5-anhydro-2-deoxy-2-iodo-D-*arabino*-hex-1-enitol (2-iodo-D-glucal). Since 2-fluoro-D-glucal is a known compound [8] and since addition of iodine is likely to be more facile, the former route was followed. 2-Deoxy-2-fluoro-2-iodo-D-mannose (2-FIM; **2**) was prepared in 60% yield from 2-fluoro-D-glucal by iodination with KI in the presence of aqueous hydrogen peroxide [9]. 2-Deoxy-2-fluoro-2-iodo-D-glucose (2-FIG; **1**) was prepared by iodohydration of 3,4,6-tri-*O*-acetyl-2-fluoro-D-glucal in aqueous acetone to give a 4:3 mixture of the 3,4,6-tri-*O*-acetates of 2-FIG and 2-FIM (75%), followed by deacetylation with sodium methoxide in methanol and flash chromatography to separate the *gluco* and *manno* epimers (63% of 2-FIG). Both 2-FIM and 2-FIG were isolated and characterized by ^{19}F and ^{1}H NMR, and by elemental analysis. ^{19}F and ^{1}H NMR studies indicated that both acetylated and free sugars existed in the expected $^{4}C_{1}$ chair conformations. Thin layer chromatographic analysis of samples of 2-FIM and 2-FIG dissolved in phosphate-buffered saline at physiological (pH 7.4) and stored for several days at room temperature revealed that no decomposition had occurred indicating that the geminal fluorine indeed stabilized these 2-iodo sugars. ^{19}F NMR studies confirmed these findings.



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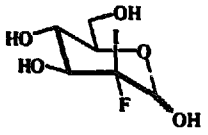

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2-FIM was also prepared in a ^{123}I-labelled form since the one-step synthesis of this compound was particularly amenable to the radiolabelling procedure. As described in the Experimental section, the reaction was complete within 1 h, and a radiochemical yield of 63%, as determined by TLC, was obtained. This is comparable to the chemical yield for the synthesis of the nonlabelled material.

Both 2-FIM and 2-FIG were evaluated as potential substrates of yeast or bovine heart hexokinase, using a pyruvate kinase–lactate dehydrogenase coupled assay system. In this system, the ADP produced upon phosphorylation of the sugar is monitored via the oxidation of NADH, which produces a change in absorbance at 340 nm. As a control experiment, hexokinase was first added to a sample containing 0.40 mM D-glucose as the limiting reagent. Rapid reaction was observed, with the consumption of a full equivalent of NADH. Similar treatment of 5.8 mM 2-FIM or 18 mM 2-FIG resulted in only a very limited reaction, at a very low rate. The total absorbance changes observed with both 2-FIM and 2-FIG represented consumption of less than 10% of the available NADH, which therefore was not limiting. Incomplete reaction was not due to inactivation of hexokinase or the coupling enzymes by the iodo sugars as addition of glucose to such a mixture resulted in a rapid absorbance decrease of the expected size. The final rates were not increased by additional enzyme and were not significantly different from background. Thus, the initial changes in absorbance observed with both 2-FIM and 2-FIG almost certainly arise from contaminants in the samples (possibly D-glucose or 2-FDG) comprising ~3 and ~0.5%, respectively, of the iodo sugars present, and do not represent reaction of the dihalo sugars. These results indicate that neither 2-FIM nor 2-FIG are detectably phosphorylated by either the yeast or heart enzymes.

2. Conclusions

2-FIM and 2-FIG were synthesized and shown to be stable in physiological saline for several days at room temperature. However, neither compound was found to be a substrate for hexokinase from either yeast or bovine heart in vitro. Unlike 2-FDG, they would not therefore be expected to be phosphorylated in vivo, precluding metabolic trapping. They are therefore not likely to be useful as ^{123}I-labelled analogues of 2-FDG for imaging of D-glucose metabolism but may, however, be useful probes of glucose transport across cell membranes. Since 2-FDG, 2-FDM, and 2,2-DFG are good substrates for hexokinase, the significantly greater size and/or lipophilicity of the iodine atom relative to the fluorine atom in either the *gluco* or *manno* configurations is likely to be responsible for the lack of activity of 2-FIG or 2-FIM with the enzyme.

3. Experimental

General methods.—Melting points (mp) were determined on a Laboratory devices Meltemp II apparatus and are uncorrected. Solvents and reagents used were either reagent grade, certified, or spectral grade. Reactions were monitored by TLC using Merck Kieselgel 60 F_{254} analytical plates and visualized by charring with 10% H_2SO_4 in CH_3OH. Flash chromatography was performed using silica columns of Kieselgel 60 (230–400 mesh). ^1H and ^{19}F NMR spectra were recorded on a 200-MHz Bruker AC-200 spectrometer. ^1H NMR chemical shifts (δ) recorded for samples in $CDCl_3$ or D_2O were measured against internal $(CH_3)_4Si$ or external 4,4-dimethyl-4-silapentane-1-sulfonate, respectively. ^{19}F NMR chemical shifts (δ) were measured against CF_3CO_2H as an external standard and are quoted relative to $CFCl_3$. Optical rotations were measured at 25 ± 0.5°C.

