junction with other oxidases to further develop this new class of amperometric biosensor.

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1,1-Difluoroalkyl Glucosides: A New Class of Enzyme-Activated Irreversible Inhibitors of α-Glucosidases

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There is presently an upsurge of interest in glycosidase inhibitors. Such compounds have been shown to be important tools in mechanistic studies on glycohydrolases,[1] as well as having promising therapeutic applications.[2] However, only a few examples of enzyme-activated irreversible inhibitors of glycosidases have been described. They include conduritol epoxides,[3] glycosylmethyltriazenes,[4] and the aziridine triol derivatives of piperidine.[5] These compounds are activated in the first protonation step of the enzyme-assisted glycoside hydrolysis. Due to their high intrinsic chemical reactivity, insufficient specificity could limit their use as drugs. Inhibitors activated only after or during enzymic cleavage of the glycosidic bond should be more selective. The first examples of this new class are 2-deoxy-2-fluoroglycosides.[6] These inhibitors are believed to be cleaved before inactivating the enzyme by the formation of a covalent glycosyl–enzyme complex. We now wish to report a novel enzyme-activated irreversible inhibitor of yeast α-glucosidase based on the activation, following hydrolytic cleavage, of the aglycon moiety of the molecule.

α-Glucosidases catalyze the hydrolysis reaction shown in Scheme I where R can be either a glycosidic residue or an aglycon leaving group. Our approach toward irreversible inhibition of α-glucosidase was to design R in such a way that the glucosidase **1** would be a stable substrate, while the alcohol **2** would be spontaneously and rapidly converted into a reactive alkylating agent.

1,1-Difluoroalkyl glucosides **3** (Scheme II) were chosen to illustrate this concept. The α,α-difluoro alcohols **4** (products of the α-glucosidase-catalyzed hydrolysis of **3**) are known[7] to rapidly lose HF and to be transformed into acid fluorides **5**. These acylating agents are then expected to form a covalent adduct **6** with a nucleophilic residue of the enzyme-active site.[8]

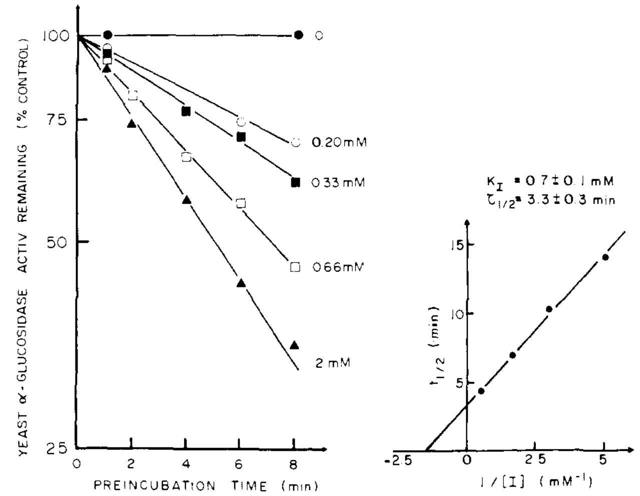
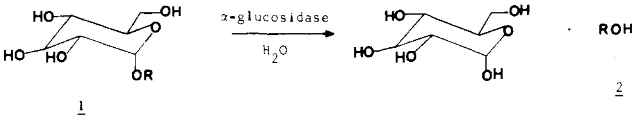
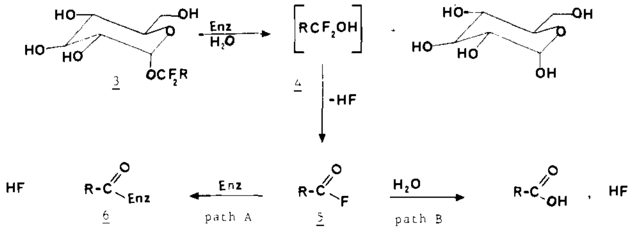


Figure 1. Time- and concentration-dependent inhibition of yeast α-glucosidase by **7**. The enzyme was incubated at 37 °C with 0.2 M phosphate buffer (pH 6.9) and various concentrations of inhibitor. At given time intervals, aliquots were withdrawn and assayed for the remaining activity according to ref 14. In the right-hand part of the figure, the times of half-inactivation ($t_{1/2}$) are plotted against the reciprocal of the **7** concentrations, according to Kitz and Wilson.[15]

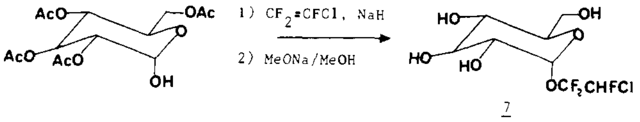
Scheme I



Scheme II



Scheme III



In order to evaluate this concept, we prepared 2-chloro-1,1,2-trifluoroethyl α-D-glucopyranoside **7** (Scheme III) in two steps from 2,3,4,6-tetraacetyl-α-D-glucopyranose.[11] The latter is first regioselectively condensed with trifluorochloroethylene[12] (DMF, −20 °C, 4 h) in the presence of a catalytic amount of NaH; then the acetyl groups are removed (MeONa/MeOH) to give 2-chloro-1,1,2-trifluoroethyl α-D-glucopyranoside as a 3/1 mixture of α/β anomers in 65% overall yield. The two anomers were separated by flash chromatography on silica gel, and compound **7** was isolated in pure (>95%) α-anomeric form as a mixture of 2 epimers.[13]

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[1] Lalégerie, P.; Legler, G.; Yon, J. M. *Biochimie* **1982**, *64*, 977.

