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## Lysosomal β-galactosidases of rat kidney

Fractionations of homogenates of rat liver[1] and various organs of the mouse[2] have provided evidence that β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) is a lysosomal enzyme. However, in rat kidney the enzyme has a bimodal distribution[3,4], being found in the lysosomal and supernatant fractions. This work shows that rat kidney lysosomal β-galactosidase is resolved by DEAE-cellulose column chromatography into four peaks of activity that have differences in $K_m$ values, inactivation by heat, inhibition by *p*-chloromercuribenzoate (PCMB) and ATP, and to some extent in their pH optima.

Kidney lysosomes were prepared according to the method of SHIBKO AND TAPPEL[5]. β-Galactosidase and α-glucosidase activities were measured as described elsewhere[3].

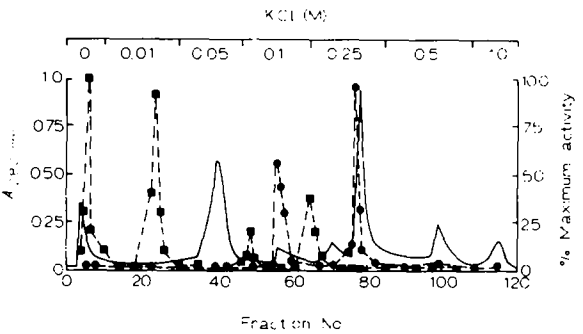



Fig. 1. Chromatography of soluble lysosomal fraction on DEAE-cellulose with 5 mM potassium phosphate buffer (pH 6.8). Protein ( ) was eluted stepwise with KCl as indicated. Maximum specific activity of β-galactosidase (■) was obtained in Fraction 6 in which the enzyme was purified 280-fold over the homogenate. The protein in fractions that contained β-galactosidase activity was pooled as follows (peak numbers, fraction numbers): I, 5–7; II, 23–25; III, 47–50; and IV, 63–67. α-Glucosidase (●) activity.

DEAE-cellulose column chromatography (Fig. 1) of a lysosomal fraction solubilized by freeze-thaw treatment separated β-galactosidase activity into four peaks. The distribution of total activity in these peaks was 31, 61, 2, and 6%, respectively, in Peaks I, II, III, and IV. α-Glucosidase activity was well separated from β-galactosidase activity. For further studies, pooled fractions from each of the peaks were used. $K_m$ values, obtained with *p*-nitrophenyl-β-D-galactopyranoside as substrate, were determined from Lineweaver Burk plots and were 6.7, 6.5, 3.4, and 0.25 mM, respectively, for Peaks I, II, III, and IV. These $K_m$ values show that the β-galactosidases of Peaks I and IV apparently differed in their affinity for the substrate. All the activities were inhibited by substrate concentrations greater than 17 mM. The pH profiles of Peaks I and IV and the soluble lysosomal fraction are shown in Fig. 2. The pH activity curves for Peaks I and IV are similar above pH 4.5, but they are markedly different below pH 4.5. The pH activity curve for Peak II was similar to that of Peak I; that of Peak III showed more resemblance to Peak IV.

Abbreviation: PCMB, *p*-chloromercuribenzoate.

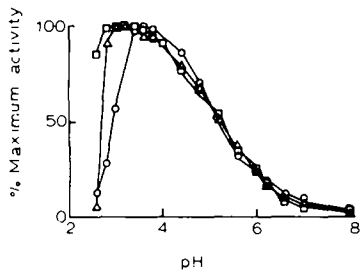
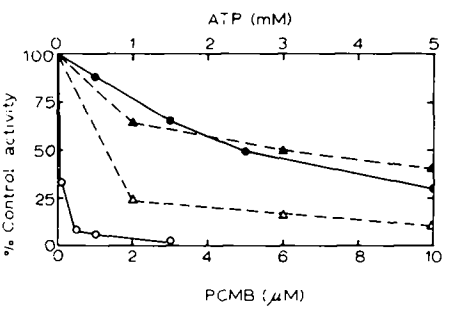



Fig. 2. $\beta$-Galactosidase activity as a function of pH. Substrate used was 10 mM $p$-nitrophenyl-$\beta$-D-galactopyranoside in 80 mM citrate–phosphate buffer. □, soluble lysosomal fraction; △, Peak I; ○, Peak IV.

Fig. 3 shows the inhibition of the $\beta$-galactosidase activities of Peaks I and IV by ATP and PCMB. It is seen that the activity in Peak I was more strongly inhibited than the activity in Peak IV. Fig. 4 shows that more than 80% of the activity in Peak I was inactivated by a 10-min heat treatment at 65° (pH 3.6), while the same treatment caused only 30% inactivation of the activity in Peak IV. Inactivation by heat and inhibition by PCMB and ATP of the activities in Peaks II and III were at levels intermediate between those obtained for Peaks I and IV. Glucono(1–4)-lactone and galactono(1–4)lactone inhibited all four of the activities with no significant differences among the inhibition patterns. All four peaks hydrolyzed lactose.



