Spontaneous Hydrolysis of Glycosides

Richard Wolfenden,* Xiangdong Lu, and Gregory Young

Department of Biochemistry and Biophysics
University of North Carolina
Chapel Hill, North Carolina 27599

Received April 16, 1998

Polysaccharides account for much of the carbon in the biosphere. The stability of these polymers in water presumably renders them suitable for the storage of metabolic energy in the α-linked polymers amylose and glycogen, and for the maintenance of physical structures supported by the β-linked polymers cellulose and chitin. The O-glycosidic bonds joining these polymers are readily hydrolyzed in the presence of acids or glycosidases,[1] but the rate of uncatalyzed hydrolysis of a simple, unactivated, glycoside does not appear to have been determined.[2] That information would allow assessment of the rate enhancements produced by glycosidases as catalysts, and their potential susceptibility to inhibition by transition state analogue inhibitors.[3] Here we show that the spontaneous hydrolysis of unactivated O-glycosides proceeds very much more slowly than the hydrolysis of bonds joining other biological polymers. The results identify glycosidases as exceptionally sensitive targets for inhibitor design, and may have a significant bearing on the conservation of paper documents.

In these experiments, methyl glucopyranosides and methyl ribofuranosides (0.05−0.1 M) were dissolved in potassium acetate, phosphate, or carbonate buffers (0.05−0.2 M) at room temperature, sealed in quartz tubes (1 mm wall thickness, 2 mm internal diameter) under vacuum, and then incubated at elevated temperatures[4] for various time intervals. When the tubes had cooled, the contents were analyzed by proton NMR after 100-fold dilution with D_2O, to which pyrazine had been added as an integration standard. In each case, hydrolysis proceeded to completion with satisfactory first-order kinetics, as indicated by the disappearance of glycoside and the release of methanol.[5]

Rates of glycoside hydrolysis are so slow that they are easily observed only at very high temperatures. β-Methyl D-glucopyranoside undergoes hydrolysis at 220 °C with rate constants that decrease with increasing pH, approaching a value that remains constant at pH values above 7 as expected for an uncatalyzed reaction with water (Figure 1), nor does that rate vary significantly with changing buffer concentration (0.05−0.2 M). When hydrolysis was conducted at pH 10 in the presence of $H_2{}^{18}O$ (70 atom % excess), GC−MS analysis of the methanol produced showed that this reaction occurs >99% by cleavage between the glycosidic oxygen atom and C-1 of glucose (exchange of ^{18}O from water to methanol does not occur to a detectable extent under these conditions). Arrhenius plots of rate constants obtained from 180 to 260 °C (Figure 2), in potassium carbonate buffer (0.1 M, pH 10.6 measured at room temperature), indicate that the enthalpy of activation for spontaneous hydrolysis of β-methyl glucopyranoside is 29.7 ± 1.5 kcal/mol. Unlike the acid-catalyzed hydrolysis of β-methyl glucopyranoside (which exhibits a positive entropy of activation as expected for unimolecular decomposition of the conjugate acid),[6] the uncatalyzed hydrolysis of β-methyl glucopyranoside proceeds with a negative entropy of activation (−24 cal deg^{-1} mol^{-1}), consistent with bimolecular attack by water.

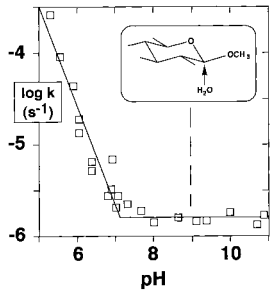

Figure 1. Apparent first-order rate constants for hydrolysis of β-1-methylglucopyranoside (0.05 M) in potassium acetate, phosphate, and carbonate buffers (0.2 M) at 220 °C, plotted as a function of pH determined at 25 °C.

