The Separation and Characterization of Marmoset Kidney β -D-Galactosidase and β -D-Glucosidase

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β -D-Galactosidase and β -D-glucosidase activities were determined in homogenates of marmoset kidney by using the appropriate 4-methylumbelliferyl glycoside. β -D-Galactosidase activity was separated into two main components by ion-exchange chromatography on DEAE-cellulose, starch-gel electrophoresis, isoelectric focusing and gel filtration on Sephadex G-200. One form designated A had a pI of 5.1, was loosely bound to DEAE-cellulose at pH 7.0, remained near the origin on starch-gel electrophoresis at pH 7.0 and had an apparent molecular weight of 160 000. The second β -D-galactosidase component, designated B, was associated with the total β -D-glucosidase activity, had a pI of 4.3, was firmly bound to DEAE-cellulose, migrated rapidly towards the anode on starch-gel electrophoresis and had an apparent molecular weight of 50 000. The optimum pH values of β -D-galactosidase A and B were 4.5 and 6.0 respectively. β -D-Galactosidase A was activated by 0.1 M-NaCl but the activity of the B form was inhibited by 1 M-NaCl at pH 4.5. β -D-Galactosidase had a bimodal distribution, the A form being recovered in the lysosomal fraction whereas the B form was present in the soluble fraction, as was the major portion of the β -D-glucosidase activity. The lysosomal and soluble forms were further characterized by DEAE-cellulose chromatography.

Interest in β -D-glucosidase and β -D-galactosidase has been stimulated by the finding that both these enzymes have elevated activities in the urine of rats during kidney damage (Ellis & Price, 1975) and that their excretion can be used for the detection of renal damage and in early warning of renal-transplant rejection (Wellwood *et al.*, 1973). A further understanding of the biochemical characteristics of the enzymes in the kidney is an essential prerequisite to the understanding of the changes occurring in renal damage. A comparative study of marmoset kidney enzymology was undertaken as part of a wider investigation of the marmoset as a model for kidney damage and disease with particular reference to drug testing. Since it is a primate, the marmoset is possibly a more relevant model for man than are other laboratory animals such as rodents, and further, because of its small size, is more easily handled than other primates.

Multiple forms of β -D-glucosidase and β -D-galactosidase activities have been described in a variety of species, including rat kidney (Price & Dance, 1967; Glew *et al.*, 1976), pig kidney (Price &

Robinson, 1966; Abrahams & Robinson, 1969), human kidney (Dance *et al.*, 1969), human liver (Ho & O'Brien, 1969) and monkey (*Macaca mulatta*) liver and intestine (Swaminathan & Radhakrishnan, 1969).

Two major forms of β -D-galactosidase activity are present in all the species studied. One form, sedimenting in the lysosomal fraction, had no β -D-glucosidase activity. A second form with a neutral pH optimum was found in the soluble fraction and was active towards β -D-glucoside substrates. The lysosomal acid β -D-galactosidases are able to hydrolyse the terminal β -D-galactoside residue from GM₁ ganglioside (Ho *et al.*, 1973). These activities are absent in GM₁ gangliosidosis, which is an inherited disease characterized by the accumulation of GM₁ ganglioside (Okada & O'Brien, 1969). The soluble-fraction enzyme has a broad specificity, and in the rat kidney is active towards β -D-galactoside, β -D-glucoside, β -D-fucoside and α -L-arabinoside (Glew *et al.*, 1976). In the present paper we report some investigations of the isoenzymic forms of β -D-galactosidase and β -D-glucosidase in marmoset

kidney separated on the basis of charge, molecular size and subcellular distribution, and their properties are further characterized.

Materials and Methods

Tissues

Common marmosets (*Callithrix jacchus*) bred at I.C.I. Pharmaceuticals Ltd., Alderley Park, Cheshire, U.K., were killed under diethyl ether anaesthesia and their kidneys removed and either frozen at -20°C or used immediately for subcellular studies. Frozen tissue was homogenized with a hand-rotated Potter-Elvehjem homogenizer with a close-fitting pestle (diameter clearance 0.25 mm). Homogenates (5%, w/v) were prepared in water and were then centrifuged at 37000g for 30 min in an MSE18 centrifuge with an 8×50 ml angle head (r_{av} , 4.2 cm). The pellet was discarded and the supernatant was then used in subsequent studies.

