Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXI, No. 12

Synthesis of 2-Deoxy-2-[^{18}F]-Fluoro-β-Mannosyl [^{18}F]-Fluoride as a Potential Imaging Probe for Glycosidases

[a]John D. McCarter, *[b]Michael J. Adam and [a]Stephen G. Withers

[a]*Department of Chemistry, University of British Columbia, Vancouver, B.C., Cananda, V6T 1Y6*

[b]*TRIUMF, 4004 Wesbrook Mall, Vancouver, B.C., Canada, V6T 2A3*

Abstract

The mechanism-based glycosidase inhibitor 2-deoxy-2-[^{18}F]-fluoro-β-mannosyl [^{18}F]-fluoride was synthesized and its covalent binding to *Agrobacterium* β-glucosidase was demonstrated *in vitro*.

Key Words: β-glycosidase, glycosyl fluoride, fluorine-18

Introduction

Various 2-deoxy-2-fluoro glycosides have been shown to act as covalent, mechanism-based inhibitors of "retaining" β-glycosidases by forming a relatively stable 2-deoxy-2-fluoro glycosyl-enzyme intermediate.[1,2] The normal mechanism of this class of enzymes involves the formation and hydrolysis of a glycosyl-enzyme intermediate *via* transition states with substantial oxocarbonium ion character. The fluorine at C-2 of the inhibitor is proposed to inductively destabilize these positively charged transition states, slowing the rates of both glycosyl-enzyme formation and hydrolysis. In addition, the limited hydrogen bonding capability of fluorine would be expected to result in loss of significant transition state binding interactions, further slowing these processes. The presence of a good leaving group at C-1 increases only the rate of glycosyl-enzyme formation, thereby resulting in accumulation of the intermediate and inactivation of the enzyme. Indeed, the existence of a covalent 2-deoxy-2-fluoro-α-D-mannosyl-enzyme

0362–4803/92/121005–05$07.50

Received 10 July, 1992
Revised 24 August, 1992

intermediate has been demonstrated by ^{19}F nmr spectroscopy with *Agrobacterium* β-glucosidase[3] and the identity of the enzymic nucleophile determined.[4]

There are many diseases which are associated with specific genetic deficiencies in cerebral glycosidases. For example, the neurological damage in Tay-Sachs disease is caused by an accumulation of GM_2 ganglioside in neuronal lysosomes due to a deficiency in hexosaminidase A, which cleaves a β(1,4) glycosidic bond in GM_2 to give N-acetylgalactosamine and a shorter ganglioside. There are other diseases characterized by deficiencies in various gluco-, manno- and galactocerebrosidases.[5]

We undertook the labelling of some of the above described glycosidase inhibitors with ^{18}F for use as potential diagnostic agents to image glycosidase activity in the brain with positron emission tomography (PET). This class of inhibitors would be expected to be well suited to PET imaging since the labelled enzyme is later reactivated by hydrolysis or transglycosylation of the 2-deoxy-2-fluoro glycosyl-enzyme.[4] Thus the label would not remain covalently bound to the enzyme permanently. It has been previously reported that 2-deoxy-2-halo halides can cross the blood-brain barrier in rats.[6] These agents may potentially find use in monitoring the effectiveness of enzyme replacement therapy for lysosomal storage diseases in the brain, as well as in diagnosis.

Experimental

General

Acetonitrile (BDH) was spectrophotometric grade. Diethyl ether was *not* anhydrous. 3,4,6-Tri-O-acetyl-D-glucal (Aldrich) was deacetylated with sodium methoxide in methanol according to standard protocols. ^1H and ^{19}F spectra were recorded at 200 MHz (Bruker AC-200). High Performance Liquid Chromatography (HPLC) was carried out on a Waters system equipped with a W-Porex GP-300 gel permeation column (4.6 mm x 250 mm) (Phenomenex, Inc.) using aqueous sodium phosphate buffer (50 mM Na_2HPO_4 and NaH_2PO_4, pH 6.8) as eluant at a flow rate of 1.0 mL/min.