[2] (a) Truscheit, E.; Frommer, W.; Junge, B.; Müller, L.; Schmidt, D. D.; Wingender, W. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 744. (b) Gruters, R. A.; Neefjes, J. J.; Tersmette, M.; de Goede, R. E. Y.; Tulp, A.; Huisman, H. G.; Miedema, F.; Ploegh, H. L. *Nature (London)* **1987**, *350*, 74. (c) Sunkara, P. S.; Bowlin, T. L.; Liu, P. S.; Sjoerdsma, A. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 206.

[3] Legler, G. *Methods Enzymol.* **1977**, *46*, 308.

[4] Marshall, P. J.; Sinnott, M. L.; Smith, P. J.; Widdows, D. *J. Chem. Soc., Perkin Trans. 1* **1981**, 366.

[5] Tong, M. K.; Ganem, B. *J. Am. Chem. Soc.* **1988**, *110*, 312.

[6] (a) Withers, S. G.; Street, I. P.; Bird, P.; Dolphin, D. H. *J. Am. Chem. Soc.* **1987**, *109*, 7530. (b) Withers, S. G.; Rupitz, K.; Street, I. P. *J. Biol. Chem.* **1988**, *263*, 7929.

[7] For example, trifluoromethanol undergoes exothermic loss of HF at temperatures above −20 °C according to: Seppelt, J. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 322.

[8] The rapid formation of acyl fluorides from α-difluoro alcohols suggested use of their esters as suicidal substrates for esterases and phosphatases.[9,10]

[9] (a) Ortiz de Montellano, P. R.; Vinson, W. A. *J. Am. Chem. Soc.* **1979**, *101*, 2222. (b) Vinson, W. A.; Prickett, K. S.; Spahic, B.; Ortiz de Montellano, P. R. *J. Org. Chem.* **1983**, *48*, 4661.

[10] Walsh, C. *Adv. Enzymol.* **1983**, *55*, 197.

[11] Fiandor, J.; Garcia-Lopez, M. T.; De Las Heras, F. G.; Mendez-Castrillon, P. P. *Synthesis* **1985**, 1121.

[12] The base-catalyzed addition of alcohols to fluoroolefins was described by: England, D. C.; Melby, L. R.; Dietrich, M. A.; Lindsey, R. V., Jr. *J. Am. Chem. Soc.* **1960**, *82*, 5116. For a more recent example, see: Krespan, C. G.; Smart, B. E. *J. Org. Chem.* **1986**, *51*, 320.

The α-glucoside **7** is a stable compound that decomposes only very slowly in water solution with a half-life of 680 h. Incubation of yeast α-glucosidase[14] with **7** resulted in a time-dependent loss of enzyme activity that followed pseudo-first-order kinetics (Figure 1). A Kitz and Wilson[15] replot of the data indicated that saturation was attained. The K_I value for **7** is 0.7 ± 0.1 mM, and the k_{inact} value is 0.21 ± 0.02 min^{-1}. Phenyl α-D-glucopyranoside, a substrate of the enzyme, as well as α-D-glucose and tris(hydroxymethyl)aminomethane ("Tris"), two competitive inhibitors, protected the enzyme from inactivation. These results demonstrate that the inactivation takes place in the active site. Furthermore, addition of dithiothreitol (5 mM) in the preincubation medium had no effect on the rate of inactivation, indicating that the species responsible for inactivation was not released from the active site.[16] Incubation with 5 mM **7** for 7 min at 37 °C resulted in 80% inactivation of the enzyme. Prolonged dialysis of this inactivated enzyme for 24 h at 4 °C did not regenerate any enzyme activity, suggesting the formation of a covalent linkage of the inhibitor to the enzyme-active site (Scheme II, path A; R = CHFCl).

Contrary to what was observed with yeast α-glucosidase, **7** was found to be a substrate of the sucrase–isomaltase complex purified from rat small intestine.[17] The enzyme-catalyzed hydrolysis of **7** resulted in the liberation of two molecules of HF for one molecule of glucose[18,19] according to Scheme II, path B. One possible reason that would explain why the mammalian glucosidases, contrary to the yeast enzyme, are not inactivated by **7** might lie in a difference of nucleophilic residues involved in the respective active site of these enzymes.[20]

In conclusion, we have shown for the first time that a 1,1-difluoroalkylglucoside is an enzyme-activated irreversible inhibitor of yeast α-glucosidase. All the biochemical data reported in this paper are in agreement with a novel process of enzyme-activated inhibition due to the inactivatory property of the leaving group released during the glucosidase-catalyzed hydrolysis.

Work is in progress in our laboratories to extend this approach to the inhibition of other hydrolytic enzymes of therapeutic interest.