Subcellular fractionation

Tissue homogenates (5%, w/v) were made from freshly removed kidneys by using a hand-rotated Potter-Elvehjem homogenizer (diameter clearance 0.4 mm) in 0.45 M-sucrose containing 1 mM-EDTA and adjusted to pH 7.4 with 1 M-NaOH. The homogenate was filtered through two layers of muslin and centrifuged at 4°C in an MSE18 centrifuge in an 8×50 ml angle rotor for 2 min at 1000g, 1 min at 10000g and 20 min at 12000g to sediment the nuclear (N), lysosomal-mitochondrial (LM) and mitochondrial-microsomal (MM) fractions. The resulting supernatant was then centrifuged for 1 h at 75800g in an MSE 25 centrifuge with an 8×50 ml angle rotor (r_{av} , 4.2 cm) to sediment the microsomal (Mic) fraction. The final supernatant was termed the soluble (S) fraction. The sediments produced at each stage were resuspended in 10 ml of 0.45 M-sucrose containing 1 mM-EDTA, pH 7.4. Marker enzymes assayed were acid phosphatase (EC 3.1.3.2), succinate-neotetrazolium reductase (EC 1.3.99.1) and glucose 6-phosphatase (EC 3.1.3.9) for lysosomes, mitochondria and microsomal fraction respectively (Price & Dance, 1967).

Glycosidase assays

β -D-Galactosidase (EC 3.2.1.23) and β -D-glucosidase (EC 3.2.1.21) activities were assayed by the following method. The enzyme preparation (0.2 ml) was added to 0.2 ml of 1 mM-4-methylumbelliferyl β -D-galactopyranoside or glucopyranoside (Koch-Light Laboratories, Colnbrook, Bucks., U.K.) dissolved in 0.1 M-sodium phosphate/0.05 M-citric acid buffer, pH 4.5 or 6.0. The mixture was incubated

at 37°C for 15 or 30 min depending on the activity of the enzyme preparation and the reaction terminated by the addition of 0.5 M-glycine/NaOH buffer, pH 10.4 (1.6 ml). Fluorescence was measured at 440 nm by using a Locarte LF 5 fluorimeter with an F2 filter on the excitation side. Alternatively β -D-galactosidase and β -D-glucosidase activities were measured by using a modification of the automated assay developed by Tucker *et al.* (1975) for the assay of *N*-acetyl- β -D-glucosaminidase.

In determinations of pH optima, 2 mM-substrate solutions in water were prepared and diluted 1:1 (v/v) with 0.2 M- NaH_2PO_4 /0.2 M- Na_2HPO_4 or sodium phosphate/citric acid buffers of the appropriate pH range.

The latency of enzyme activities in the subcellular fractions was assayed at 25°C for 10 min. Substrate solutions in 0.45 M-sucrose in the appropriate buffer were used for measurement of free activity, and in 0.45 M-sucrose plus 0.1% Triton X-100 for assay of the total activity.

Gel filtration

Gel filtration was carried out in a column ($40\text{ cm} \times 2.5\text{ cm}^2$ diam.) of Sephadex G-200 [fine grade, Pharmacia (G.B.) Ltd., London, W.5, U.K.] equilibrated with 10 mM-sodium phosphate buffer, pH 6.0. The column was calibrated by the method of Andrews (1965) by using Blue Dextran, bovine serum albumin, urease, horseradish peroxidase [Sigma (London) Chemical Co., Kingston, Surrey, U.K.] and cytochrome *c* (BDH, Poole, Dorset, U.K.) as molecular-size markers. The loading and elution of enzyme samples (1.0 ml) was carried out at 4°C with a flow rate of 42 ml/h. Fractions (3.5 ml) were collected with an LKB fraction collector.

Ion-exchange chromatography

Supernatants (1 ml) prepared from whole marmoset kidney homogenates were dialysed against 1 litre of 10 mM-sodium phosphate buffer, pH 7.0, for 2 h and then applied to DEAE-cellulose (Whatman DE 23; Whatman Chemical Co., Maidstone, Kent, U.K.) previously packed at 4°C into the barrel of 10 ml disposable syringes containing a porous Vyon disc [Portland Plastics (Portex), Hythe, Kent, U.K.]. Buffer (10 mM-sodium phosphate, pH 7.0) was pumped on to the column at 60 ml/h and ten fractions (5 ml) were collected before elution with the same buffer containing a 200 ml linear concentration gradient of NaCl up to 0.3 M.

