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PURIFICATION AND PROPERTIES OF LACTASE FROM MONKEY KIDNEY

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SUMMARY

1. Lactase* activity is present in the particulate fraction obtained upon centrifugation of a homogenate of monkey kidney at 100 000 × *g* for 60 min and could be solubilized by treatment with papain. The solubilized enzyme has been purified about 60-fold, and its properties have been studied.

2. Lactose was the most active among a variety of substrates tested. Cellobiose and salicin showed, respectively, 20 and 16% of the activity using lactose. Other hetero-β-glucosides and -β-galactosides showed negligible activity. The purified lactase is strongly inhibited by glucono-(1→5)-lactone, while galactone-(1→4)-lactone inhibited to a much smaller extent.

INTRODUCTION

Previous studies[1] on the disaccharidases of monkey intestine and kidney showed that maltase, invertase and trehalase activities of kidney were much lower than those of the intestinal mucosa. It was subsequently found that monkey kidneys possess a powerful lactase* activity which is comparable to that of the intestine[2]. The possible presence of lactase in the rat kidney has been shown by homogenate assay[3] or by histochemical procedures[4]. However, in a recent report BINKLEY AND KING[5] were unable to demonstrate the presence of lactase in the rat kidney, either in a homogenate or in the isolated subcellular fractions. The presence of a powerful lactase activity in the monkey kidney prompted us to isolate the enzyme and to study its characteristics which are reported in the present paper.

MATERIALS AND METHODS

Chemicals

p-Nitrophenyl-β-D-glucoside, *p*-nitrophenyl-β-D-galactoside, *o*-nitrophenyl-β-

* To avoid confusion in nomenclature the authors prefer to use the term 'lactase' instead of 'β-galactosidase' (β-D-galactoside galactohydrolase, EC 3.2.1.23). Lactase is essentially a disaccharidase. It has little activity towards commonly employed hetero-β-galactosides and hetero-β-glucosides, acts on cellobiose and is strongly inhibited by gluconolactone. Also, in intestine there are two other hetero-β-galactosidases distinguishable from lactase and one of them has no action on lactose at all.

galactoside, 6-bromo-2-naphthyl-β-D-galactoside, 6-bromo-2-naphthyl-β-D-glucoside, salicin (saligenin-β-D-glucoside), β-methylglucoside, glucose oxidase, glucono-δ-lactone, galactono-γ-lactone, peroxidase, *o*-dianisidine and 6-bromo-2-naphthol were purchased from Sigma Chemical Co. Other chemicals were obtained as indicated below. Lactose and glucose (British Drug Houses), Triton X-100 was a gift from Rohm and Haas; 3,5-dinitrosalicylate (E. Merck), crystalline papain (Mann), diazonapthanil blue B (Dajac Labs., U.S.A.) and Sephadex G-200 and DEAE-Sephadex (Pharmacia, Sweden).

Methods

Lactase activity using lactose as substrate and hetero-β-galactosidase and -β-glucosidase activities using nitrophenyl-substituted sugars were determined as mentioned earlier[2] at pH 5.5. When 6-bromo-2-naphthylglycosides were used 6-bromo-2-naphthol formed was estimated according to the procedure of DAHLQVIST *et al.*[6]. Salicin hydrolysis was measured by following the formation of reducing sugar using 3,5-dinitrosalicylate reagent of SUMNER[7].

Enzyme unit. One unit of enzyme activity is defined as the amount of enzyme required to hydrolyze 1 μmole of substrate per min at 37°. The specific activity is expressed as enzyme units/mg protein.

Protein. Protein was determined according to the procedure of LOWRY *et al.*[8].

RESULTS

Purification of kidney lactase

Preparation of homogenate. It was initially found that the specific activity of lactase was about the same in both the medulla and cortex regions of the kidney. Hence, for the following purification procedure whole kidneys were used as such.

Kidneys obtained from adult monkeys of the *Makac mullata* or *Makac radiata* species were collected after sacrifice under Nembutal anesthesia and were frozen at −20° until used. The frozen kidneys were thawed and homogenized with 4 vol. of 0.01 M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 100 000 $\times g$ for 60 min. The bulk of the lactase activity (over 90%) and a fraction (25%) of the hetero-β-galactosidase activity were present in the sediment fraction. The supernatant was discarded, and the sediment was suspended in 0.01 M sodium phosphate buffer (pH 7.0) and was made up to the original volume of the homogenate.