Radiosynthesis and Enzyme Inactivation

The synthesis of the mixture of 2-deoxy-2-fluoro-α-glucosyl- and β-mannosyl fluorides was achieved by modification of previously reported syntheses of 2-deoxy-2-fluoro-D-glucose and 2-deoxy-2-fluoro-D-mannose,[7] but the glycosyl fluorides were separated without subsequent hydrolysis. [^{18}F]-F_2 was bubbled at a flow rate of 150 mL/min through a solution of D-glucal (~ 20 mg) in acetonitrile (10 mL). The resulting 2:1 mixture of 2-deoxy-2-fluoro-α-glucosyl- and β-mannosyl fluorides was evaporated to dryness and the residue chromatographed on silica gel with diethyl ether as the eluant. Each compound was chromatographically homogenous and ^1H and ^{19}F nmr spectra were fully consistent with literature values.[7,8] The fraction containing the β-mannosyl fluoride was evaporated, dissolved in sodium phosphate buffer and an aliquot incubated with a

solution of *Agrobacterium* β-glucosidase (2.7 mg/mL) for 15 minutes at 37°C., sufficient to ensure > 99.9% inactivation of the enzyme at the inhibitor concentration used (~ 1 mM). This rate is consistent with the inactivation parameters determined previously for the unlabelled compound (K_i = 1.29 mM, k_i = 5.6 min^{-1}).[1] The enzyme/inhibitor mixture was then chromatographed on an HPLC aqueous gel permeation column.

Results and Discussion

The radiochemical yield of the 2-deoxy-2-fluoro-β-mannosyl fluoride was 12% and the overall time from end of bombardment (EOB) for synthesis and purification was ~ 40 min. The radiochromatogram from the HPLC column of the enzyme/inhibitor mixture showed two peaks (Figure 1). The first, smaller peak eluted at the same time as uninhibited enzyme (detected by UV absorbance at 214 nm) and therefore comprised the radiolabelled inhibited enzyme, while the second, larger peak corresponded to excess unbound inhibitor. The labelled enzyme peak was collected to monitor turnover of the isolated 2-deoxy-2-[^{18}F]-fluoro mannosyl-enzyme.

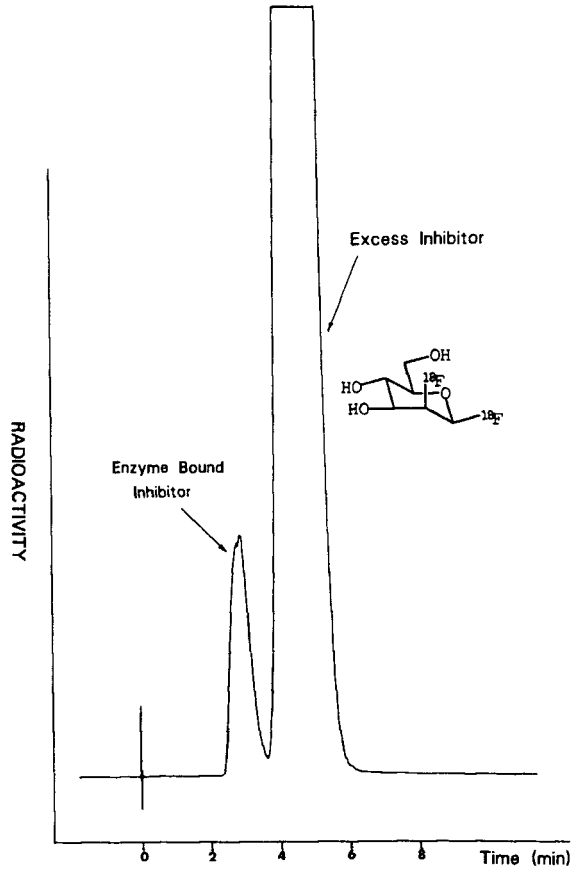

Figure 1. Radiochromatogram from gel permeation HPLC column of incubated mixture of excess 2-deoxy-2-[^{18}F]-fluoro-β-mannosyl [^{18}F]-fluoride and *Agrobacter* β-glucosidase.

The glycosyl-enzyme complex was incubated in buffer at 37°C. in the absence and presence of 197 mM 1-deoxy-β-D-glucosyl benzene, which promotes reactivation *via* a facile transglycosylation reaction yielding a disaccharide product.[4] Aliquots were removed at intervals and re-injected onto the HPLC column. The decrease in radioactivity of the glycosyl-enzyme peak and concomitant *relative* increase in radioactivity of the released label (which co-eluted with a 2-deoxy-2-fluoro mannose standard) were measured. The 2-deoxy-2-[^{18}F]-fluoro mannosyl moiety was released from the enzyme considerably faster *via* transglycosylation with glucosyl benzene (k_{react} = 5.0 x 10^{-3} min^{-1}) than *via* hydrolysis (k_{react} = 7.4 x 10^{-4} min^{-1}) as expected (Figure 2). In both cases, the labelled hydrolysis or transglycosylation products eluted significantly later than the glycosyl-enzyme complex, as would be anticipated for small molecules such as mono- or disaccharides on a gel permeation column. These data are in good agreement with turnover rates previously obtained by assaying activity of the reactivated enzyme with p-nitrophenyl β-D-glucopyranoside (k_{react} = 5.7 x10^{-3} min^{-1} for transglycosylation and k_{react} = 10 x 10^{-4} min^{-1} for hydrolysis).[9]