Isoelectric focusing

The enzyme preparation (0.2–0.5 ml of the supernatant from a whole kidney homogenate) was placed

in one of the two central fractions of a sucrose density gradient of 24 steps (0–1.17 M) containing Ampholines giving a pH range of 3.5–10 or of 4–6. The gradient was contained in an LKB 8100 isoelectric-focusing column (capacity 110 ml) used as described by Vesterberg & Svensson (1966). Focusing was for 48 h at 600 mW and 4°C. The column contents were then run out at 60 ml/h and collected in 1.4 or 2.5 ml fractions.

Starch-gel electrophoresis

Starch gels were prepared from hydrolysed starch (Connaught Medical Research Laboratories, Toronto, Canada) suspended in 4 mM-sodium phosphate buffer, pH 7.0, as described by Gammack *et al.* (1960). Electrophoresis was carried out for 4 h at 200 V and 20 mA and the temperature maintained at 4°C. Enzyme bands were detected by applying either 4-methylumbelliferyl- β -D-glucoside (1 mM) in sodium phosphate buffer, pH 6.0, or 4-methylumbelliferyl- β -D-galactoside (1 mM) in sodium phosphate/citric acid buffer, pH 4.5, to Whatman no. 1 filter paper on the cut surface of the gel and incubating at 37°C. Fluorescent bands were visible after about 10 min and were intensified by placing filter paper soaked in 0.5 M-glycine/NaOH buffer, pH 10.4, on the gel surface. Alternatively after electrophoresis the gel was sliced into 0.25 cm pieces from the sample origin. These pieces were then suspended in 2 ml of water, left overnight at 40°C, centrifuged at 800g for 5 min and the enzyme activity was assayed in the supernatant.

Protein determination

Protein was measured by the Folin method (Lowry *et al.*, 1951), with bovine serum albumin as standard.

Results

The pH-activity curves for β -D-galactosidase and β -D-glucosidase are shown in Fig. 1. The peak of β -D-galactosidase activity occurs at pH 4.5–5.0, and there is a pronounced shoulder on the activity curve between pH 6 and pH 7, above which the activity decreases more rapidly. The curve for β -D-glucosidase, in contrast, shows a single peak of activity, with a maximum at pH 6–6.5. Subsequent assays were performed at pH 6 for β -D-glucosidase and at pH 4.5 for β -D-galactosidase. Mean specific activities for these enzymes in the kidney were 1.1 μ mol/h per mg of protein (30 μ mol/h per g wet wt. of tissue, $n = 3$) for β -D-glucosidase and 1.3 μ mol/h per mg of protein (35 μ mol/h per g wet wt. of tissue, $n = 3$) for β -D-galactosidase.

Fig. 1. pH-activity curves of marmoset kidney β -D-glycosidases

Activity was assayed as described in the text. Δ , β -D-Glucosidase (0.1 M-sodium phosphate/0.05 M-citric acid buffer); $\blacktriangle$, β -D-glucosidase (0.1 M-sodium phosphate buffer); $\square$, β -D-galactosidase (sodium phosphate/citric acid buffer); $\blacksquare$, β -D-galactosidase (sodium phosphate buffer).

Gel filtration

Profiles of β -D-galactosidase and β -D-glucosidase activities eluted from Sephadex G-200 in the presence and absence of NaCl are shown in Figs. 2(a) and 2(b). When β -D-galactosidase was assayed at pH 4.5 in the fractions from the column run in the presence of 5 mM-NaCl a broad peak of activity was recovered. The peak fractions (peak 2) corresponded to a molecular weight of about 50 000. When the NaCl concentration of the buffer was increased to 1 M a change in pattern occurred which might be due to aggregation. This could also be the result of decreased interaction with the Sephadex gel, since 50 mM-NaCl is required to suppress the ion-exchange capacity of the Sephadex (Andrews, 1970). A major peak occurred with an apparent molecular weight of 160 000 (I) but was very broad. The assay of the fractions for β -galactosidase at pH 6.0 in fact resolved this peak into two components, one having the same molecular weight as peak I and the other coinciding with the peak of β -D-glucosidase activity (peak II). Fractions from columns run in the absence of salt showed a sharp fall in β -D-galactosidase activity (Fig. 2b) and a change in elution profile. A small peak occurred at a molecular weight of 160 000 (peak I), and after a gradual decline in activity, a

