Purification and characterization of a lysosomal form and a variant form of β -glucuronidase from the rat basophil leukaemia tumour

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Two isoenzymes of β -glucuronidase from a rat basophil leukaemia tumour were co-purified 4067-fold by $(\text{NH}_4)_2\text{SO}_4$ precipitation and sequential chromatography on concanavalin A–Sephadex, Sephadex G-200, DEAE-cellulose, CM-cellulose and phosphocellulose. The purity of the mixture was established by the coincidence of the peaks of enzyme activity and protein at a molecular weight of 300000 on Bio-Gel P-300, the presence of only two protein bands, both of them enzymically active, in polyacrylamide gels after electrophoresis under non-denaturing conditions, and the presence of a single subunit species, of mol.wt. 75000, after electrophoresis in polyacrylamide gels under a denaturing condition. The major isoenzyme co-migrated with the L form from rat liver during electrophoresis in alkaline polyacrylamide gels, whereas the minor isoenzyme migrated more rapidly than either the lysosomal form or the rat liver microsomal form and was designated the tumour (T) isoenzyme. A mixture of the purified isoenzymes from two preparations had an average specific activity of 1389 units/mg for phenolphthalein β -D-glycopyranosiduronic acid. The L and T isoenzymes, which had pI 5.9 and 5.7 respectively, could be obtained free of cross-contamination by isoelectric focusing and had similar specific activities. Although the T isoenzyme could be a catabolic product of the M or the L form, it could also be a unique tumour product, because it was not detected in extracts of normal rat tissues.

β -Glucuronidase is an acid exoglycosidase with two major isoenzyme forms, lysosomal (L) and microsomal (M), as defined in rat (de Duve *et al.*, 1955; Mameli *et al.*, 1972) and mouse (Paigen, 1961) liver. Both rat liver isoenzymes are tetramers composed of polypeptides with similar amino acid compositions (Himeno *et al.*, 1978; Tulsiani *et al.*, 1978) and molecular weights of about 75000 (Stahl & Touster, 1971; Himeno *et al.*, 1976; Owens & Stahl, 1976), and are derived from the same structural gene (Paigen, 1961). These isoenzymes are distinguished by their subcellular localizations, electrophoretic mobilities and isoelectric points, heat stabilities (Tsuji *et al.*, 1977), carbohydrate compositions (Himeno *et al.*, 1978; Tulsiani *et al.*, 1978) and the capacity of the M form to bind a microsomal-membrane-associated protein (Strawser & Touster, 1979). The lysosomal isoenzyme is the only form of β -glucuronidase detected in mouse brain and spleen (Lusis & Paigen, 1976) and in the specialized secretory granule of rat serosal mast cells

(Schwartz *et al.*, 1979). β -Glucuronidase has been purified and characterized from a rat basophil leukaemia tumour because this tumour has immunoglobulin membrane receptors (Kulczycki & Metzger, 1974), a characteristic of rat mast cells (Conrad *et al.*, 1975), and is available in large amounts. Both lysosomal (L) and tumour-specific (T) isoenzymes of β -glucuronidase were present in the rat basophil leukaemia tumour.

Experimental

Phenolphthalein β -D-glycopyranosiduronic acid, α -methyl mannoside, naphthol AS-BI-D-glucuronide and Fast Garnet GBC (Sigma Chemical Co., St. Louis, MO, U.S.A.), DEAE-cellulose (DE-52), CM-cellulose (CM-52) and phospho-cellulose (P-11) (Whatman, Maidstone, Kent, U.K.), phosphorylase b, bovine serum albumin, ovalbumin, aldolase, α -chymotrypsinogen, ferritin, catalase, thyroglobulin, concanavalin A–Sephadex and Sephadex G-200 (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.), Bio-Gel P-300, ampholytes (pH 5–7), acryl-

Abbreviation used: SDS, sodium dodecyl sulphate.

amide, bisacrylamide, ammonium persulphate, *NNN'*-tetramethylethylenediamine, SDS and Coomassie Brilliant Blue (Bio-Rad Laboratories, Richmond, CA, U.S.A.) were obtained as indicated.

