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Labelling of the active centre of a β-glucosidase

An important method in the study of the amino acid side chains directly involved in enzymatic reactions is the covalent labelling of the active centre with compounds that react specifically at the desired position. For glycosidases no suitable compounds seem to have been described. Experiments with conduritol B epoxide (3,5/4,6-cyclohexenetetrol oxide) had indicated that this compound might be suitable for certain enzymes of this group.

Conduritol B epoxide reacts with β-glucosidases (β-D-glucoside glucohydrolase, EC 3.2.1.21) from different sources under specific, irreversible deactivation[1]. The investigation of this reaction with a pure β-glucosidase from *Aspergillus wentii*[2] showed a rapid deactivation with 10^{-4} M epoxide (half-life, 22 min at pH 3.5 and 25°). The pH dependence of the deactivation rate indicated the participation of an acidic group in the protonated form with $pK_s = 6.1$. $\bar{v}_{max}$ for the hydrolysis of different substrates showed a similar sigmoid pH dependence with $pK_s = 5.35$–5.55.

For the preparation of ^{14}C-labelled conduritol B epoxide radioactive *myo*-inositol was reacted with acetyl bromide and acetic acid to give a mixture of mono- and dibromocyclohexane polyol acetates[1,3]. These were deacetylated and separated by paper chromatography. The isolated (1,2,4/3,5,6)-6-bromocyclohexanepentol was converted to conduritol B epoxide by treatment with an aqueous suspension of silver oxide. Both the bromocyclohexanepentol and the epoxide were checked for purity by paper chromatography and co-crystallisation with authentic material. The epoxide obtained in this way had a specific activity of $0.82 \cdot 10^6$ counts/min per μmole.

Deactivation of the β-glucosidase A_3 (ref. 2) with the labelled epoxide gave a radioactive protein which was shown, after exhaustive dialysis, to have a specific activity of $5.4 \cdot 10^3$ counts/min per mg. From the molecular weight of 170 000 found by gel chromatography and osmotic pressure measurements an incorporation of 1.1 mole epoxide per mole enzyme could be calculated, partial deactivation gave a correspondingly smaller incorporation of radioactivity. With completely denatured enzyme no incorporation of radioactivity was observed. On treatment of the labelled enzyme with 0.5 M hydroxylamine at pH 9.6 (12 h at 37°) all of the radioactivity was released as a compound which was identified as (+)-inositol by paper chromatography and co-crystallisation with authentic material. Treatment of the labelled enzyme with $NaHCO_3$–Na_2CO_3 buffer of the same pH was without effect.

It may therefore be assumed that the deactivation of this β-glucosidase by conduritol B epoxide proceeds by an acid-catalysed *trans*-opening of the epoxide ring by a carboxylate ion to give an ester of (+)-inositol. If one assumes a similar mechanism for both this deactivation and glucoside hydrolysis, these observations would point to an acid catalysed cleavage of the glucosyl–oxygen bond with the formation of a glucosyl enzyme, in which the glucose is bound at C-1 to a carboxyl group. This acyl glucoside is then hydrolysed to free enzyme and glucose. An implication of this double displacement mechanism is retention of configuration at C-1 of the glucose, which is supported by the observation that during hydrolysis the glucose is initially released in the β-form[2]. Mechanisms of the double displacement type were originally suggested by KOSHLAND[4] to explain the retention of configuration with certain glucosidases and other enzymes. A mechanism similar to the one suggested here was recently proposed by PHILLIPS[5] for the action mechanism of lysozyme; he assumes, however, a carboxylate ion-stabilized glycosyl ion as intermediate instead of the glycosyl enzyme. A certain difficulty with the mechanism proposed here is the extremely low pK_s value required for the carboxylate group acting as glucosyl acceptor. No decrease of hydrolytic activity is observed even at pH 2.0.

A detailed account of this work will shortly be published[6].

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1 G. LEGLER, *Z. Physiol. Chem.*, 345 (1966) 197.
2 G. LEGLER, *Z. Physiol. Chem.*, 348 (1967) 1359.
3 G. E. MCCASLAND AND E. HORSWILL, *J. Am. Chem. Soc.*, 75 (1955) 4020.
4 D. E. KOSHLAND, JR., *Biol. Rev. Cambridge Phil. Soc.*, 28 (1953) 416.
5 D. C. PHILLIPS, *Sci. Am.*, 215 ,No. 5 (1966) 78.
6 G. LEGLER, *Z. Physiol. Chem.*, in the press.

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