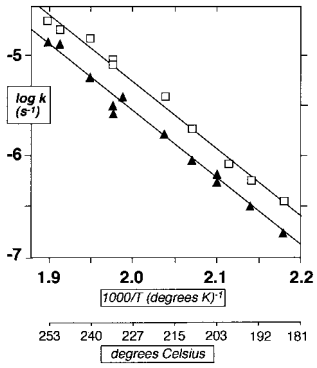

Figure 2. Hydrolysis of β- (squares) and α- (closed triangles) 1-methylglucopyranosides in potassium carbonate buffer (0.1 M, pH 10.6), plotted as a function of reciprocal temperature (K).

Despite this difference in mechanism, the uncatalyzed and acid-catalyzed reaction rates respond similarly to changes in glycoside structure. Thus, the β-anomer is roughly twice as reactive as the α-anomer in both the ribofuranosides and glucopyranosides, and removal of the 2′-hydroxyl group enhances the rate of spontaneous hydrolysis by a factor of $\sim 10^3$ (Table 1), as in the acid-catalyzed reactions.[1] Ribofuranosides are somewhat more reactive than glucopyranosides. This effect may be associated with ring strain in the transition state, as a similar difference is also apparent if one compares rate constants that have been reported for phenoxide departure from tetrahydrofuran[7] and tetrahydropyran.[8]

When rate constants for spontaneous cleavage of glycosides are compared with other covalent bonds in biological polymers (Figure 3), the glycosidic bonds that join polysaccharides appear to be more stable to spontaneous hydrolysis than the phosphodiester bonds that join the nucleotides of DNA,[9] by roughly 2 orders of magnitude (Figure 3). In contrast, proteins[10] are less stable to hydrolysis than DNA by roughly 2 orders of magnitude in neutral solution (Figure 3), and RNA is even less stable.[11] Thus, at

(1) For reviews, see: Capon, B. *Chem. Rev.* **1969**, *69*, 407−499. BeMiller, J. N. *Adv. Carbohydr. Chem.* **1969**, *74*, 24−108. Sinnott, M. L. In *The Chemistry of Enzyme Action*; Page, M. I., Ed.; Elsevier: Amsterdam, 1984; pp 389−432. Legler, G., *Adv. Carbohydr. Chem*, **1990**, *48*, 319−384.

(2) 2,4-Dinitrophenyl β-galactopyranoside, for example, undergoes spontaneous hydrolysis at 25 °C with $t_{1/2} \approx 40$ h (Cocker, D.; Sinnott, M. L. *J. Chem. Soc., Perkin Trans. 2* **1975**, 1392−1395).

(3) Wolfenden, R. *Nature* **1969**, *223*, 704−707.

(4) The acid dissociation constants of buffers, and of solvent water, vary with temperature (Edsall, J. T.; Wyman, J. *Biophysical Chemistry*; Academic Press: New York, 1958; pp 452−453. These complications are not expected to interfere with the determination of the rates of the uncatalyzed reactions, which are pH-independent.

(5) At high temperatures, the hydrolysis products ribose and glucose are degraded rapidly, with $t_{1/2} \approx 80$ min and 40 h, respectively, at pH 7.4 and 100 °C (Larralde, R., Robertson, M. P.; Miller, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8158−8160).

(6) $\Delta S^{\ddagger} = +16.5$ cal deg^{-1} mol^{-1} at 60 °C (Overend, G.; Rees, C. W.; Sequeira, J. *J. Chem. Soc.* **1961**, 3429−3436).

(7) Lönnberg, H.; Pohjola, V. *Acta Chem. Scand. A* **1975**, *30*, 669−670.

(8) Craze, G.-E.; Kirby, A. J. *J. Chem. Soc., Perkin Trans. 2* **1975**, 354−356.