Rat basophil leukaemia tumour was maintained by mincing solid 2–3-week-old tumours and injecting about 10^5 cells in 0.1 ml of Hanks balanced salt solution (M. A. Bioproducts, Walkersville, MD, U.S.A.) into the subcutaneous nuchal region of 1-day-old Wistar rats.

DEAE-cellulose, CM-cellulose and phosphocellulose were suspended directly in their respective equilibration buffers, which were 10 mM- NaH_2PO_4 titrated to pH 6.0 with 10 mM- Na_2HPO_4 (sodium phosphate buffer, pH 6.0) for DEAE-cellulose and phosphocellulose, and 10 mM- Na_2HPO_4 titrated to pH 4.8 with 0.1 M-citric acid for CM-cellulose. The resins were washed with their equilibration buffers until the conductivity and pH of their effluents were the same as the respective buffers, and they were stored in 0.02% NaN_3 at 4°C. Sephadex G-200 and Bio-Gel P-300 were swollen in 10 mM-sodium phosphate buffer, pH 6.0, containing 0.2 M- NaCl as described in the manufacturer's instructions, and stored with 0.02% NaN_3 at 4°C. Standards employed for calibration of the filtration columns included thyroglobulin (mol.wt. 660 000), ferritin (mol.wt. 440 000), catalase (mol.wt. 250 000), aldolase (mol.wt. 158 000), rat basophil-leukaemia-tumour arylsulphatase A (mol.wt. 116 000), bovine serum albumin (mol.wt. 67 000) and ovalbumin (mol.wt. 43 000).

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Absorbance measurements in the visible range were performed with a Gilford (Oberlin, OH, U.S.A.) 300-N-Micro-Sample spectrophotometer. Salt concentrations were measured with a Radiometer (Copenhagen, Denmark) CDM3 conductivity meter after 100-fold dilutions with water.

Assay for β -glucuronidase

β -Glucuronidase activity was quantified by release of phenolphthalein from phenolphthalein β -D-glycopyranosiduronic acid at 37°C for 30 min in 0.5 ml reaction mixtures containing 0.6 mM substrate and 2–50 μ l of enzyme solution in 0.2 M-sodium acetate adjusted to pH 5.0 with 0.2 M-acetic acid, unless stated otherwise. The incubation time was shortened with highly concentrated solutions of enzyme in order to be in the linear range of the assay. Reactions were stopped by the addition of 1.5 ml of 0.2 M-glycine adjusted to pH 10.7 with 10 M- NaOH ; chromogen release was quantified by A_{552} for phenolphthalein. One unit of enzyme liberated 1 μ mol of phenolphthalein in 1 h at 37°C.

Analytical procedures

Purified β -glucuronidase was assessed for charge

heterogeneity by isoelectric focusing. Enzyme that had been dialysed against distilled water was mixed into an ice-cold linear 10–40% (w/v) sucrose density gradient containing 2% (w/v) ampholytes, pH 5.0–7.0, in a 1.25 cm $\times$ 15.5 cm plastic column (Medical Research Apparatus, Clearwater, FL, U.S.A.) at 4°C. Isoelectric focusing was conducted at a constant power of 800 V per two gradients for 3–5 h until the current fell to 2 mA, at which time a constant current of 2 mA was used until the voltage was stable for 1 h. Gradient fractions (0.5 ml) were collected by aspiration from the bottom of the column and assessed for pH and enzyme activity.