Solubilization with papain. The particulate kidney lactase was solubilized by papain as employed for intestinal lactase[2,9–11]. To the particulate fraction suspended in 0.01 M sodium phosphate buffer (pH 7.0) was added crystalline papain to give a ratio of homogenate protein to papain of 200:1. β-Mercaptoethanol was added to give a final concentration of 0.01 M, and the suspension was incubated at 37° for 2 h with constant shaking. The solution was centrifuged at 100 000 $\times g$ for 60 min. The supernatant fraction was dialyzed against 0.005 M sodium phosphate buffer (pH 7.0) for 36 h, with several changes of the same buffer. The contents of the bag were lyophilized and dissolved in a small volume of water (volume of fraction, 6.0 ml). The undissolved material was removed by centrifuging at 10 000 $\times g$ for 15 min.

Gel filtration on Sephadex G-200. An aliquot of the lyophilized fraction (5.3 ml) was loaded on a column of Sephadex G-200 (2.2 cm × 27 cm) equilibrated with 0.01 M

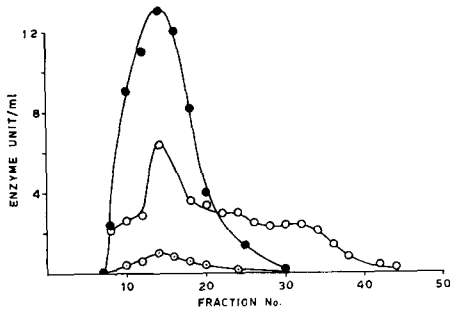


Fig. 1. Elution profile of kidney lactase on Sephadex G-200. 3 ml fractions were collected. The void volume as determined by use of blue dextran was 36 ml. ●—●, lactase; ○—○, hetero-β-galactosidase; ○—○, protein.

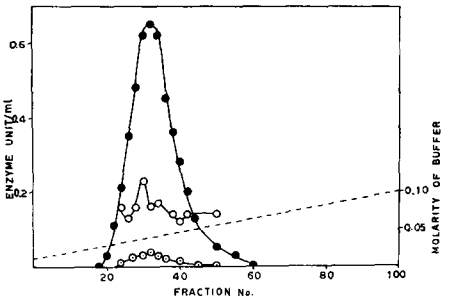


Fig. 2. Elution profile of kidney lactase on DEAE-Sephadex A-50. The linear gradient was obtained by use of two chambers of the Varigrad device containing 150 ml in each of 0.01 M and 0.1 M potassium phosphate buffer, respectively. 3-ml fractions were collected. ●—●, lactase; ○—○, hetero-β-galactosidase; ○—○, protein.

sodium phosphate buffer (pH 7.0) and was eluted with the same buffer. Lactase activity emerged as a single peak near the void volume (Fig. 1) and the active fractions were pooled.

Fractionation on DEAE-Sephadex. The pooled fractions were charged on a column of *DEAE*-Sephadex A-50 (1 cm × 19 cm) equilibrated with 0.01 M sodium phosphate buffer (pH 7.0) and washed with 3 bed vol. of the same buffer. The proteins were then eluted out with a linear sodium phosphate buffer gradient (0.01–0.10 M) using the Varigrad device (Technicon). The enzyme was eluted between 0.025–0.045 M buffer concentration (Fig. 2). The active fractions were pooled and used for further studies. The enzyme was thus purified about 60-fold with a recovery of 16% (Table I). It is also noted that the ratio of lactase to hetero-β-galactosidase activity increases during purification, reaching almost a constant value at the final stages.

Properties of the purified lactase

Substrate specificity. The purified enzyme acted on lactose, cellobiose and salicin

TABLE I

PURIFICATION OF LACTASE FROM MONKEY KIDNEY

Fraction	*Vol. (ml)*	*Lactase (total units)*	*Protein (mg)*	*Specific activity (units/mg protein)*	*Recovery (%)*	*Ratio**
Sediment 100 000 × *g*	200	86	3220	0.027	100	1.5
Papain digest	200	74	392	0.189	86	7.8
Sephadex G-200	40	33.2	134	0.248	45	14.0
DEAE-Sephadex (pooled fractions)	48	13.4	8.2	1.640	16	14.7

* Refers to the ratio of lactase to hetero-β-galactosidase activity measured using lactose and *o*-nitrophenyl-β-galactoside as substrates, respectively. Assay procedures as described earlier[1,2].