S0002-7863(98)01305-5 CCC: $15.00
Published on Web 06/25/1998

Table 1. Rate Constants for Spontaneous Hydrolysis of Methyl Glycosides[a]

	$k_{150\ °C}$ (s^{-1})	$\Delta G^{‡}_{25\ °C}$ (kcal)	$\Delta H^{‡}$ (kcal)	$T\Delta S^{‡}_{25\ °C}$ (kcal)	$k_{25\ °C}$ (s^{-1})
α-methylglucopyranoside	8.6×10^{-9}	37.4	30.3	−7.1	1.9×10^{-15}
β-methylglucopyranoside	2.2×10^{-8}	36.8	29.7	−7.1	4.7×10^{-15}
α-methylribofuranoside	1.5×10^{-7}	36.1	31.3	−4.8	1.9×10^{-14}
β-methylribofuranoside	2.9×10^{-7}	35.7	30.9	−4.8	3.7×10^{-14}
αβ-methyl-2-deoxyribofuranoside	3.1×10^{-6}	31.4	27.4	−4.2	3.7×10^{-11}

[a] Rate constants were obtained from the integrated intensities of the methyl protons of reactant glycoside and product methanol, after reaction in potassium carbonate buffer, (0.1 M, pH 10.6). Standard errors, estimated by linear least squares regression of Arrhenius plots, are ±1.5 kcal/mol for $\Delta H^{‡}$, ±2.2 kcal/mol for $T\Delta S^{‡}_{25\ °C}$, and ±45% for values of $k_{25\ °C}$.

physiological pH values, the two major classes of biological polymers used by organisms for long-term storage of energy and information are distinguished by their resistance to hydrolytic attack. Of additional interest (see below) is the considerably steeper dependence of reaction rate on temperature for polysaccharide hydrolysis than for hydrolysis of DNA or proteins (Figure 3).

The present results can be used to assess the rate enhancements produced by glycosidases as catalysts. Extrapolation to room temperature yields a first-order rate constant of $(1.9 \pm 0.7) \times 10^{-15}$ s^{-1} for the uncatalyzed hydrolysis of α-methyl glucopyranoside at 25 °C. Comparison of that first-order rate constant with values of k_{cat} (∼1360 s^{-1}) and K_m (7×10^{-5} M), reported for crystalline sweet potato β-amylase (1,4-α-D-glucan maltohydrolase; EC 3.2.1.2) generating maltose from starch[12] by an "inverting" mechanism involving direct water attack,[13] indicates that this enzyme enhances the rate of glycoside hydrolysis by a factor (k_{cat}/k_{non}) of more than 10^{17}. Combining this K_m value with the rate enhancement, one is led to infer that the dissociation constant of the ES‡ complex, in the transition state, is not greater than 10^{-22} M.[14] These results identify glycosidases, along with phosphatases, phosphodiesterases, and orotidine 5′-phosphate decarboxylase,[9] as exceptionally proficient catalysts and sensitive potential targets for inhibitor design. Remarkably, β-amylase differs from these other enzymes in achieving this catalytic proficiency as a pure protein,[15] without the assistance of metals or other cofactors.[16]

These findings may have an unexpected bearing on efforts to preserve paper books and other documents against deterioration with time, a serious problem in major libraries and rare book collections. Of special interest from this standpoint is the finding that β-methyl glycoside hydrolysis exhibits a larger enthalpy of activation (∼30 kcal/mol) than the hydrolyses of other biological polymers (compare slopes in Figure 3), corresponding to a 6-fold decrease in rate as the temperature is lowered from 30 to 20 °C. The unusual magnitude of that Q_{10} value implies that refrigeration may furnish a particularly effective means of preserving valuable documents against deterioration with time.