β -Glucuronidase preparations were subjected to electrophoresis on polyacrylamide gels under non-denaturing and denaturing conditions. Electrophoresis under a non-denaturing condition was performed at 4°C by layering either tissue extracts or purified preparations of enzyme containing Bromophenol Blue and 5% sucrose directly on to the 3% stacking and 5% resolving polyacrylamide gels, which were constructed by the method of Maizel (1971), and subjected to a constant current of 1.5 mA/gel for 2–4 h until the Bromophenol Blue dye front reached the anodal end of the gel. When gels were stained directly for enzyme activity, the entire gel was incubated at 37°C for 30–120 min in a solution containing 10 mg of naphthol-AS-BI β -D-glucuronide and 100 mg of Fast Garnet GBC per 100 ml of 0.2 M-sodium citrate adjusted to pH 4.2 with 0.2 M-citric acid. The bands with enzyme activity were identified by the appearance of the purple reaction product, and 10% (v/v) acetic acid was then added to stop the enzyme reaction and to store the gels. Enzyme activity bands either were photographed or were scanned at 550 nm with a Gilford linear transport device on a Gilford spectrophotometer. β -Glucuronidase isoenzymes extracted from the rat liver served as reference standards. Replicate gels were stained for protein as described for SDS/polyacrylamide gels, except that the fixation step was omitted.

Samples to be denatured were concentrated, if necessary, by freeze-drying after dialysis against distilled water or by trichloroacetic acid precipitation (Schwartz & Roeder, 1974). Phosphorylase b (mol.wt. 94 000), bovine serum albumin (mol.wt. 67 000), ovalbumin (mol.wt. 43 000) and α -chymotrypsinogen (mol.wt. 25 700) served as reference proteins and were treated in parallel. Concentrated material was dissolved in 25–50 μ l of SDS sample buffer, consisting of 2.5 mM-Tris/19.2 mM-glycine, pH 8.3, containing 1% (w/v) SDS, 10% sucrose and 0.005% Bromophenol Blue, in the presence and in the absence of dithiothreitol, and heated at 90°C for 5 min. This solution was layered on to a 0.2 ml 3% polyacrylamide/SDS stacking gel, which rested on a SDS-containing resolving gel of

the appropriate polyacrylamide concentration (Laemmli, 1970). Electrophoresis was performed at 25°C with 1 mA/gel for 2–5 h, until the dye front reached the anodal end of the gel. Gels were severed at the dye front and fixed in 12% (w/v) trichloroacetic acid/50% (w/v) ethanol for 15 min at 80°C. They were then stained in 10% (w/v) trichloroacetic acid/50% (v/v) ethanol/0.1% Coomassie Brilliant Blue for 10–16 h at 23°C and then for 1 h at 70°C. Gels were destained at 90°C with 10% acetic acid, and stained bands either were photographed or were scanned spectrophotometrically at 600 nm.

Isolation of β -glucuronidase from rat basophil leukaemia tumour

In the preparation outlined in Table 1, 150 g of tumour excised from the backs of 65 rats was placed in 1500 ml of ice-cold distilled water and homogenized in 200 ml portions with a Polytron PCU-2 homogenizer (Polytron Kinematica, Luzern, Switzerland) at power 10 for three 15 s intervals. In the homogenate 9000 units of β -glucuronidase activity were detected. For five consecutive experiments the mean activity ($\pm$ S.D.) was found to be 58 ± 20 units/g of tumour. This amount was about twice the quantity per gram found in rat lung, spleen and kidney and was similar to that found in liver. All subsequent purification procedures were performed at 0–4°C unless stated otherwise. After centrifugation of the tumour homogenate at 23 000 g for 60 min, 90% of the β -glucuronidase activity remained in the supernatant. Solid $(\text{NH}_4)_2\text{SO}_4$ was added to 25% saturation to precipitate contaminating proteins, which were then removed by centrifugation at 23 000 g for 60 min. The enzyme was then precipitated by adding $(\text{NH}_4)_2\text{SO}_4$ to 63% saturation, sedimented by centrifugation at 23 000 g for 60 min, and resuspended in and dialysed extensively against 10 mM-sodium phosphate buffer, pH 6.0. Insoluble material appearing during dialysis was removed by centrifugation at 48 000 g for 30 min and extracted once with a small volume of 0.4 M-NaCl in 10 mM-sodium phosphate buffer, pH 6.0.