TABLE II

SUBSTRATE SPECIFICITY

The assay mixtures consisted of buffer, pH 5.5, purified enzyme and substrate (saturating concentration in each case as indicated) and the rest of the procedure as described in text. Under these conditions β-methylglucoside, melibiose and gentiobiose were not hydrolyzed.

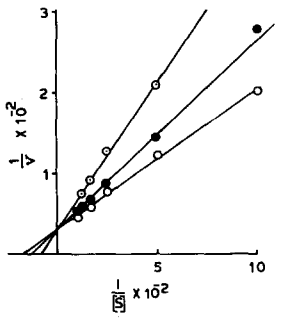
Substrate	*Final concn. (mM)*	*Relative activity (%)*
Lactose	50	100
Cellobiose	50	20.0
Salicin	50	16.2
o-Nitrophenyl-β-galactoside	10	8.9
p-Nitrophenyl-β-galactoside	10	6.3
p-Nitrophenyl-β-glucoside	10	6.3
6-Bromo-2-naphthyl-β-galactoside	0.2	0.1
6-Bromo-2-naphthyl-β-glucoside	0.2	0.1

(Table II). The nitrophenylgalactose and nitrophenylglucose derivatives were much less active while bromonaphthyl analogues showed very little activity. β-Methylglucoside, melibiose and gentiobiose were not hydrolyzed under the same conditions.

K_m values. The K_m value with lactose as substrate was $2 \cdot 10^{-2}$ M and with *o*-nitrophenyl-β-galactoside, $8 \cdot 10^{-3}$ M though the rate of hydrolysis of *o*-nitrophenyl-β-galactoside was only 1/15th of that of lactose under saturating conditions (Table II). Lactose competitively inhibited *o*-nitrophenyl-β-galactoside hydrolysis (Fig. 3). The K_m for lactose hydrolysis was similar to the K_i of the lactose inhibition of *o*-nitrophenyl-β-galactoside. The inhibition of lactose hydrolysis by a nitrophenyl-β-galactoside was not studied because of interference with the glucose estimation by the glucose oxidase procedure.

pH optima. The pH optimum for the hydrolysis of lactose was 5.5 (Fig. 4).

Inhibition by lactones. Gluconolactone was a powerful inhibitor of lactase activity as compared to galactonolactone. At a concentration of 0.001 M, the former inhibited the activity by 77% while the latter inhibited it only by 34% (Table III).



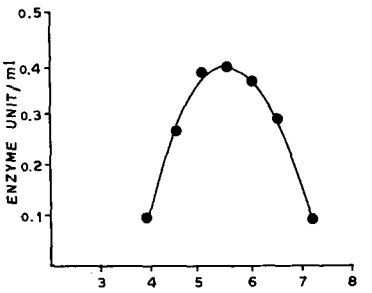


Fig. 3. Competitive inhibition between lactose and *o*-nitrophenyl-β-galactoside. The effect of lactose on the hydrolysis of *o*-nitrophenyl-β-galactoside was studied. The assay conditions as cited in text. ○—○, with *o*-nitrophenyl-β-galactoside alone; ●—●, with *o*-nitrophenyl-β-galactoside + lactose (10 mM); ○—○, with *o*-nitrophenyl-β-galactoside + lactose (20 mM).

Fig. 4. pH optimum for lactose hydrolysis. Assay conditions as cited in text. 0.1 M citrate–0.2 M phosphate buffer of the indicated pH was used throughout.

TABLE III

INHIBITION OF MONKEY KIDNEY LACTASE BY GLUCONO- AND GALACTONOLACTONES

The standard reaction mixture in a total volume of 0.2 ml contained lactose (10 μmoles), acetate buffer, pH 5.4 (10 μmoles), enzyme and lactone at the indicated final concentration. The percentage inhibition was calculated with respect to control tubes in which lactone was omitted.

