DNA can be attributed to an increase in solution viscosity, while the larger changes in both $E^{0'}$ and i_{pc} for $Co(phen)_3^{3+}$ are attributed to interactions with the DNA duplex.

CV experiments were carried out in which the ratio of DNA to $Co(phen)_3^{3+}$ was varied. The ratios are reported in terms of R = [nucleotide phosphate]/[cobalt(III)].[14] At $R = 0$, the diffusion coefficient of the free $Co(phen)_3^{3+}$, D_f, was obtained from the $i_p/v^{1/2}$ data ($5 \le v \le 200$ mV/s) as $(3.6 \pm 0.8) \times 10^{-6}$ cm^2/s. At $R = 304.5$, the apparent diffusion coefficient of the bound metal complex D_b, obtained by differential pulse voltammetry, was $(3.1 \pm 1.6) \times 10^{-7}$ cm^2/s.

A titration of 30.2 μmol nucleotide phosphate with $Co(phen)_3^{3+}$ while measuring the total cathodic peak current (i_T) as a function of μmol metal chelate added (C_T) (or R) gave the results shown in Figure 2. Two limiting regions are found. At large R the current is attributed primarily to $Co(phen)_3^{3+}$ intercalated to DNA (characterized by a concentration, C_b, and D_b), while at very small R the main contribution to i_T is free $Co(phen)_3^{3+}$ in solution (concentration, C_f and D_f). The total current at any R is

$$i_T = B[D_f^{1/2}C_f + D_b^{1/2}C_b] \quad (1)$$

where $B = 2.69 \times 10^5$ n$^{3/2}$ Av$^{1/2}$ for CV of a Nernstian wave at 25°.[16] C_b is related to the total metal added, C_T, with the assumption of control by the equilibrium binding of the chelate by DNA by

$$C_b = \{b - (b^2 - 2K^2C_T[NP]/n_s)^{1/2}\}/2K$$

$$b = 1 + KC_T + K[NP]/2n_s \quad (2)$$

where K is the intrinsic binding constant of the 3+ species, n_s is the number of pairs required per $Co(phen)_3^{3+}$, and [NP] is the nucleotide phosphate concentration. Equation 2 is valid for noncooperative, nonspecific binding with the existence of one type of discreet binding site. An analogous treatment could be used for a more complicated type of binding interaction.[17] The small viscosity changes that occur upon addition of the metal ion solution are also neglected. A regression analysis of the data in Figure 2 to eq 1 and 2 yield the following: $K = 5.8 \times 10^3$ M^{-1}, $n_s = 6.7$ base pairs, $D_f = 4.1 \times 10^{-6}$ cm^2/s, $D_b = 1.2 \times 10^{-7}$ cm^2/s. The value for n_s found here is somewhat larger than that reported for $Ru(phen)_3^{2+}$ ($n_s = 4$ bp). K is close to that for the $Ru(phen)_3^{2+}$ ($K = 6.2 \times 10^3$ M^{-1}).[1a] The positive 40-mV shift in the peak potential for bound complex compared to free $Co(phen)_3^{3+}$ unequivocally shows the +2 species (binding constant, K') is bound more strongly than the +3 species with $K'/K = 4.8$; $K' = 2.8 \times 10^4$ M^{-1}. This stronger binding of +2 might be explained by the importance of hydrophobic interactions, in addition to electrostatic ones.[1f,g] Similar effects have been found, for example, in the interaction of viologens and metal chelates with micelles[18] and perfluorosulfonated (Nafion) films.[19]

The results given here demonstrate that rather straightforward electrochemical methods can be employed to characterize the intercalative interaction between a metal complex or other electroactive species and DNA to yield estimates of the binding constants and binding site sizes. The electrochemical oxidation and reduction of selected bound species on DNA can also be carried out and in favorable circumstances may allow chemical changes in the DNA, e.g., strand scission.

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2-Deoxy-2-fluoroglucosides: A Novel Class of Mechanism-Based Glucosidase Inhibitors

Stephen G. Withers,* Ian P. Street, Paul Bird, and David H. Dolphin

Department of Chemistry
University of British Columbia
Vancouver, British Columbia, Canada V6T 1Y6

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Glucosidase inhibitors are of interest in the treatment of diabetes and obesity due to their potential in controlling blood glucose levels.[1] Currently available glucosidase inhibitors include the noncovalent, naturally occurring inhibitors such as acarbose[2] and nojirimycin[3] and covalent, mechanism-based inhibitors such as the conduritol epoxides[4] and glucosylmethyltriazenes.[5] This paper describes a novel mechanism-based glucosidase inhibitor based on a strategy which has not previously been exploited for this class of enzymes.

