Evidence for Catalysis other than Acidic in β-Galactosidase-catalysed Hydrolyses

By **Michael Maybury** and **Michael L. Sinnott,*** Department of Organic Chemistry, University of Bristol, BS8 1TS

Although acid-catalysed hydrolyses of t-butyl, 1,1-diethylpropyl, and diphenylmethyl β-D-galactopyranosides proceed by partial alkyl–oxygen fission, no products derivable from this process are detectable in the hydrolysis of these substrates catalysed by the wild-type β-galactosidase of *Escherichia coli*.

It has been shown that the conjugate acids of the β-D-galactopyranosides of 3-ethylpentan-3-ol, 2-methylpropan-2-ol, and diphenylmethanol react unimolecularly by both alkyl–oxygen and glycosyl–oxygen fission. These processes occur to a comparable extent.[1] As with most glycosidases,[2] there is considered to be some form of acidic catalysis acting on the exocyclic oxygen atom of the substrate during hydrolyses catalysed by the wild-type β-galactosidase of *Escherichia coli*.[3] If this is the only form of catalysis available on the enzyme surface, then alkyl–oxygen fission should also be observed in enzyme-catalysed hydrolyses of the three galactosides mentioned above. In the case of the 1,1-diethylpropyl galactoside, this would be manifested by the production of 3-ethylpent-2-ene; it could be detected in the cases of the other two glycosides by trapping of the cation with methanol. The accessibility of methanol to the active site of the enzyme is established.[4]

EXPERIMENTAL

The substrates have been described.[1] β-Galactosidase from Boehringer, Ltd., Uxbridge was obtained as a 0·5% slurry in ammonium sulphate. Hydrolyses were carried out in 0·100M-sodium phosphate buffer, pH 7·0, 1·0 mM in magnesium chloride, at 22°. The ammonium sulphate slurry was centrifuged, the supernatent discarded, and the enzyme dissolved in buffer before use. Enzyme substrate ratios of *ca.* 1 : 5 (w/w) were used, at substrate concentrations of *ca.* 10 mg/ml and reactions were carried out in sealed glass ampoules for 2—14 days. The hydrolyses of the diphenylmethyl and t-butyl glycosides were carried out in 10% (v/v) methanol. Some g.l.c. techniques have been described:[1] diphenylmethanol and diphenylmethyl methyl ether were separated on an 18 m SCOT OV-17 column at 180° (relative retention times 1·7 : 1). The volatility of 2-methylpropan-2-ol and methyl t-butyl ether precluded their successful g.l.c. separation from the extracting solvent, so analyses were carried out by direct injection of the aqueous methanolic solution onto a 1 m Phasepak column at 100°; this gave retention times (relative to methanol = 1) of 2·8 for 2-methylpropan-2-ol and 3·4 for its *O*-methyl derivative.

RESULTS AND DISCUSSION

All three glycosides were stable indefinitely in the buffer used. 1,1-Diethylpropyl β-D-galactopyranoside gave 100 ± 5% of 3-ethylpentan-3-ol and less than 0·05% 3-ethylpent-2-ene on β-galactosidase-catalysed hydrolysis. This contrasts with the 8% olefin formed on acid-catalysed hydrolysis.[1] Less than 1 and 0·1%, respectively, of the *O*-methyl derivatives of 2-methylpropan-2-ol and diphenylmethanol are formed in the enzymic hydrolysis of their galactosides in 10% (v/v) methanol. On the assumption that the trimethyl- and diphenyl-carbonium ions are effectively non-specific between water and methanol (*cf.* ref. 5), *ca.* 5% methoxy-compound is expected from complete alkyl–oxygen fission in the hydrolysis of the galactosides.

The aglycon binding site in β-galactosidase is hydrophobic;[6] reduction of solvent polarity would increase the proportion of olefin from the triethylcarbonium ion, and favour production of the highly delocalised diphenylcarbonium ion, *i.e.* increase the probability of observing products from alkyl–oxygen fission. Therefore the change in the cleavage site of these galactosides must be a reflection of the operation of other forms of catalysis than acidic in the enzyme-active site. These account for a rate acceleration of at least 10^2 of glycosyl–oxygen fission relative to alkyl–oxygen fission. This is not great, and could be caused by either conformational distortion of the pyranose ring, or the action of a nucleophile. It is, however, difficult to ascribe *selective* acceleration of glycosyl–oxygen fission to electrostatic stabilisation of a galactopyranosyl cation (*cf.* lysozyme[7]), since such stabilisation would also be available to only a slightly lesser extent to an alkyl cation, because of the variation of stabilisation energy with the inverse of the displacement between point charges.

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[1] D. Cocker, L. E. Jukes, and M. L. Sinnott, *J.C.S. Perkin II*, 1973, 190.
[2] B. Capon, *Chem.Rev.*, 1969, **69**, 407.
[3] M. L. Sinnott, *Biochem. J.*, 1971, **125**, 717.
[4] O. M. Viratelle, J. P. Tenu, J. Garnier, and J. Yon, *Biochem. Biophys. Res. Comm.*, 1969, **37**, 1036.
[5] L. C. Bateman, E. D. Hughes, and C. K. Ingold, *J. Chem. Soc.*, 1938, 881.
[6] F. Jacob and J. Monod, *J. Mol. Biol.*, 1961, **3**, 318.
[7] D. M. Chipman and N. Sharon, *Science*, 1969, **165**, 454.