slightly larger peak (III) was reached, thus corresponding to the peak of β -D-glucosidase activity in the absence of NaCl (Fig. 2a, peak III). When 5 mM-NaCl was included in the elution buffer a single β -glucosidase peak with an apparent molecular weight of 50000 was eluted (Fig. 2a, peak II). The behaviour of the β -D-glucosidase activity could be clearly distinguished from β -D-galactosidase, since in contrast with β -D-galactosidase no salt-dependent

increase in apparent mol.wt. occurred with 1 M-NaCl. The activity was decreased at high salt concentrations, indicating inhibition of β -D-glucosidase by the NaCl. Peaks I and III separated by gel filtration were pooled and if necessary concentrated by vacuum dialysis and used for subsequent studies. Various kinetic parameters were investigated by using the enzymes from these separated peaks. The enzymes in peaks I and III had similar K_m values and could not be distinguished by the effects of inhibitors (Table 1). Glucono- δ -lactone is a more potent inhibitor of the β -D-galactosidase activity of peak I than is galactono- γ -lactone. Similarly glucono- δ -lactone was the more potent inhibitor of both the enzyme activities of peak III. The β -D-galactosidases and β -D-glucosidases were heat-labile, and 50 % of the total activity was lost within 1 min at 50°C. Only 5 % of the β -D-glucosidase activity remained after 20 min, whereas the β -D-galactosidase activities of peaks I and III were completely destroyed after 20 min. Incubation of the isoenzymes with NaCl at various concentrations showed that the β -D-galactosidase activity of peak I was increased by 100 % with 0.1 M-NaCl, but that higher concentrations had no further effect. In contrast the β -D-galactosidase activity of peak III was unaffected by NaCl concentrations below 0.1 M, but was decreased by 27 % by 1 M-NaCl.

Fig. 2. Separation of marmoset kidney β -D-glycosidases on Sephadex G-200

The sample was applied in 1 ml of 0.01 M-sodium phosphate buffer, pH 7.0 (containing 5 mM-NaCl, 1 M-NaCl or without NaCl) to a column (40cm $\times$ 2.5cm) and eluted with the same buffer. (a) β -D-Glucosidase activity assayed at pH 6.0 as described in the text. Δ , No NaCl in elution buffer; $\blacktriangle$, 5 mM-NaCl in elution buffer; $\circ$, 1 M-NaCl in elution buffer. (b) β -D-Galactosidase activity assayed at pH 4.5 as described in the text. $\square$, No NaCl in elution buffer; $\blacksquare$, 5 mM-NaCl in elution buffer; $\bullet$, 1 M-NaCl in elution buffer. V_0 , Void volume; V_t , total volume of column.

Ion-exchange chromatography

The elution profile of the kidney supernatant from DEAE-cellulose, pH 7, is shown in Fig. 3. A small proportion (6%) of recovered activity sometimes failed to bind to the column. Two major components, 1 and 2, were displaced by the salt gradient. Peak 1 of β -D-galactosidase activity was eluted by 25 mM-NaCl and contained 39 % of the activity, whereas peak 2 was eluted by 0.1 M-NaCl and contained 55 % of the activity. A single peak of β -D-glucosidase activity coinciding with peak 2 was incompletely resolved and showed prolonged tailing. The tube fractions corresponding to peaks 1 and 2 were pooled separately for further investigations.

The pH-activity profiles for the isoenzymes are shown in Fig. 4. The unadsorbed activity had a

Table 1. Some properties of β -D-galactosidase peaks separated on Sephadex G-200

Abbreviation: n.d., not done.

Peak	K_m (mM)		K_i (mM)			
			β -D-Glucosidase		β -D-Galactosidase	
	β -D-Glucosidase	β -D-Galactosidase	Glucono- δ -lactone	Galactono- γ -lactone	Glucono- δ -lactone	Galactono- γ -lactone
I	0.14	0.2	n.d.	n.d.	0.4	23
II	0.2	0.17	1.5	8.5	2.8	6.2

Fig. 3. DEAE-cellulose chromatography of marmoset kidney β -D-glycosidases

The sample (1 ml) in 0.01 M-sodium phosphate buffer, pH 7.0, was applied to the column (5 cm $\times$ 2 cm; 10 ml) equilibrated in the same buffer and eluted with an NaCl gradient (—) in 0.01 M-sodium phosphate buffer, pH 7.0, up to 0.3 M. Fractions (5 ml) were assayed for β -D-glucosidase at pH 6.0 (Δ) and β -D-galactosidase at pH 4.5 ($\square$) as described in the text.

