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PURIFICATION AND CHARACTERIZATION OF RAT LIVER MICROSOMAL β-GLUCURONIDASE

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Summary

β-Glucuronidase (EC 3.2.1.31) has been isolated from rat-liver microsomes by a novel chromatographic method employing antibody to rat preputial gland β-glucuronidase coupled to Sepharose. The purified enzyme, homogeneous by several methods, was purified some 1700-fold. The microsomal β-glucuronidase has been characterized with respect to catalysis, stability, and molecular weight. The purified enzyme is a tetramer of 290 000 daltons. Comparative studies with lysosomal β-glucuronidase indicate that while these two enzymes are electrophoretically distinct, they are catalytically and immunologically identical and have indistinguishable molecular dimensions. The results suggest that microsomal and lysosomal β-glucuronidase are charge isomers.

Introduction

β-Glucuronidase (EC 3.2.1.31), commonly described as a lysosomal hydrolase, displays a unique subcellular distribution in rat liver tissue with activity associated with both lysosomes and endoplasmic reticulum [1]. Earlier work has established that rat lysosomal β-glucuronidase is a large glycoprotein enzyme which is easily released from lysosomes by osmotic shock [2]. Rat microsomal β-glucuronidase, on the other hand, appears to be a true membrane enzyme which is released from microsomal membrane fractions only by treatment with detergents [3]. Moreover, β-glucuronidase from lysosomes and microsomes can be readily separated by ion-exchange chromatography. The biosynthetic relationships between lysosomal and microsomal β-glucuronidase, while of considerable interest in relationship to the biogenesis of lysosomes, have not been clarified.

β-Glucuronidase, isolated from various mammalian tissues, has been the subject of many studies [2—8]. Unfortunately, a good deal of the work has been

done without regard to the intracellular origin of the enzyme [4,5]. β-Glucuronidase, from lysosomes of rat liver [2], mouse kidney [5] and mouse liver [7], has been isolated and rather fully characterized. Microsomal β-glucuronidase from any tissue, has not been isolated and characterization has been obtained on semi-purified preparations [8]. We now report the purification of rat microsomal β-glucuronidase to apparent homogeneity with comparative characterization experiments using purified rat liver lysosomal β-glucuronidase. The results demonstrate that the β-glucuronidase from these two organelles, while having distinct isoelectric points, are catalytically and immunologically very similar, if not identical.

Methods and Materials

Female Wistar rats (200 g) obtained from National Laboratory Animals, O'Fallon, Mo. were used for all experiments. Bio-Gel A-1.5 was obtained from Bio-Rad Laboratories, Richmond, Calif. Acrylamide and bis-acrylamide were purchased from Eastman, Rochester, N.Y. Ampholine was obtained through LKB, Inc., Chicago, Ill. and ultra-pure urea from Schwarz-Mann, Orangeburg, N.Y. All other reagents were obtained from Sigma Chemical Co., St. Louis, Mo. or local vendors.

Enzyme assays. β-Glucuronidase was assayed using phenolphthalein-β-D-glucuronide, *p*-nitrophenyl-β-D-glucuronide and 4-methylumbelliferyl-β-D-glucuronide. The substrates were varied from 0.025 to 2 mM in 50 mM acetate, pH 5.0, for double reciprocal plots. Buffers for the pH optima were all 50 mM acetate. Routinely, the enzyme was assayed using 1 mM phenolphthalein-β-D-glucuronide as described by Stahl and Touster [2]. One unit is 1 μmol substrate cleaved per h. Protein was assayed according to Miller [9].

Subcellular fractionation, chromatography and electrophoretic methods. Rat liver microsomes were prepared by the calcium-precipitation technique of Kamath and Rubin [10] as modified by Owens et al. [3]. The method has been outlined in detail in earlier work from this laboratory [3]. Briefly, a post-mitochondrial-lysosomal supernatant, prepared by centrifuging liver homogenate at $1 \cdot 10^5 \times g \cdot$ min, was made to 0.008 M $CaCl_2$. Within a few minutes, the membranes (i.e. microsomes) agglutinate, allowing for sedimentation and washing by low speed centrifugation. Hypotonic buffers (0.005 M Tris · HCl, pH 7.5) were used in the membrane washing to assure rupture of lysosomes contaminating the preparation. Antibody-Sepharose, specific for β-glucuronidase, was prepared by using rabbit antibody to pure rat preputial gland β-glucuronidase. Rabbit IgG was isolated and coupled to Sepharose 4B as described earlier [3]. Ouchterlony double-diffusion analysis and antibody-precipitation curves were set up as described in Ref. 11. Concanavalin A-Sepharose, purchased from Sigma Chemical Co., St. Louis, was employed as an alternate method for purification of microsomal and lysosomal β-glucuronidase. The enzyme, applied to a concanavalin A-Sepharose column in 0.1 M NaCl containing 0.020 M Tris/phosphate, pH 7.8, was completely adsorbed and was eluted from the column with 0.75 M α-methylmannoside containing 0.05 M EDTA and 0.02 M Tris/phosphate, pH 7.8. When crude extracts of microsomal and lysosomal β-glucuronidase were passed over concanavalin A-Sepharose, in