The possible fate of 2-deoxy-2-fluoro glycosides *in vivo* is unclear. It is unlikely that an intact glycosyl fluoride would be phosphorylated by hexokinase since it has been shown that both α- and β-glucopyranosyl fluorides are exceedingly poor substrates for yeast hexokinase.[10] Upon hydrolysis, the resulting 2-deoxy-2-fluoro sugar could then be phosphorylated.[10] However, the spontaneous hydrolysis of a 2-deoxy-2-fluoro glycoside would be expected to be slow relative to its unsubstituted parent due to the inductive effect of the C-2 fluorine and enzymic hydrolysis would presumably result in the desired labelling of the enzyme.

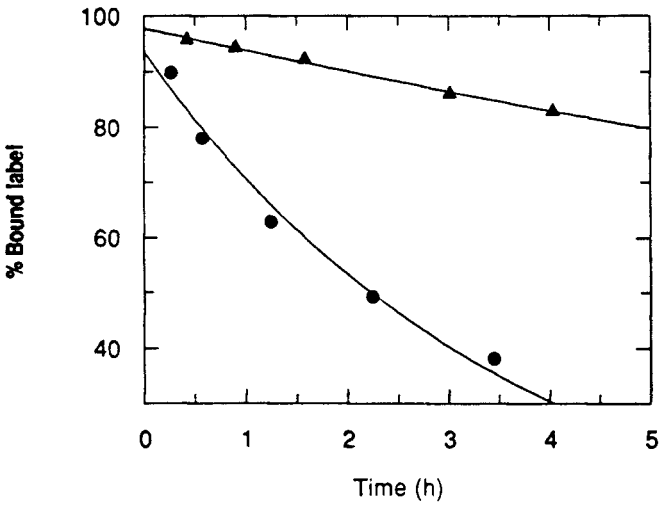

Figure 2. Plots of % enzyme-bound label remaining *vs.* time for isolated 2-deoxy-2-[^{18}F]-fluoro mannosyl-enzyme in buffer (triangles) and in presence of 197 mM glucosyl benzene (circles). Activity corrected for decay of ^{18}F ($t_{1/2}$ = 110 min).

In summary, we have demonstrated for the first time the covalent incorporation of a [^{18}F]-fluorinated carbohydrate inhibitor into a glycosidase. Turnover of the 2-deoxy-2-[^{18}F]-fluoro glycosyl-enzyme can be readily monitored *in vitro* by following release of radioactivity from the enzyme. Animal studies are underway to evaluate the biodistribution and *in vivo* stability of 2-deoxy-2-[^{18}F]-fluoro glycosides.

Acknowledgements

We gratefully acknowledge the Natural Sciences and Engineering Research Council of Canada for financial support and Salma Jivan for expert technical assistance.

References

1. Withers, S.G., Rupitz, K. and Street, I.P., *J. Biol. Chem.*, **263**, 7929 (1988).

2. McCarter, J.D., Adam, M.J. and Withers, S.G., *Biochem. J.*, (1992). In press.

3. Withers, S.G. & Street, I.P., *J. Am. Chem. Soc.*, **110**, 8551 (1988).

4. Withers, S.G., Warren, R.A.J., Street, I., Rupitz, K., Kempton, J., Aebersold, R., *J. Am. Chem. Soc.*, **112**, 5887 (1990) .

5. Watts, R.W.E. & Gibbs, D., *Lysosomal Storage Diseases: Biochemical and Clinical Aspects*, Taylor and Francis, London, 1986.

6. Homma, Y., Murase, Y. & Ishii, M., *J. Radioanal. Chem.*, **76**(2), 283 (1983).

7. Satyamurthy, N., Bida, G.T., Padgett, H.C., Barrio, J.R., *J. Carb. Chem.*, **4**, 489 (1985).

8. Diksic, M. & Jolly, D., *J. Carb. Chem.*, **4**, 265 (1985).

9. Street, I.P., Kempton, J.B. & Withers, S.G. *Biochemistry*, (1992). In press.

10. Bessell, E.M., Foster, A.B. & Westwood, J.H. *Biochem. J.*, **128**, 199 (1972).