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(13) **7**: ^{1}H NMR (CD_3OD, 360 MHz, TMS as reference) large d (6.6 ppm, CHFCl, $^2J_{H-F}$ = 48 Hz); d (5.85 ppm, anomeric H, J_{H-H} = 3.6 Hz); m (3.6–3.95 ppm, m, 6 H), s (5,1 ppm, 4 H, O*H*). ^{19}F NMR (CD_3OD, 338 MHz, CF_3CO_2H as reference) −11 ppm (m, CF_2, 2 AB parts of ABX; $^2J_{FF}$ = 144 Hz, $^3J_{FF}$ = 12.6 Hz); −78.5 ppm (2 dt, CHF_2). MS (CI/NH_3) 314 (MNH_4^+), 212, 180. Elemental analysis found for ($C_8H_{12}O_6F_3Cl$): C: 33.00; H, 4.24. Calcd: C, 32.39; H, 4.08.

(14) α-Glucosidase, type III, from yeast (Sigma). The enzyme activity was measured at 37 °C in 0.2 M sodium phosphate buffer (pH 6.9), using *p*-nitrophenyl α-D-glucopyranoside (PNPG) as the substrate, according to: Halvorson, H. *Methods Enzymol.* **1966**, *8*, 559.

(15) Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245.

(16) Rando, R. R. *Biochem. Pharmac.* **1974**, *23*, 2328.

(17) Danzin, C.; Ehrhard, A. *Arch. Biochem. Biophys.* **1987**, *257*, 472.

(18) Liberation of fluoride ions was measured with an Orion 96-09 fluoride specific electrode connected to a Metler semiautomatic titration system (C. Gaget, unpublished results). Glucose formation was measured according to the method of Dahlqvist (ref 19).

(19) Dahlqvist, A. *Anal. Biochem.* **1964**, *7*, 18.

(20) For instance, an essential thiol group has been found in the yeast α-glucosidase by Halvorson, 1966 (see ref 14), but not in the mammalian sucrase–isomaltase complex (ref 21).

(21) Kolinska, J.; Semenza, G. *Biochim. Biophys. Acta* **1967**, *146*, 181.

Additions and Corrections

Carbon and Proton Basicity [*J. Am. Chem. Soc.* **1988**, *110*, 5611–5613]. JOHN I. BRAUMAN* and CHAU-CHUNG HAN

This paper showed that proton basicity and methyl cation affinities are better correlated than might have been expected from earlier work, particularly if one used a non-unit-slope linear correlation of heats of formation of HA and CH_3A molecules. The equations derived in the paper are mathematically correct, but the non-unit-slope correlation between heats of formation of HA and CH_3A is only a statistical one that has no physical significance.

The relationship between proton affinity and methyl cation affinity of various A^- depends only on the heats of formation of the HA and CH_3A species

$$\mathrm{MCA}(A^-) - \mathrm{PA}(A^-) = \Delta H_f^\circ(CH_3^+) - \Delta H_f^\circ(H^+) - \Delta H_f^\circ(CH_3A) + \Delta H_f^\circ(HA) \qquad (1)$$

where MCA(A^-) is the methyl cation affinity, and PA(A^-) is the proton affinity, of A^-. The specific values of the heats of formation of the various species, however, have no physical content and depend on the arbitrary assignment of $\Delta H_f^\circ = 0$ for the elements in their most stable states at 298 K. The differences in heats of formation of reactants and products in any reaction are independent of the choice of the standard states of the elements. Similarly, the differences in heats of formation on the right-hand side of eq 1 are independent of the choice of standard states.

The suggestion that the correlation of MCA and PA could be improved by allowing a non-unit-slope correlation of heats of formation of HA and CH_3A is thus true in a statistical sense only. Although we can improve the correlation of the heats of formation by allowing a non-unit slope, the slope, and the standard deviation of the correlation, will depend on the arbitrary choice of standard states of the elements.

In short, the correlation of heats of formation of CH_3A and HA, Figure 2 and eq 4 of this paper, is simply a statistical one. The correlation, and the equation derived from it for calculating MCA from PA, eq 5 of this paper, has neither predictive content nor chemical pertinence.

It remains true, however, that there is a rough unit-slope correlation between heats of formation of HA and CH_3A which is equivalent to that between PA and MCA for the various A^-. The deviations from these unit-slope correlations, as originally pointed out by Hine and Weimer,[1] can, however, be quite large. Other relationships of proton affinities with metal cation affinities that have been reported recently[2] were found to have unit slope so that their significance is not compromised by the arguments presented here.

Acknowledgment. We are indebted to Professor Calvin D. Ritchie for discovering this problem and his helpful advice regarding it.

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(1) Hine, J.; Weimer, R. D., Jr. *J. Am. Chem. Soc.* **1965**, *87*, 3387.

(2) Bryndza, H. E.; Fong, L. K.; Paciellow, R. A.; Tam, W.; Bercaw, J. E. *J. Am. Chem. Soc.* **1987**, *109*, 1444. Labinger, J. A.; Bercaw, J. *Organometallics* **1988**, *7*, 926.