After $(\text{NH}_4)_2\text{SO}_4$ fractionation, the β -glucuronidase was loaded in batches on to concanavalin A–Sephadex (6 g of protein/100 ml of resin); the resin was washed twice with 300 ml of 10 mM- Na_2HPO_4 titrated to pH 7.0 with 10 mM- NaH_2PO_4 (sodium phosphate buffer, pH 7.0), containing 0.5 M-NaCl and 1 mM- CaCl_2 , and poured into a 2.6 cm $\times$ 19 cm column at room temperature. The resin was washed again with 250 ml of the same buffer, and the β -glucuronidase was eluted with 0.3 mM- α -methyl mannoside/0.5 M-NaCl/1 mM- CaCl_2 in 10 mM-sodium phosphate buffer, pH 7.0, at room temperature into ice-cold tubes. Essentially all of the β -glucuronidase bound to the concanavalin A–Sephadex column, 77% was recovered, and an 18-fold purification was achieved. The recovered enzyme was concentrated by ultrafiltration with an Amicon UM 10 filter to 10 ml and centrifuged at 48 000 g for 30 min to remove insoluble material; the supernatant, which contained all of the β -glucuronidase activity, was applied to the bottom of a 2.6 cm $\times$ 100 cm column of Sephadex G-200, eluted with 10 mM-sodium phosphate buffer, pH 6.0, containing 0.2 M-NaCl by upward flow with 10 cm H_2O pressure, and collected in 5 ml fractions. β -Glucuronidase was eluted in one symmetrical peak of 50 ml at a molecular weight of about 300 000, and a 2.5-fold purification was achieved. This material was dialysed against 10 mM-sodium phosphate buffer, pH 6.0, to less than 20 mM-NaCl and applied to a 2.1 cm $\times$ 8.5 cm column containing DEAE-cellulose. The column was washed with 60 ml of 10 mM-sodium phosphate buffer, pH 6.0, and then a 120 ml linear gradient of 0–0.4 M-NaCl in 10 mM-sodium phosphate buffer, pH 6.0, was applied and the eluate collected in 5 ml fractions. All of the β -glucuronidase adhered and was eluted at about 80 mM-NaCl in 35 ml with a 2-fold purification. This fraction was dialysed against 2 litres of 10 mM-sodium phosphate buffer, pH 6.0, for 4 h, titrated to pH 4.8 with ice-cold 0.1 M-citric acid, and applied to a 6.5 cm $\times$ 2 cm column containing CM-cellulose. The column was washed with 60 ml of the equilibration buffer, and eluted with a 120 ml linear gradient of 0–

Table 1. *Purification of β -glucuronidase from the rat basophil leukaemia tumour*
For experimental details see the text.

	Protein (mg)	Activity (units)	Specific activity (units/mg)	Yield (%)
Homogenate	30 000	9000	0.3	100
$(\text{NH}_4)_2\text{SO}_4$ precipitation	6000	6200	1.0	69
Concanavalin A–Sephadex	263	4800	18.3	53
Sephadex G-200	81	3552	43.9	39
DEAE-cellulose	25	2550	102.0	28
CM-cellulose	5	1083	216.6	12
Phospho-cellulose P-11	0.82	1000	1220	11
Phospho-cellulose P-11 peak fraction	0.20	265	1325	—