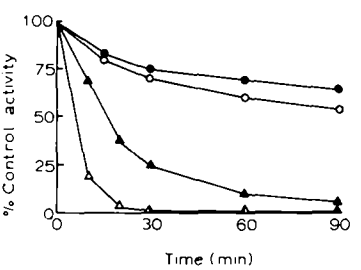




Fig. 3. Inhibition of $\beta$-galactosidase activity by ATP (triangles) and PCMB (circles). The reaction mixtures contained enzyme and the inhibitors in 80 mM citrate-phosphate buffer (pH 3.6) and were incubated at 37° for 10 min prior to addition of the substrate. Open symbols, Peak I; solid symbols, Peak IV.

Fig. 4. Inactivation of $\beta$-galactosidase activity at 55° (circles) and 65° (triangles). The reaction mixtures contained protein from either Peak I (open symbols) or Peak IV (solid symbols) in 10 mM citrate-phosphate buffer (pH 3.6). Samples were taken at intervals and were immediately cooled in ice water (0°); the enzyme assays were done at 37°.

The results described here show that the $\beta$-galactosidase activity of rat kidney lysosomes has at least two components that differ considerably in some of their properties. From rat small intestine, ASP AND DAHLQVIST[6] separated "acidic" (pH 3.4) $\beta$-galactosidase activity into two components which differed in their kinetic properties. Multiple forms of other lysosomal enzymes have been demonstrated[7,8].

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1 O. Z. SELLINGER, H. BEAUFAY, P. JAQUES, A. DOYEN AND C. DE DUVE, *Biochem. J.*, 74 (1960) 450.
2 J. CONCHIE AND G. A. LEVVY, *Biochem. Soc. Symp.*, 23 (1963) 86.
3 V. PATEL AND A. L. TAPPEL, *Biochim. Biophys. Acta*, 191 (1969) 653.
4 R. G. PRICE AND N. DANCE, *Biochem. J.*, 105 (1967) 877.
5 S. SHIBKO AND A. L. TAPPEL, *Biochem. J.*, 95 (1965) 731.
6 N. G. ASP AND A. DAHLQVIST, *Biochem. J.*, 110 (1968) 143.
7 B. V. PLAPP AND R. D. COLE, *Biochemistry*, 6 (1967) 3676.
8 M. IGARASHI AND V. P. HOLLANDER, *J. Biol. Chem.*, 243 (1968) 6084.

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## Role of histidine in the active site of alkaline protease from *Aspergillus flavus* derived from kinetic data

In the preceding paper[1] we reported the specificity of the alkaline protease from *Aspergillus flavus* which contains an active serine residue. From the results of our experiments with the cleavage of the B-chain of oxidized insulin and of certain synthetic substrates we have been able to conclude that the protease[2] isolated by us belongs to the group of serine proteases with a broad specificity[3]. Another protease which falls into this group is subtilisin which has the same amino acid sequence around the serine active center[4,5] as our protease. The aim of this paper is to report the determination of kinetic constants and the effect of pH and temperature on the catalysis of the hydrolysis of *N*-benzoyl-L-arginine ethyl ester (BAEE) by our protease. From the results of these experiments the role of histidine in the active site can be deduced.

*Material.* The protease preparation used for the hydrolysis was obtained by the method described elsewhere[2] with the exception that the protease was precipitated first from the fraction obtained by chromatography on DEAE-Sephadex in acetone (−10°; 1:1, v/v) and then, after having been dissolved in water, lyophilized. The preparation thus obtained showed the presence of only one N-terminal group (glycine) and was free from low molecular weight peptide material. Chromatographically pure *N*-benzoyl-L-arginine ethyl ester hydrochloride was purchased from Fluka, A.G. (Buchs, SG; A51073, substrate for trypsin determination).

*Determination of esterase activity.* The rate of hydrolysis of the substrate was determined in a Model TTT1a pH-stat (Radiometer, Copenhagen) equipped with a titrating device (TTA31) and a temperature-controlled reaction vessel. The temperature of the reaction mixture in the vessel was kept constant with an accuracy of ± 0.1°. The volume of the reaction mixture was 2 ml. All solutions were in 0.1 M KCl

Abbreviations used: BAEE, *N*-benzoyl-L-arginine ethyl ester; ATEE, acetyl-L-tyrosine ethyl ester.