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1 N. B. DAS, *Biochem. J.*, 31 (1937) 1116.
2 A. B. PARDEE AND V. R. POTTER, *J. Biol. Chem.*, 176 (1948) 1085.
3 D. V. DERVARTANIAN AND C. VEEGER, *Biochim. Biophys. Acta*, 92 (1964) 233.
4 T. Y. WANG, C. L. TSOU AND Y. L. WANG, *Sci. Sinica Peking*, 5 (1956) 73.
5 A. B. WOJTCZAK, in J. M. TAGER, S. PAPA, E. QUAGLIARIELLO AND E. C. SLATER, *Regulation of Metabolic Processes in Mitochondria*, BBA Library, Vol. 7, Elsevier, Amsterdam, 1966, p. 539.
6 L. WOJTCZAK, A. B. WOJTCZAK AND L. ERNSTER, *Abstracts Third Meeting of the Federation of European Biochemical Societies, Warsaw, 1966*, Academic Press, London, and PWN, Warsaw, 1966, p. 124.
7 D. V. DERVARTANIAN, W. P. ZEYLEMAKER AND C. VEEGER, in E. C. SLATER, *Flavins and Flavoproteins*, BBA Library, Vol. 8, Elsevier, Amsterdam, 1966, p. 183.

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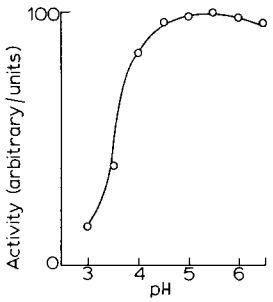
β-D-Xylosidase in pig kidney

It has recently been reported[1] that rat liver possesses β-xylosidase activity. Little is known of the distribution of this enzyme in other mammalian tissues, although highly active preparations have been demonstrated in bacteria, plants and fungi[2,3]. It is possible that the enzyme occurs in a number of organs where the cleavage of the link between xylose and serine in chondroitin sulphate–protein complexes may be an important step in the catabolism of the mucoid residues, but lack of a suitably sensitive method of assay has so far hampered investigation of this enzyme.

By using the fluorigenic substrate, 4-methylumbelliferyl β-D-xyloside[4], we have been able to detect and examine a β-xylosidase in pig kidney. The substrate (1 ml of 1 mM solution) in 0.2 M phosphate–citrate buffer is incubated with 1 ml of a 0.04% (w/v) homogenate of the tissue in water for 30 min before adding 3 ml of 0.5 M glycine–NaOH buffer (pH 10.4) to stop the reaction and develop the fluorescence of the liberated aglycone. Fluorescence was measured on a Locarte LF.5 fluorimeter at 440 mμ, (activation at 340–380 mμ).

Under these conditions the enzyme had a broad pH optimum of 5–6 (Fig. 1), the rapid loss of activity in more acid conditions being caused by irreversible denaturation. The working substrate concentration used does not allow complete saturation of the enzyme (Fig. 2), but higher concentrations are precluded by its low solubility.

It has been shown that the rat liver enzyme is sedimented in isotonic sucrose homogenates along with the acid phosphatase-rich particles, and the β-xylosidase activity is latent until released by detergent or hypotonic conditions[1]. If thus appears



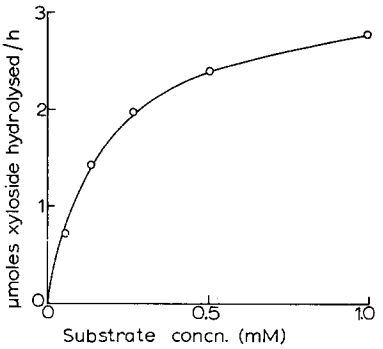


Fig. 1. Variation of pig kidney β-xylosidase with pH, assayed in phosphate–citrate buffer (0.2 M) with 0.5 mM 4-methylumbelliferyl β-D-xyloside as described in the text.

Fig. 2. Effect of substrate concentration on β-xylosidase activity, using the assay method described in the text.

to be a typical lysosomal enzyme. In the pig kidney, however, centrifugal fractionation of a 0.25 M sucrose homogenate left 97% of the β-xylosidase in the 240 000 × *g*·min supernatant, and no latency was observed (Table I). The lysosomal particles in these preparations were mainly intact as shown by the large amount of latent acid phosphatase activity which could be demonstrated in the particulate fraction and it is concluded that the β-xylosidase is normally present as a component of the cytoplasm that is not sedimented under these conditions.