profile identical with that of the first retained peak 1 of β -D-galactosidase activity (Fig. 4a). Although the pH optimum of peak 2 of β -D-galactosidase activity was the same as that of the peak 1 (pH 4.5), this peak was asymmetrical, with a shoulder between pH 5 and 7 (Fig. 4b). In contrast, the pH profile of the single β -D-glucosidase peak had a pH optimum of 6.0–6.5 (Fig. 4c).

Starch-gel electrophoresis

Two major bands of β -D-galactosidase activity at pH 4.5 were detected by staining of the cut gel surface after electrophoresis of the whole homogenate (Fig. 5). The slower-moving band was diffuse, and some activity remained. The fast-moving band was sharper and corresponded to the single band of β -D-galactosidase activity stained at pH 6 (Fig. 5b), the slow-moving band being absent at this pH. The fast-moving band also corresponded to the band of β -D-glucosidase activity (Fig. 5e).

When peaks 1 and 2 from DEAE-cellulose chromatography were concentrated by vacuum dialysis and subjected to electrophoresis, different patterns were obtained. Peak 2 gave a very faint fast-moving band of β -D-galactosidase activity and an intense slow-moving band (Fig. 5c). This peak also gave a faint fast-moving band of β -D-glucosidase (Fig. 5f). In contrast, peak 2 eluted from DEAE-cellulose showed a faint slow-moving band of β -D-galac-

Fig. 4. pH-activity curves for the β -D-galactosidase isoenzymes separated by DEAE-cellulose chromatography

Enzyme activities were assayed in 0.1 M-sodium phosphate/0.05 M-citric acid buffer as described in the text. (a) β -D-Galactosidase activity of peak 1 ($\square$); (b) β -D-galactosidase activity of peak 2 ($\square$); (c) β -D-glucosidase activity of peak 2 (Δ).

tosidase and a strong fast-moving band (Fig. 5d). A strong band of β -D-glucosidase activity from this peak was also present (Fig. 5g).

Pre-treatment of the homogenate with neuraminidase produced no apparent effect on the patterns visible on the stained gel surface. When other gels were sliced and the eluted activity was assayed, some changes (caused by neuraminidase) were discernible in the slow-moving band of β -D-galactosidase, with some decrease in mobility. The fast-moving band was unaffected, however.

Fig. 5. Starch-gel electrophoresis of β -D-glycosidases from marmoset kidney shown diagrammatically

A continuous line indicates a strong band of activity and a broken line a weak band. The separation was carried out at 4°C in 0.04M-sodium phosphate buffer, pH 7.0, for 4h and the bands were developed as described in the text. (a) β -D-Galactosidase activity of the homogenate at pH 4.5; (b) β -D-galactosidase activity of the homogenate at pH 6.0; (c) β -D-galactosidase activity of peak 1 from DEAE-cellulose chromatography (pH 4.5); (d) β -D-galactosidase activity of peak 2 (pH 4.5); (e) β -D-glucosidase activity of the homogenate (pH 6.0); (f) β -D-glucosidase activity of peak 1 from DEAE-cellulose chromatography (pH 6.0); (g) β -D-glucosidase activity of peak 2 separated by DEAE-cellulose chromatography (pH 6.0).

Isoelectric focusing

Focusing of the whole kidney homogenate in the pH range 4–6 produced three major peaks. Two of these peaks were close together, with pI values of 5.6 and 5.1, and had no β -D-glucosidase activity. The third peak, focusing at pI 4.3, possessed 34% of the recovered β -D-galactosidase activity and coincided with the single peak of β -D-glucosidase activity.

Subcellular distribution of glycosidases

The major proportion (75%) of β -D-glucosidase activity is recovered in the soluble fraction (Fig. 6b). In contrast, β -D-galactosidase showed a bimodal distribution of the specific activity, with a major component present in the LM fraction but 36% of the total activity recovered in the soluble fraction (Fig. 6a). When relative specific activities were plotted, the highest value of β -D-galactosidase was in the LM fraction and the bimodal distribution was masked (Fig. 6c).