The enzymic hydrolysis of glucosidases likely proceeds through a glucosyl enzyme intermediate via oxocarbonium ion-like transition states as shown in Scheme I.[6] Therefore substitution of an electronegative fluorine atom for a hydroxyl group adjacent to the reaction center, at C-2, should destabilize these transition states and decrease both the rates of glycosylation (k_1) and deglycosylation (k_2). Indeed, we have synthesized several 2-deoxy-2-fluoroglucosides and glucosyl phosphates and found them to be very slow substrates for their respective glucosidases or glucosyl transferases, with K_m values generally similar to those for the normal substrate. A similar approach has been employed previously[7] in studies of terpene biosynthetic enzymes where reaction proceeds via carbocationic intermediates.

The incorporation of a highly reactive leaving group as the aglycone into such deactivated substrates might increase the glycosylation rate sufficiently to permit trapping of the 2-fluorodeoxyglucosyl enzyme intermediate, therefore inhibiting the enzyme in a temporary covalent fashion. We describe here the synthesis[8] and testing of such an inhibitor, 2,4-dinitrophenyl

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(8) Hydrolysis of 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide[9] afforded the anomeric mixture of protected hemiacetals. Treatment of this mixture with 1-fluoro-2-4-dinitrobenzene in the presence of DABCO[10] gave a mixture of the α- and β-dinitrophenyl glycoside peracetates which was separated by fractional crystallization. Deprotection (NaOMe/MeOH) of the β-anomer afforded crystalline **1**. Satisfactory spectral and analytical data were obtained for all compounds.

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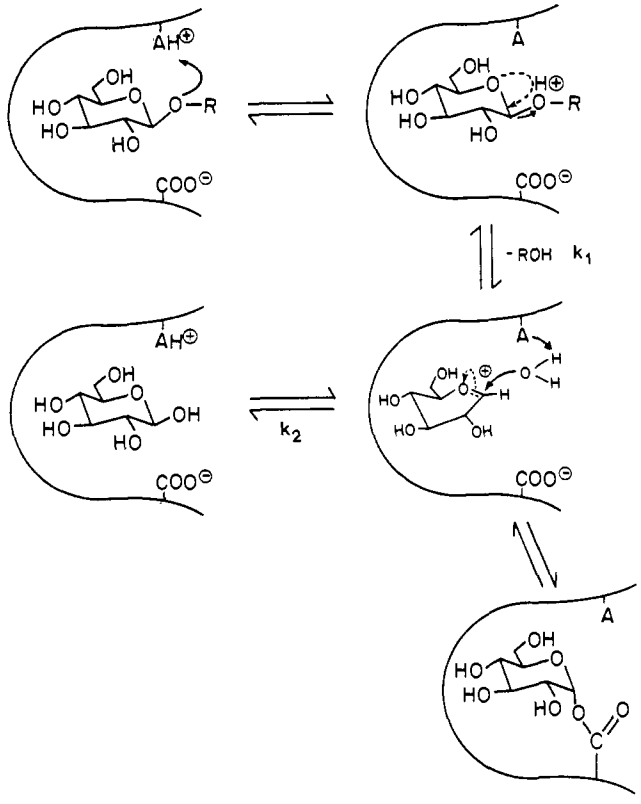
Scheme I



2-deoxy-2-fluoro-β-D-glucopyranoside (**1**).

Incubation of *A. faecalis* β-glucosidase[11,12] with **1** resulted in a rapid time-dependent loss of enzyme activity. Inactivation followed pseudo-first-order kinetics (Figure 1a) with a dissociation constant (K_i) of 0.05 mM and a rate constant (k_i) of 25 min^{-1}, measured at 37 °C, in 50 mM sodium phosphate buffer, pH 6.8 (Figure 1b). The inactivation rate was sufficiently high that measurements could not be made at concentrations approaching saturation since the half-life of this process, under saturating conditions, is 1.6 s. The value of K_i is therefore imprecise but interestingly quite similar to the K_m value of 0.03 mM measured previously[12] for 2,4-dinitrophenyl β-D-glucopyranoside suggesting that fluorine substitution at C-2 has negligible effects on binding.