Lactone concn. (mM)	*Inhibition (%)*	
	Glucono-lactone	*Galactono-lactone*
0.1	26	13
1.0	77	34

DISCUSSION

Monkey kidney possesses a lactase activity which is comparable to that found in the intestinal mucosa[2]. The kidney enzyme is also in the particulate fraction and can be solubilized by papain digestion. A solubilized kidney preparation has been partially purified about 60-fold, and its properties have been studied.

Although the small intestine has been recognized as the main site of hydrolysis of dietary lactose, evidence for the likely presence of lactase in other tissues are available. For instance the presence of lactase has been demonstrated in rat liver by LANGLEY[21] and its possible presence in kidney[4] by histochemical techniques. While DAHLQVIST AND BRUN[3] reported lactase activity in rat kidney homogenates, BINKLEY AND KING[5] could not show its presence.

The purified lactase from monkey kidney behaved like a true disaccharidase, as demonstrated by its substrate specificity. It acted on hetero-β-galactosides and -β-glucosides only at a small rate, while lactose, cellobiose and salicin were the most effective substrates in that order. This enzyme is different from the kidney β-galactosidases studied by ROBINSON *et al.*[12]. They separated two hetero-β-galactosidases by gel filtration of a soluble fraction of rat kidney homogenate on Sephadex columns. The enzyme in the present study was particulate and was solubilized by treatment with papain. The ratio of lactase activity to the hetero-β-galactosidase activity increased during purification and attained almost a constant value in the final stages. Further we have shown earlier[2] that the rate of heat inactivation at 55° of kidney hetero-β-galactosidase was much faster than that of lactase showing that hydrolysis of lactose is catalyzed by a separate enzyme.

The kidney lactase resembles the intestinal enzyme in several respects. Like the intestinal lactase the kidney enzyme is also particulate, hydrolyzes lactose predominantly, shows only very little hetero-β-glucosidase or -β-galactosidase activity. The kinetic properties also closely parallel the intestinal enzyme (N. SWAMINATHAN AND A. N. RADHAKRISHNAN, unpublished results). It is also interesting to note that the lactase to cellobiase ratio is the same as reported for the intestinal lactase[2,10]. It has been well established that intestinal lactase is a brush border enzyme. The exact subcellular localization of kidney lactase is not yet determined.

The inhibition of glycosidase activities by corresponding aldonolactones has

been studied by CONCHIE *et al.*[13,14]. Thus, in general, β-glucosidases were inhibited by gluconolactones and β-galactosidases by galactonolactones. Both lactones were inhibitory when the enzyme exhibited β-glucosidase as well as β-glactosidase activity. In the present study with the purified enzyme it was found that both lactones were inhibitory*. Gluconolactone was more inhibitory than galactonolactone. This would indicate that the enzyme is active towards both β-glucosides and β-galactosides as evidenced from the substrate specificity. A similar observation has also been made with almond emulsin[14], plant[15] β-galactosidases and monkey intestinal lactase (N. SWAMINATHAN AND A. N. RADHAKRISHNAN, unpublished results).

The functional significance of this enzyme in the kidney poses interesting questions. The site of dietary disaccharide hydrolysis has been shown to be the intestinal mucosa. Under normal physiological conditions only negligible amounts of unhydrolyzed disaccharide enters the blood. Parenterally injected lactose is largely excreted in the urine[16–18]. However, in some pathological states, like malabsorption syndrome, in which a deficiency of intestinal disaccharidases has been demonstrated, intact disaccharides are excreted in the urine[19–20]. The kidney lactase can then be visualized as playing an important role in the hydrolysis of a portion of lactose in the blood. On the other hand, the kidney lactase normally may have a function in the hydrolysis of more complex glucoside or galactoside compounds which are physiologically important. Hydrolytic removal of galactose from α_1-acid glycoprotein by liver lactase fraction has recently been shown[21]. This preparation does not act on *o*-nitrophenyl-β-galactoside.

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* After submission of this manuscript the paper by MALATHI AND CRANE[22] appeared. They have also shown that the hamster intestinal lactase and cellobiase activities are affected by both glucono- and galactonolactones.

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