All the preparations showed a considerable amount of β-glucosidase activity, from which the β-xylosidase could not be separated, either by DEAE-cellulose chromatography or by starch-gel electrophoresis. Both these enzymes were competitively inhibited by glucono-(1→4)-lactone, a specific inhibitor of β-glucosidase[5], and non-competitively by NaCl, the effect being pH dependent and most marked at pH 4.0 when 0.25 M NaCl produced 90% inhibition. These results suggest that in the pig kidney the β-glucosidase and β-xylosidases activities may be attributed to a single enzyme species of wide substrate specificity, namely β-D-glucoside glucohydrolase, EC 3.2.1.21. Almond emulsin has been shown to have 2 xylosidase components, both able to hydrolyse β-glucosides[1], while preparations of *Aspergillus oryzae* and „hemi-

TABLE I

CELLULAR DISTRIBUTION OF β-XYLOSIDASE IN PIG KIDNEY

Differential centrifugation of a 0.25 M sucrose homogenate of pig kidney followed by enzyme assay as described in the text. Latency (in parentheses) is the percentage of the activity in a given fraction that can only be estimated after alternately freezing and thawing the homogenate 10 times.

	% Total activity in each fraction		
	Nuclear (1600 × g·min)	*Lysosomes and mitochondria (240 000 × g·min)*	*Microsomes and supernatant (unsedimented)*
β-Xylosidase	0.2 (0)	2.9 (0)	96.9 (0)
β-Glucosidase	0.6 (17)	5.6 (0)	93.8 (0)
Acid phosphatase	14.3 (37)	51.0 (70)	34.7 (0)

cellulase" have been shown to contain specific β-xylosidases which can be separated from the β-glucosidase activity which is also present[6].

The function of β-xylosidase in the kidney requires investigation. It could, however, account for the production of acid mucopolysaccharides which occur in normal urine by releasing them from the protein complexes found in the plasma.

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1 D. FISHER, M. HIGHAM, P. W. KENT AND P. PRITCHARD, *Biochem. J.*, 98 (1966) 46P.
2 Y. MORITA, *J. Biochem. Tokyo*, 43 (1956) 7.
3 F. G. LOONTIENS AND C. K. DE BRUYNE, *Naturwiss.*, 19 (1963) 614.
4 C. K. DE BRUYNE AND F. G. LOONTIENS, *Naturwiss.*, 24 (1965) 661.
5 J. CONCHIE AND G. A. LEVVY, *Biochem. J.*, 65 (1957) 389.
6 F. G. LOONTIENS AND C. K. DE BRUYNE, *Naturwiss.*, 21 (1964) 509.

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Competitive inhibition of adenosine deaminase by urea, guanidine, biuret, and guanylurea

Many enzymes are inhibited by low concentrations of urea and guanidine[1–3]. The most extensive study is that of RAYAGOPALAN, FRIDOVICH AND HANDLER[1] who have studied a number of enzymes; according to their findings urea can be a competitive or a non-competitive or an uncompetitive inhibitor of different enzymes. When the inhibition is competitive 1 or 2 molecules of urea per molecule of enzyme are involved in the formation of enzyme–urea complexes.

Adenosine deaminase (EC 3.5.4.4) from calf intestinal mucosa is not irreversibly denatured by urea at concentrations lower than 6 M (ref. 4). However, urea at relatively low concentrations inhibits the enzyme activity. A kinetic analysis of the inhibition of adenosine deaminase by urea is reported in this paper. The inhibition of the enzyme is competitive and 2 molecules of urea are involved in the formation of an enzyme–urea complex. We have also observed that the enzyme is inhibited by guanidine, biuret, and guanylurea.

Adenosine deaminase was purified from calf intestinal mucosa[5]; enzymatic activity was determined spectrophotometrically according to KALCKAR[6].

The reversibility of the inhibition by the reagents assayed was demonstrated by means of a procedure whereby the enzyme was first exposed to a given concentration of inhibitor which was then lowered by dilution. Prolonged incubation (60 min) of the enzyme in 3 M urea or guanidine sulphate, as well as in 10 mM biuret or guanylurea sulphate, at pH 7.5 and 25° resulted in no detectable inactivation of the enzyme.