Further proof that the observed inhibition is due to reaction at the active site is the protection against inactivation afforded by the competitive inhibitor isopropylthio β-D-glucopyranoside (K_i = 4 mM) (Figure 1c).

Since the inactivation process represents the first step of very slow substrate turnover, the inactivated enzyme should gradually hydrolyze liberating free enzyme which could be assayed under appropriate conditions. Inactivated β-glucosidase was therefore freed of excess inhibitor by gel filtration (Sephadex G10), incubated at 30 °C, and assayed for regain of activity. Surprisingly no reactivation was observed unless the enzyme was incubated in the presence of substrate, *p*-nitrophenyl β-D-glucopyranoside or other glucosides such as isopropylthio β-D-glucopyranoside. Under these conditions first-order reactivation kinetics were observed with a half life of ≈ 85 min (at 10 mM *p*-nitrophenyl β-D-glucopyranoside), and full activity was regained. In the absence of added glucoside the inactivated enzyme was stable for several weeks at 4 °C but could then be fully reactivated in the presence of substrate. This requirement for added glucosides was unexpected and is being investigated further but will be very useful in future attempts to identify the active site nucleophile and in planned ^{19}F NMR experiments to probe the 2-fluoroglucosyl enzyme intermediate.

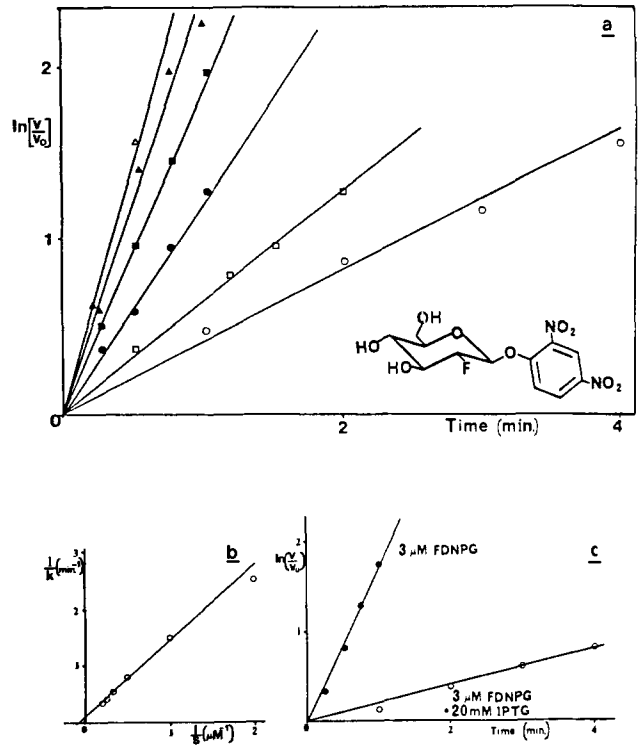


Figure 1. Inactivation of *A. faecalis* β-glucosidase with **1**. (a) β-glucosidase incubated with the following concentrations of **1** and aliquots assayed against *p*-nitrophenyl β-glucopyranoside at the times shown: ○ = 0.5 μM, □ = 1.0 μM, ● = 2.0 μM, ■ = 3.0 μM, ▲ = 4.0 μM, Δ = 5.0 μM). (b) Replot of first-order rate constants from **1a**. (c) Protection against inhibition given by isopropylthio β-D-glucopyranoside.

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HPLC Separation of DNA Adducts Based on Hydrogen Bonding[‡]

Binyamin Feibush,*[†] Manasi Saha, Kay Onan, Barry Karger,* and Roger Giese*

Barnett Institute and Departments of Chemistry and Medicinal Chemistry Northeastern University Boston, Massachusetts 02115

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Exposure of humans to genotoxic chemicals increases the risks of carcinogenesis and mutagenesis. Since these risks tend to correlate with the extent of chemical damage to DNA, i.e., with "DNA adducts", detection of such adducts in exposed humans is important.[1,2] However, extraordinary sensitivity is required: one adduct in 10^9 DNA base pairs or less may need to be determined, corresponding to about one adduct per cell. Highly sensitive techniques are therefore being developed for this purpose, e.g., radioenzymatic labeling with [^{32}P]phosphate[3] or derivatization

[‡]Contribution No. 322 from the Barnett Institute.
[†]Current address: Supelco Inc., Bellefonte, PA 16823.
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