0.4M-NaCl in the equilibration buffer, in 2.5ml fractions. Active fractions were eluted at about 0.15M-NaCl and were combined, diluted with 1 vol. of 10mM-sodium phosphate buffer, pH 6.0, and applied to a 1.1cm $\times$ 4cm column containing phospho-cellulose equilibrated in 10mM-sodium phosphate buffer, pH 6.0. This column was washed with 2 vol. of 0.1M-NaCl in 10mM-sodium phosphate buffer, pH 6.0, and eluted with a 40ml linear gradient of 0.1–0.9M-NaCl in 10mM-sodium phosphate buffer, pH 6.0. Fractions (1.5 ml) were collected, and β -glucuronidase appeared in the gradient fractions at 0.15M-NaCl, coincident with the earlier of two protein peaks. The β -glucuronidase had an average specific activity of 1220 units/mg of protein and a peak specific activity of 1325 units/mg of protein, representing a 4417-fold purification over the initial homogenate. A second preparation of 280g of tumour containing 17500 units of β -glucuronidase in the initial homogenate yielded 1130 units of purified enzyme with a specific activity of 1557 units/mg. No contaminants were detected by polyacrylamide-gel electrophoresis under non-denaturing conditions with the use of up to 5 μ g of protein.

Results

Recognition and characterization of the L and T isoenzymes of β -glucuronidase

Crude extracts of rat basophil leukaemia tumours and rat liver were prepared and analysed for β -glucuronidase heterogeneity by electrophoresis in polyacrylamide gels at alkaline pH. The two recognized electrophoretic forms of rat liver β -glucuronidase (Mameli *et al.*, 1972) were detected (Fig. 1c), namely a slower-migrating microsomal (M) and a faster-migrating lysosomal (L) isoenzyme. Two forms were present in the rat basophil-leukaemia-tumour extract and the one with the faster electrophoretic mobility than either of the liver isoenzymes (Fig. 1a) accounted for one-third of the total enzyme activity by a scan of the stained gel. In a mixture of the tumour and liver extracts (Fig. 1b) there were three bands with distinct electrophoretic mobilities, corresponding to the M form from the liver, the L form from both, and the most rapidly migrating minor band from the tumour, designated the T isoenzyme. The T isoenzyme was not detected when normal rat tissue extracts other than liver were examined, including lung, spleen, kidney and purified serosal mast cells. Purified β -glucuronidase in a fraction collected from the phospho-cellulose column gave on electrophoresis two bands of enzyme activity (Fig. 2b), which coincided with two bands of protein (Fig. 2a) and with the L and T electrophoretic forms present in the crude rat basophil-

Fig. 1. Electrophoresis under non-denaturing conditions of 0.02 ml of rat basophil-leukaemia-tumour extract with 7.8 units of β -glucuronidase/ml (a), of 0.02 ml of rat liver with 7.4 units of β -glucuronidase/ml (c) and of 0.04 ml of a mixture of equal volumes of the two extracts (b) in parallel alkaline 5% polyacrylamide gels stained for enzyme activity

M, L and T refer to microsomal, lysosomal and tumour subtypes of β -glucuronidase respectively. Migration was towards the anode at the bottom of the gels. For experimental details see the text.

leukaemia-tumour tissue extracts. The L-form/T-form protein and enzyme-activity ratios were each about 1.5:1. No contaminant protein was detected with purified β -glucuronidase. The L-form/T-form activity ratios increased from about 1:1 to 4:1 in β -glucuronidase fractions of increasing ionic strength across the peak of enzyme activity and were similar in each case to the relative amounts of L-form and T-form protein.

Fig. 2. Electrophoresis under non-denaturing conditions of (a) 30 units and (b) 7 units of purified β -glucuronidase (1500 units/mg) in alkaline polyacrylamide gels, stained for protein (a) and enzyme activity (b)

Migration was towards the anode at the bottom of the gels. For experimental details see the text.

To determine any size difference between the L and T isoenzymes, phosphocellulose-purified β -glucuronidase from the second preparation was subjected to Bio-Gel P-300 chromatography (Fig. 3). A symmetrical peak of enzyme activity appeared at a molecular weight of about 300000 and was coincident with the single peak of protein. Specific activity in the peak tube was 1566 units/mg of protein, and averaged 1473 units/mg of protein across the peak of enzyme activity. Polyacrylamide-gel electrophoresis at alkaline pH was performed with fractions from Bio-Gel P-300 and showed the presence of both L and T isoenzymes, thereby indicating no significant difference in the molecular weights of the holoenzymes.