The lysosomal fraction previously dialysed against column buffer was applied to a DEAE-cellulose column (pH 7.0) and the β -D-galactosidase activity was eluted from the column at the start of the salt gradient (Fig. 7a). There was also a small amount of β -D-glucosidase activity present, which was eluted at a salt concentration of 0.10–0.14M. The soluble fraction gave a different elution profile, with one peak eluted at 0.1M-salt containing both glycosidase activities (Fig. 7b), and only a small peak of β -D-galactosidase activity eluted at a low (0.06M) salt concentration without β -D-glucosidase activity. The

β -D-galactosidase in the lysosomal fraction had a pH optimum of 4.5 and a shoulder between pH 6 and 7, a similar pattern to that of the β -D-galactosidase of the peak 2 separated by ion-exchange chromatographs (Fig. 4b). The pH-activity curves for the soluble-fraction β -D-galactosidase and β -D-glucosidase were similar and resembled the profile found for the β -D-glucosidase activity of peak 2 obtained by ion-exchange chromatography (Fig. 4c). Both enzymes from the soluble fraction showed symmetrical pH-activity curves, with optima at pH 6.0 for β -D-galactosidase and pH 6.5 for β -D-glucosidase. The soluble β -D-galactosidase had negligible activity at pH 3.0, whereas 20% of the maximum activity of the lysosomal enzyme is expressed at this pH.

Discussion

Two major forms of β -D-galactosidase activity are present in marmoset kidney, which can be separated on the basis of charge. A β -D-galactosidase with an acid pH optimum was predominantly present in the lysosomal fraction. This form had an apparent molecular weight of 160000 as estimated by Sephadex gel filtration (peak I, Fig. 2) and was eluted from DEAE-cellulose by low salt concentrations (peak 1, Fig. 3). The second form was present in the soluble fraction, had a neutral pH optimum, an apparent lower molecular weight of 50000 (peak II, Fig. 2a) and was more firmly bound to DEAE-cellulose (peak 2, Fig. 3). Inhibition studies (Table 1) indicated that the β -D-glucosidase and β -D-galactosidase activities of peak III (Fig. 2) were probably associated with the same enzyme protein. We have applied to the isoenzymes of β -D-galactosidase from marmoset kidney the nomenclature devised by Cheetham & Dance (1976) for those from human liver. That isoenzyme which is loosely bound to DEAE-cellulose, migrates slowly on starch-gel electrophoresis and possesses an acid pH optimum we have designated as the A form. The isoenzyme that also exhibits β -D-glucosidase activity, binding more firmly to DEAE-cellulose, migrating more rapidly on electrophoresis and having a neutral pH optimum, we have called the B form. On isoelectric focusing of the whole homogenate the A form was further subdivided into peaks with pI values of 5.1 and 5.6, whereas the B form focused as a single peak (pI 4.3).

Studies on human kidney β -D-galactosidase (Dance *et al.*, 1969) demonstrated the presence of soluble and lysosomal forms in this tissue. The β -D-glucosidase activity was present in the soluble fraction and was demonstrable as a rapidly migrating band on starch-gel electrophoresis. The major proportion of the β -D-galactosidase activity present in the cytosol was associated with the β -D-glucosidase component. There are many similarities between the isoenzymes of β -D-galactosidase and β -D-glucosidase

Fig. 6. Subcellular distribution of marmoset kidney β -D-glycosidases

Specific activities are given as $\mu\text{mol/h}$ per mg of protein and relative specific activities as percentage of the total specific activity in each fraction divided by the percentage of the total protein in that fraction. The abscissa gives the percentages of total protein in each fraction in order of elution: N, nuclear fraction; LM, lysosomal-mitochondrial fraction; MM, mitochondrial-microsomal fraction; Mic, microsomal fraction; S, soluble fraction. Enzymes were assayed as described in the text: total activity (—); free activity (---). (a) β -D-Galactosidase; (b) β -D-glucosidase, specific activity; (c) β -D-galactosidase; (d) β -D-glucosidase, relative specific activity.

present in human kidney and those reported in the present paper for the marmoset.