*2-Deoxy-2-fluoro-2-iodo-*D*-glucose* (**1**).—3,4,6-Tri-*O*-acetyl-1,5-anhydro-2-deoxy-2-fluoro-D-*arabino*-hex-1-enitol [8] (100 mg, 0.345 mmol) and silver nitrate (41 mg, 0.24 mmol) were dissolved in acetone (7.5 mL) to which a few drops of water had been added. Iodine (62 mg, 0.24 mmol) was added and the mixture stirred in the dark at room temperature. An additional 4–5 equiv of silver nitrate and iodine were added in portions over 90 h. The mixture was filtered, evaporated, dissolved in CH_2Cl_2, washed with satd sodium thiosulphate, satd $NaHCO_3$, water, and then dried ($MgSO_4$). Flash chromatography (2:1 EtOAc–petroleum ether) afforded an inseparable 3:4 mixture of *manno* and *gluco* hemiacetal products (112 mg, 75%) as a pale yellow foam; ^{19}F NMR ($CDCl_3$): δ −43.48 (d, $J_{3,F}$ 10.9 Hz, F-2α *manno*), −50.81 (dd, $J_{3,F}$ 26.0, $J_{1,F}$ 4.6 Hz, F-2α *gluco*), −71.05 (dd, $J_{3,F}$ 25.4, $J_{1,F}$ 15.1 Hz, F-2β *gluco*, α/β ratio ~ 13:1). The mixture of products was dissolved in dry MeOH (10 mL), a catalytic amount of NaOMe was added and the solution was stirred under N_2 for 1.5 h. The solution was passed through a pad of silica gel, evaporated, and chromatographed (27:2:1 EtOAc–CH_3OH–H_2O) to afford first the *gluco* sugar **1** as a colourless oil (28 mg, 63%) which later solidified; mp 133–136°C (dec with liberation of I_2); $[\alpha]_D$ +13.8° (*c* 0.85, H_2O); ^1H NMR (D_2O): δ 5.60 (d, $J_{1,F}$ 5.1 Hz, H-1α), 5.17 (d, $J_{1,F}$ 17.5 Hz, H-1β), 4.1–3.0 (m, H-3,4,5,6,6'); ^{19}F NMR (D_2O): δ −52.85 (dd, $J_{3,F}$ 26.5, $J_{1,F}$ 5.1 Hz, F-2α), −72.50 (dd, $J_{3,F}$ 26.3, $J_{1,F}$ 17.5 Hz, F-2β, α/β ratio ~1:1). Anal. Calcd for $C_6H_{10}FIO_5 \cdot 0.5CH_3OH$: C, 24.09; H, 3.73. Found: C, 24.12; H, 3.60. The later-eluting *manno* compound **2** was not isolated.

*2-Deoxy-2-fluoro-2-iodo-*D*-mannose* (**2**).—1,5-Anhydro-2-deoxy-2-fluoro-D-*arabino*-hex-1-enitol [8] (100 mg, 0.609 mmol) was dissolved in citrate-phosphate buffer (pH 3.0, 7 mL) containing KI (285 mg, 1.71 mmol) and 30% H_2O_2 (140 mL). Additional H_2O_2 (140 mL) was added after 2 h and the mixture was stirred at room temperature for a further 18 h. Saturated sodium thiosulphate solution was added, and the mixture was evaporated and chromatographed (27:2:1 EtOAc–CH_3OH–H_2O) to afford a white foam (112 mg, 60%) which was crystallized from EtOH–ether–petroleum ether to give **2** as a white powder; mp 137–142°C (dec with liberation of I_2); $[\alpha]_D$ −10.5° (*c* 1.0, H_2O); ^1H NMR (D_2O): δ 5.96 (d, $J_{1,F}$ 4 Hz, H-1β), 5.74 (d, $J_{1,F}$ 1.4 Hz, H-1α), 4.1–3.0 (m, H-3,4,5,6,6'); ^{19}F NMR (D_2O): δ −42.42 (dd, $J_{3,F}$ 12.4, $J_{1,F}$ 4 Hz, F-2β), −43.75 (br d, $J_{3,F}$ 13.4 Hz, F-2α, α/β ratio ~4:1). Anal. Calcd for $C_6H_{10}FIO_5 \cdot 0.5H_2O$: C, 22.73; H, 3.50. Found: C, 22.93; H, 3.26.

Radiosynthesis of 2-FIM.—2-Fluoro-D-glucal (3 mg, 18 mmol) was dissolved in citrate–phosphate buffer (pH 3, 0.5 mL) in a Reactivial (Pierce). [^{123}I]NaI (2.9 mCi,) was added followed by 30% H_2O_2 (20 mL), and the vial was sealed. The mixture was stirred at room temperature, monitored by TLC (27:2:1 EtOAc–CH_3OH–H_2O) using an authentic standard of 2-FIM (R_f of β anomer ~0.52; R_f of α anomer ~0.38) as reference. The TLC plate was cut into four portions and each part counted separately with a Beckman gamma 8000 scintillation gamma counter. The radiochemical yield was calculated by dividing the radioactivity on the portion of the plate co-eluting with authentic 2-FIM by the total radioactivity on the plate.

Enzyme kinetics.—Activity of 2-FIM or 2-FIG with hexokinase from bovine heart and yeast (Sigma) was evaluated using a lactate dehydrogenase–pyruvate kinase coupled assay [10]. All assays were carried out in 0.1-cm quartz cells at 30°C in 50 mM Tris buffer (pH 7.4) containing 5 mM ATP, 10 mM $MgCl_2$, 1 mM phosphoenol pyruvate, 1 mM NADH, 50 mM KCl, and appropriate concentrations of either D-glucose, 2-FIM, or 2-FIG. The coupling enzymes (2–2.5 U) were added and the reaction was initiated by the addition of bovine heart or yeast hexokinase (~0.25–1 U) to the cell. The decrease in absorbance at 340 nm was monitored using a Pye-Unicam UV/Visible spectrometer.

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