Charge differences between the L and T isoenzymes of purified β -glucuronidase were analysed by isoelectric focusing of an early fraction from phosphocellulose P-11 containing a mixture of L and T isoenzymes with similar amounts of each as assessed by a 1:1 ratio of enzyme active bands in a polyacrylamide gel after electrophoresis at alkaline pH. A total of 68 units (75 μ g) of activity was

Fig. 3. Chromatography of 1 ml (600 units and 400 μ g) of phosphocellulose-purified β -glucuronidase on Bio-Gel P-300

The column (1.6 cm $\times$ 100 cm) was equilibrated with 0.2 M-NaCl in 10 mM-sodium phosphate buffer, pH 6.0, and eluted at 8 ml/h. For experimental details see the text. ●, β -Glucuronidase; —, protein.

Fig. 4. Isoelectric focusing of 1 ml (68 units and 75 μ g) of purified β -glucuronidase

For experimental details see the text. ●, β -Glucuronidase; —, pH.

focused into two overlapping areas of activity with similar apparent peak activities, at pH values of 5.7 and 5.9 (Fig. 4). The total enzyme recovered in the two peaks was 35 units. Similar results were obtained with the second preparation of purified rat basophil-leukaemia-tumour β -glucuronidase containing 180 units (115 μ g). Fractions from the second isoelectric-focusing gradient were analysed by polyacrylamide-gel electrophoresis at alkaline pH. Enzyme that focused on the more acidic side of the pH 5.7 peak migrated with the electrophoretic mobility of the T isoenzyme (Fig. 5b), whereas enzyme that focused on the less acidic side of the

Fig. 5. Electrophoresis in 5% polyacrylamide gels under non-denaturing conditions of L (0.65 unit) (a) and T (0.65 unit) (b) subtypes of purified β -glucuronidase, which were stained for enzyme activity

Migration was towards the anode at the bottom of the gels. For experimental details see the text.

pH 5.9 peak corresponded to the L isoenzyme (Fig. 5a).

The subunit composition of purified β -glucuronidase was analysed by polyacrylamide-gel electrophoresis in the presence of SDS. Material purified through phospho-cellulose, which contained both L and T isomers, showed one major band with a molecular weight of 75 000, representing more than 98% of the Coomassie Brilliant Blue-stained protein (Fig. 6a). The same result was obtained after reduction and alkylation. Purified isoenzymes L (16 units) (Fig. 6c) and T (10 units) (Fig. 6b), separated by collecting the more-acidic and less-acidic shoulders of enzyme activity after isoelectric focusing, each gave one detectable band of protein, with molecular weights of about 75 000 by polyacrylamide gel electrophoresis in the presence of SDS. Thus both L and T isoenzymes are tetramers with subunits of identical molecular weight. Furthermore, in each case the ratio of applied activity to protein as estimated by Coomassie Brilliant Blue staining was the same $\pm 10\%$, suggesting that both L and T forms have the same specific activity.

Discussion

β -Glucuronidase extracted in distilled water from the rat basophil leukaemia tumour was purified to homogeneity by $(\text{NH}_4)_2\text{SO}_4$ precipitation and

Fig. 6. Electrophoresis in parallel 10% polyacrylamide gels under denaturing conditions with SDS of β -glucuronidase forms T and L (12 units and 9 μg) purified by phospho-cellulose P-11 chromatography (a), and of β -glucuronidase form T (10 units) (b) and form L (16 units) (c) obtained subsequently by isoelectric focusing. The isomers were prepared by dialysis for 12 h at 4°C against distilled water, freeze-drying and addition of SDS sample buffer. For experimental details see the text. —, A_{600} . A photograph of the gel with phospho-cellulose-purified β -glucuronidase is shown in panel (a). Migration was towards the anode at the right of the scans and gel.

