β-Galactosidase-catalysed Hydrolysis of the β-D-Galactopyranosylpyridinium Cation

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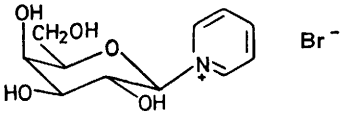
Summary The β-D-galactopyranosylpyridinium cation is a well-behaved, if slow, substrate for the β-galactosidase of *E. coli*.

MOST theories about the mechanism of action of glycosidases invoke some form of general acid[1] or electrophilic[2] catalysis of the departure of the aglycone. It is herein reported that β-D-galactopyranosylpyridinium bromide (I), in the hydrolysis of which such processes are impossible, is nonetheless hydrolysed at the active site of the β-galactosidase of *Escherichia coli*. In the case of this particular enzyme, studies on the binding of positively charged inhibitors have shown that electrophilic catalysis by the necessary Mg^{2+} ion does not operate, and that tetramethylammonium bromide does not detectably influence kinetic parameters at concentrations under 20 mM.[3]



(I)

The enzyme-catalysed hydrolysis of the pyridinium salt was examined at 25·0° in 0·100 M-sodium phosphate buffer, pH 7·00, 1·0 mM in $MgCl_2$. The products are the expected ones, D-galactose (88%, as estimated by galactose dehydrogenase[4]) and pyridine (101%, estimated by u.v. after extraction of the reaction mixture with two volumes of spectroscopic chloroform). That the hydrolysis is effected at the conventional active site of catalytically active protein is indicated by the following kinetic evidence:

(i) Michaelis–Menten kinetics are observed, with a K_m value of 1·25 ± 0·24 mM and a k_{cat} value, relative to one for the hydrolysis of 2-nitrophenyl β-D-galactopyranoside of 1000, of 0·122 ± 0·008.

(ii) The pyridinium salt (I) inhibits the hydrolysis of 4-nitrophenyl β-D-galactopyranoside in a cleanly competitive manner, with a K_i value of 1·16 mM, identical, within experimental error, to the K_m value for the hydrolysis of the salt itself.

(iii) Methyl 1-thio-β-D-galactopyranoside, a competitive inhibitor of the hydrolysis of conventional substrates of the enzyme, with a K_i value of 1·8 mM,[5] is also a competitive inhibitor of the hydrolysis of the salt (I), with a K_i value of 1·3 ± 0·5 mM.

Conformational distortion and electrostatic or nucleophilic catalysis must be responsible, therefore, for the entire catalytic effect of the enzyme on the hydrolysis of the pyridinium salt (I). Some form of partial proton-transfer to the aromatic π-electron cloud of the pyridine ring, in addition to being intrinsically unlikely, would not assist the cleavage of the glycosyl–nitrogen bond since it and the π-electron system are constructed from orthogonal orbitals.

β-Galactosidase will also cleave the glycosyl–nitrogen bond of β-D-galactopyranosyl azide (K_m = 2·8 mM, k_{cat} = 44[5]) but in this process general acid-catalysis is possible. Since the glycosyl–nitrogen bond of both substrates is constructed from nitrogen sp^2 orbitals, and since the leaving groups have comparable pK_a values (5·2[6] for the pyridinium

cation and 4·6 for hydrazoic acid), one can estimate that general acid-catalysis contributes a rate acceleration of only $10^{5/2}$ to the catalytic processes. This treatment assumes that for these very slow substrates the postulated protein conformation-change[8] is no longer rate-limiting.

β-D-Galactopyranosylpyridinium bromide, m.p. 161—162°, was made by conventional methods;[9] its aqueous solutions are stable indefinitely at room temperature.† As would be expected from the relative basicities of pyridine and trimethylamine, β-D-galactopyranosyltrimethylammonium bromide is yet more stable, its aqueous solutions being unchanged after 2 h at 100°:[10] this stability is reflected in a relative k_{cat} value for its β-galactosidase-catalysed hydrolysis of less than 0·005.[3] However, this stability also makes it difficult to understand the recent claim by West and Schuerch[11] that 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyltriethylammonium bromide is an extremely light- and moisture-sensitive gum and a significant α-glucopyranosylating agent.

I thank the S.R.C. for financial support.

(*Received, 1st June* 1973; *Com.* 787.)

† It gave correct (± ≤0·4%) elemental analyses; the β-configuration is established by the splitting (8·3 Hz) of the anomeric proton doublet (at δ 7·6 in $CDCl_3$) in the ^1H n.m.r. spectrum of the tetra-*O*-acetyl derivative.

[1] B. Capon, *Biochimie*, 1971, **53**, 145.
[2] C. R. Clark, R. W. Hay, and I. C. M. Dea, *Chem. Comm.*, 1970, 794.
[3] G. S. Case, M. L. Sinnott, and J.-P. Tenu, *Biochem. J.*, 1973, **133**, 99.
[4] K. Wallenfels and G. Kurz, *Biochem. Z.*, 1963, **335**, 559.
[5] M. L. Sinnott, *Biochem. J.*, 1971, **125**, 717.
[6] H. C. Brown and X. R. Mihm, *J. Amer. Chem. Soc.*, 1955, **77**, 1723.
[7] M. Quintin, *Compt. rend.*, 1940, **210**, 625.
[8] M. L. Sinnott and I. J. L. Souchard, *Biochem. J.*, 1973, **133**, 89.
[9] E. Fischer and K. Raske, *Ber.*, 1910, **43**, 1750; R. Lemieux and A. R. Morgan, *Canad. J. Chem.*, 1965, **43**, 2205.
[10] F. Micheel, *Ber.*, 1929, **62**, 687.
[11] A. C. West and C. Schuerch, *J. Amer. Chem. Soc.*, 1973, **95**, 1333.