Price & Dance (1967) described the presence of two forms of β -D-galactosidase in the rat kidney, one, with an acid pH optimum of 3.7, present in the lysosomes, which had low mobility on starch-gel electrophoresis. A second form present in the soluble fraction had a pH optimum of 6.0 and migrated more rapidly on starch-gel electrophoresis. The soluble β -D-galactosidase was associated with the β -D-glucosidase activity. Goldstone *et al.* (1971) used polyacrylamide-gel electrophoresis to separate the β -D-galactosidases present in rat kidney lysosomes. They found that at least three bands of activity were detectable, two of which were slow-moving and one migrated more rapidly. β -D-Glucosidase activity

migrated as a single band corresponding to the fast-moving band of β -D-galactosidase activity. Neuraminidase treatment resulted in an apparent intensification of the slow-moving bands at the expense of the fast-moving band, which Goldstone *et al.* (1971) interpreted as conversion of the faster into the slower bands. Later work (Goldstone & Koenig, 1973) also suggests that a fast-moving form of β -D-galactosidase is present in the lysosomal fraction, and indeed predominates. Price & Dance (1967) found that the rapidly migrating electrophoretic form of β -D-galactosidase predominates in the soluble fraction of the cell, whereas the slowly migrating forms are present in the lysosomes. Goldstone & Koenig (1973) attributed this finding to the possibility that endogenous lysosomal neuraminidase activity converts the

Fig. 7. DEAE-cellulose chromatography of subcellular fractions from marmoset kidney

The sample (1 ml) in 0.01 M-sodium phosphate buffer, pH 7.0, was applied to a column (5 cm $\times$ 2 cm, 10 ml) equilibrated in the same buffer. Elution was with an NaCl gradient (—) up to 0.3 M in the same buffer. Fractions (5 ml) were assayed for β -D-glucosidase activity at pH 6.0 (Δ) and β -D-galactosidase activity at pH 4.5 ($\blacksquare$). (a) Lysosomal-mitochondrial (LM) fraction; (b) soluble (S) fraction.

fast-moving band into the slow-moving forms even at -20°C . However, the rapidly migrating form of rat kidney β -D-galactosidase is unaffected by neuraminidase (Robinson *et al.*, 1967) and the studies of Glew *et al.* (1976) show that the purified enzyme from the cytosol of rat kidney is not a glycoprotein. The rapid mobility on gel electrophoresis is explicable in terms of the amino acid composition of this enzyme, which has a preponderance of aspartic and glutamic residues and only a few basic amino acid residues.

The B form of β -D-galactosidase in the soluble fraction prepared from marmoset kidney was present as a single major peak on both isoelectric focusing and ion-exchange chromatography on DEAE-cellulose. Abrahams & Robinson (1969) described the separation of two forms of soluble β -D-glucosidase from pig kidney, by stepwise elution from DEAE-cellulose, isoelectric focusing and starch-gel electrophoresis. The two isoenzymes of the β -D-glucosidase had identical kinetic properties and were both active towards 4-methylumbelliferyl β -D-galactoside, α -L-

arabinoside and β -D-xyloside, but could be differentiated by their heat-stabilities. Although the distribution of β -D-galactosidase isoenzymes in the kidneys of a number of species appear to follow a similar pattern, the situation in human liver may be more complex, since the lysosomal A form has been further subdivided into three components by Cheetham & Dance (1976).

Studies using natural substrates showed that the acidic A forms of β -D-galactosidase present in the lysosomal fraction prepared from human liver can hydrolyse the terminal β -D-galactoside residue from ganglioside GM₁ (Ho *et al.*, 1973; Norden & O'Brien, 1973). It seems probable that the lysosomal forms have a catalytic role in the breakdown of naturally occurring glycopeptides and glycoproteins. The possible function of the soluble form of the β -D-galactosidase is unknown. The enzyme present in pig kidney has no activity against several naturally occurring glycosides and disaccharides (Abrahams & Robinson, 1969; Glew *et al.*, 1976). This enzyme is probably not involved in the degradation of glycoproteins in the kidney. Distler & Jourdain (1977) purified an aryl- β -hexosidase from bovine liver which was without activity towards possible natural substrates. However, the enzyme actively transferred glycosyl residues from susceptible β -galactosides or β -glucosides to other sugars. The pig kidney soluble enzyme is sensitive to phlorrhizin (Abrahams & Robinson, 1969), a compound that inhibits Na⁺-dependent glucose transport, suggesting that the soluble β -D-glucosidase may be involved in the absorption of sugars. The marmoset kidney shows the same pattern as other species and provides an interesting model for further studies of renal damage and disease (Pierce *et al.*, 1977).

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