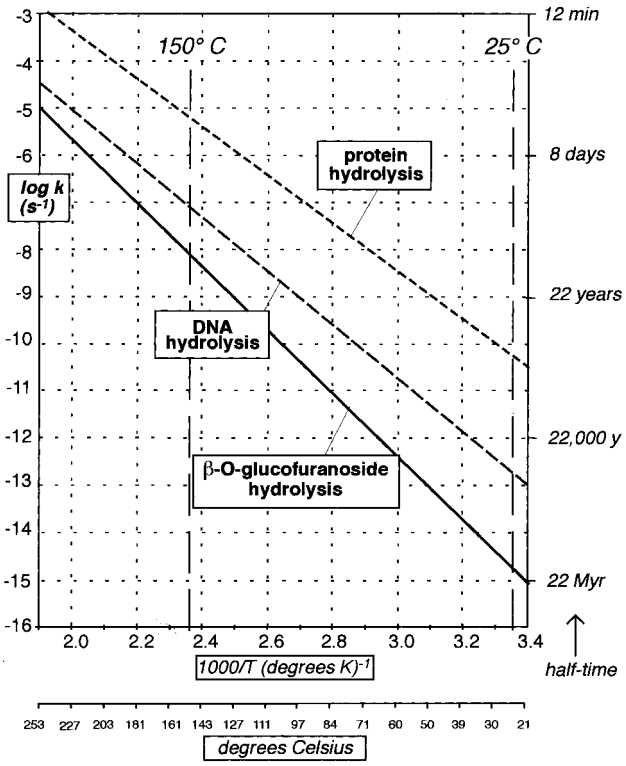

Figure 3. Arrhenius plots of the hydrolysis of individual bonds in DNA, protein, and cellulose. The line for DNA, based on observations on hydrolysis of the dimethyl phosphate anion (ref 9) yields a half-life of 140 000 years at 25 °C from pH 5 to pH 14. The line for protein, based on an average of very similar activation parameters observed for the uncatalyzed hydrolysis of gly-gly, acetyl-gly-gly, and acetyl-gly-gly *N*-methylamide (ref 10), yields a half-life of 460 years at 25 °C from pH 4 to pH 8. The line for cellulose, transposed from the data for α-1-methylglucopyranoside shown in Figure 2, yields a half-life of 4 700 000 years at 25 °C from pH 7 to pH 14.

Acknowledgment. We thank A. Ranasinghe for performing [^{18}O]-methanol analysis by GC−MS. This work was supported by NIH Grant GM-18325.

JA9813055

(9) Radzicka, A.; Wolfenden, R. *Science* **1995**, *267*, 90−93. Wolfenden, R., Ridgway, C., Young, G. *J. Am. Chem. Soc.* **1998**, *120*, 833−834. Because the pK_a of the leaving 4-OH group of glucose (∼12.4) is lower than that of methanol (15.5), the bonds joining polysaccharides are presumably somewhat less stable than those in methyl glucopyranosides.

(10) Radzicka, A.; Wolfenden, R. *J. Am. Chem. Soc.* **1996**, *118*, 6105−6109.

(11) Thompson, J. E.; Kutateladze, T. G.; Schuster, M. C.,; Venegas, F. D.; Messmore, J. M.; Raines, R. T. *Bioorg. Chem.* **1995**, *23*, 471−481.

(12) Balls, A. K.; Walden, M. K.; Thompson, R. R. *J. Biol. Chem.* **1948**, *173*, 9−20.

(13) Koshland, D. E., Jr. *Biol. Rev. Cambridge Philos. Soc.* **1953**, *28*, 416−455.

(14) That value presumably represents an upper limit, if the enzymatic and nonenzymatic reactions differ in mechanism, or if the rate of the enzyme reaction is governed by some process other than chemical cleavage of the substrate (Wolfenden, R. *Annu. Rev. Biochem. Biophys. Bioeng.* **1976**, *5*, 271−306).

(15) OMP decarboxylase, earlier believed to be metal-free, has now been found to contain Zn at its active site, as do alkaline phosphatase and several phosphodiesterases (Miller, B. G.; Traut, T. W.; Wolfenden, R. *J. Am. Chem. Soc.* **1998**, *120*, 2666−2667).

(16) Mikami, B.; Shibata, T.; Hirose, M.; Aibara, S.; Sato, M.; Katsube, Y.; Morita, Y. *J. Biochem.* **1992**, *112*, 541−546.