The Effective Charges at the Active Sites of Two Glycosidases

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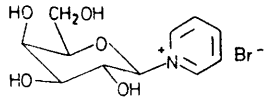
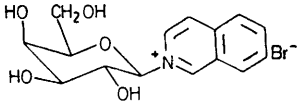
Summary At optimum pH, the active site of β-galactosidase has a slight net positive charge and that of β-glucosidase a more substantial one.

β-GALACTOSIDASE binds[1] and hydrolyses[2,3] β-D-galactopyranosyl quaternary ammonium salts. A comparison of the binding constants of these compounds with those of

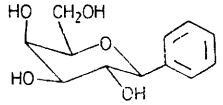
their *C*-glycoside analogues should indicate the net charge at the enzyme active site, since the two series of compounds are isoelectronic and isosteric, differing only in charge. Such *C*-glycosides have been prepared and are indeed found to be competitive inhibitors.



(I) 1·2[a]

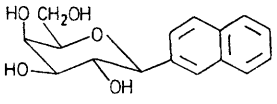


(III) 0·91[a,b]



(II) 0·47 (28)

$K_i^+ / K_i^0 = 2·6$



(IV) 0·39 (24)

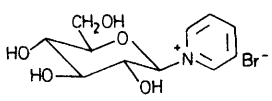
$K_i^+ / K_i^0 = 2·7$

β-Galactosidase from *Escherichia coli*. K_I/mM values, measured against 4-nitrophenyl *β*-D-galactopyranoside at 25° in 0·10M sodium phosphate buffer, pH 7·0, containing 1·0 mM $MgCl_2$, are given. Figures in parentheses are $K_m/\mu M$ values for the galactoside substrate derived from the inhibition experiment.

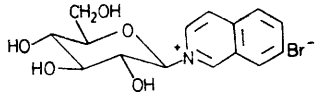
[a] Ref. 3. [b] K_m value. For this salt $K_m = K_I = K_s$ since the slow ($k_{cat} = 0·36\ s^{-1}$) bond-breaking is rate-limiting.

Data indicate a very slight positive charge on the active site of *β*-galactosidase (corresponding to a full positive charge 7·2 Å from the nitrogen atom of the bound salt in pure water [$D = 81$]). This net charge is consonant with the suggestion[4] of an anionic group at the active site of *β*-galactosidase, since the net positive charge on *β*-glucosidase is greater despite the demonstration by Legler and Hasnain[5] of the presence of an ionised carboxylate group at the active site of this enzyme.

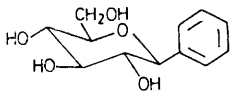
The analogy drawn between *β*-glucosidase and *β*-galactosidase[3] is strengthened by the observation of slow *β*-glucosidase-catalysed hydrolysis of the salts (V) and (VII).



(V) 345±56

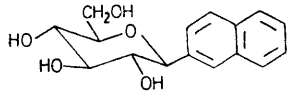


(VII) 235±59



(VI) 24±3

$K_i^+ / K_i^0 = 14$



(VIII) 8·2 ± 0·9

$K_i^+ / K_i^0 = 29$

β-Glucosidase from *Amygdalae dulces*. K_I values were measured in 0·10 M sodium acetate buffer, pH 5·2, at 25°, against 4-nitrophenyl *β*-D-glucopyranoside.

The anomeric configuration of compounds (I)—(VIII) was established by the splitting (*ca.* 8 Hz.) of the n.m.r. signal of the anomeric proton in the tetra-acetates. ^{13}C n.m.r. of the *C*-glycosides showed no acetal carbon. All compounds, and their tetra-acetates, gave satisfactory u.v. spectra and, if crystalline, elemental analyses. For inhibitors (II),[6] (V)[7], and (VI) correspondence with recorded literature properties was good. Compound (VI) could not be obtained crystalline, but was made from the crystalline tetra-acetate,[8] and gave one peak on column chromatography. Inhibition by *C*-glycosides (II) and (IV) was strictly competitive, and in the less precise measurements with compounds (V)—(VIII) an uncompetitive component of the inhibition could not be detected.

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[1] G. S. Case, M. L. Sinnott, and J.-P. Tenu, *Biochem. J.*, 1973, **133**, 99.
[2] M. L. Sinnott, *J.C.S. Chem. Comm.*, 1973, 535.
[3] M. L. Sinnott and S. G. Withers, *Biochem. J.*, 1974, **143**, 751.
[4] M. L. Sinnott and I. J. L. Souchard, *Biochem. J.*, 1973, **133**, 89.
[5] G. Legler and S. N. Hasnain, *Z. physiol. Chem.*, 1970, **351**, 25.
[6] Yu. A. Zhdanov, G. A. Korol'chenko, and G. N. Dorfeenko, *Doklady Akad. Nauk S.S.S.R.*, 1962, **143**, 852.
[7] R. U. Lemieux and A. R. Morgan, *Canad. J. Chem.*, 1965, **43**, 2205.
[8] C. D. Hurd and W. A. Bonner, *J. Amer. Chem. Soc.*, 1945, **67**, 1972.