chromatography on concanavalin A-Sepharose, Sephadex G-200, DEAE-cellulose, CM-cellulose and phospho-cellulose. The enzyme was eluted

coincident with a peak of protein at the final purification step. Criteria for purity included coincident single peaks of enzyme activity and protein on Bio-Gel P-300 gel filtration (Fig. 3), one major band of protein after SDS/polyacrylamide-gel electrophoresis (Fig. 6) and two bands of protein, both of which were enzymically active, after polyacrylamide-gel electrophoresis under a non-denaturing condition at alkaline pH (Fig. 2). The purification procedure resulted in a 4067-fold purification, 11% yield, and a final specific activity of 1220 units/mg of protein (Table 1). Given small differences in assay conditions, this specific activity is comparable with that of enzyme purified from livers of mice (Tomino & Paigen, 1975), rats (Stahl & Touster, 1971; Himeno *et al.*, 1976; Owens & Stahl, 1976), rabbits (Dean, 1974) and humans (Musa *et al.*, 1965), from mouse kidneys (Lin *et al.*, 1975) and from rat preputial glands (Ohtsuka & Wakabayashi, 1970).

Two forms of β -glucuronidase were apparent in initial tumour extracts (Fig. 1) and in the purified enzyme preparation (Fig. 2). The form migrating with the lysosomal form from rat liver during electrophoresis in polyacrylamide gels at alkaline pH accounted for two-thirds of the total activity detected in these gels. The less-abundant form migrated more rapidly than either the lysosomal form or the slower-migrating rat liver microsomal form, and was designated the tumour or T isoenzyme. The L and T holoenzymes had identical molecular weights, about 300 000, on Sephadex G-200 and Bio-Gel P-300 columns (Fig. 3). Both were tetramers, with subunits of mol.wt. about 75 000 that were identical on polyacrylamide-gel electrophoresis under denaturing conditions (Figs. 6b and 6c). That their apparent separation on alkaline polyacrylamide gels (Fig. 2) was due to charge differences was confirmed by isoelectric focusing (Fig. 4) and polyacrylamide-gel analysis at alkaline pH of the more-acidic and less-acidic activities (Fig. 5). The pI of the L isoenzyme was 5.9 and that of the T isoenzyme was 5.7. Charge difference was not sufficient to allow for complete separation and recovery of substantial amounts of L and T isoenzymes.

The L and T forms of β -glucuronidase appeared to have comparable specific activities, since the relative intensities of the enzyme-activity and protein bands of L and T isoenzymes separated by alkaline polyacrylamide-gel electrophoresis under denaturing conditions (Fig. 2) were similar. In addition, the specific activities of L and T isoenzymes were similar when the activities were determined by the standard assay after separation by isoelectric focusing and the protein was estimated by spectrophotometric scanning after electrophoresis of the individual isoenzymes under non-denaturing (Figs. 5a and 5b) and denaturing (Figs. 6b and 6c) conditions. The observation

that the specific activities of L and T isoenzymes are comparable is similar to the finding that M and L isoenzymes from rat liver (Stahl & Touster, 1971; Himeno *et al.*, 1976; Owens & Stahl, 1976) exhibit identical kinetic parameters with synthetic substrates. The T isoenzyme either could be a unique microsomal or lysosomal form of β -glucuronidase produced by the rat basophil leukaemia tumour or could be generated as a catabolic product from the typical M or L form of β -glucuronidase. Although precedence exists for chymotryptic and tryptic processing of the rat liver M isoenzyme to a variety of active subtypes distinguished by isoelectric focusing (Owens *et al.*, 1975), no size difference was detected between the rat basophil-leukaemia-tumour L and T tetrameric holoenzymes or their subunits (Figs. 3 and 6).

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