excess of 75% of the activity was recovered following elution with α-methyl-mannoside as described above with a 20—30-fold purification.

Results

Purification of β-glucuronidase

(i) Subcellular fractions. Washed microsomes were prepared as described by Owens et al. [3] using the calcium-precipitation technique. β-Glucuronidase activity associated with washed microsomes was non-latent [3] indicating only a small contamination by lysosomes. Microsomes (P_2), suspended in 5 mM Tris · HCl, pH 7.5, were made 0.2% in Triton X-100. After 5—10 min at 5°C, the suspension was centrifuged at $6 \cdot 10^6 \times g \cdot$ min. Triton X-100, as well as 0.2% sodium deoxycholate, solubilized in excess of 90% of β-glucuronidase activity from the membranes. The Triton X-100-solubilized enzyme (P_2S) was used as starting material for further purification of microsomal β-glucuronidase.

(ii) Antibody-Sepharose chromatography. Triton X-100-solubilized microsomal β-glucuronidase (P_2S) was passed over a β-glucuronidase-specific antibody-Sepharose column (1 × 20 cm). The column, eluted at about 30 ml/h, was successively washed with 200 ml 1 M NaCl containing 10 mM Tris · HCl, pH 7.5, followed by 40 ml 4 M urea containing 10 mM Tris · HCl, pH 7.5. The column was then washed with 50 ml of 8 M urea containing 10 mM Tris · HCl, pH 7.5. The enzyme (65—75% of the applied load) was recovered in the 8 M urea wash. The purification achieved by this step averaged 200-fold.

(iii) Gel filtration on Sepharose 6B in 6 M urea. The pooled fraction from the previous step was diluted to 6 M urea and concentrated to 5 ml using an Amicon ultrafiltration cell with a XM-50 membrane. The enzyme was then filtered through a 2.5 × 90 cm column of Sepharose 6B equilibrated with 6 M urea buffered with 10 mM Tris · HCl, pH 7.5, containing 50 mM NaCl and 0.2% NaN_3. The β-glucuronidase was eluted as a symmetrical peak with a 90% recovery and a 5-fold enhancement of specific activity.

(iv) Gel filtration on 8% agarose. The enzyme peak from the previous step was pooled and dialyzed against 50 mM NaCl buffered with 20 mM Tris · HCl, pH 7.5. The dialyzed enzyme was concentrated to 5 ml and chromatographed on a 2.5 × 90 cm column of Bio-Gel A-1.5 equilibrated with 50 mM NaCl containing 20 mM Tris · HCl, pH 7.5, and 0.2% NaN_3. The active fractions were pooled, dialyzed against 5 mM Tris · HCl, pH 7.5, and concentrated to about 400 units/ml. The yield of enzyme activity on A-1.5 was 80% with a 1.5-fold purification. The entire purification of microsomal β-glucuronidase is summarized in Table I. The overall yield from washed microsomes was 48% with a 1700-fold enhancement in specific activity.

Properties of purified microsomal β-glucuronidase

(i) Polyacrylamide gel electrophoresis. Purified microsomal β-glucuronidase was subjected to analytical gel electrophoresis under basic conditions as described by Davis [12] using various gel concentrations and in the presence of sodium dodecyl sulphate under neutral conditions [13]. The results shown in Fig. 1 indicate that the enzyme migrates as a single band under all the conditions tested and appears homogeneous. Gels run under basic conditions and

TABLE I

Fraction	Protein (mg)	Enzyme activity (units)	Specific activity (units/mg)	Yield (%)	Purification ratio
P_2S	1604.0	2340	1.46	100	1
Antibody column	5.09	1564	307.2	67	210
Sepharose 4B in 6 M urea	0.88	1420	1610	61	1102
8% Agarose	0.448	1118	2496	48	1709

stained for enzyme activity, revealed a pattern indistinguishable from the protein stain.

(ii) Isoelectric focusing. Earlier work has indicated that the isoelectric point of microsomal β-glucuronidase (pI = 6.7) is more basic than that of lysosomal β-

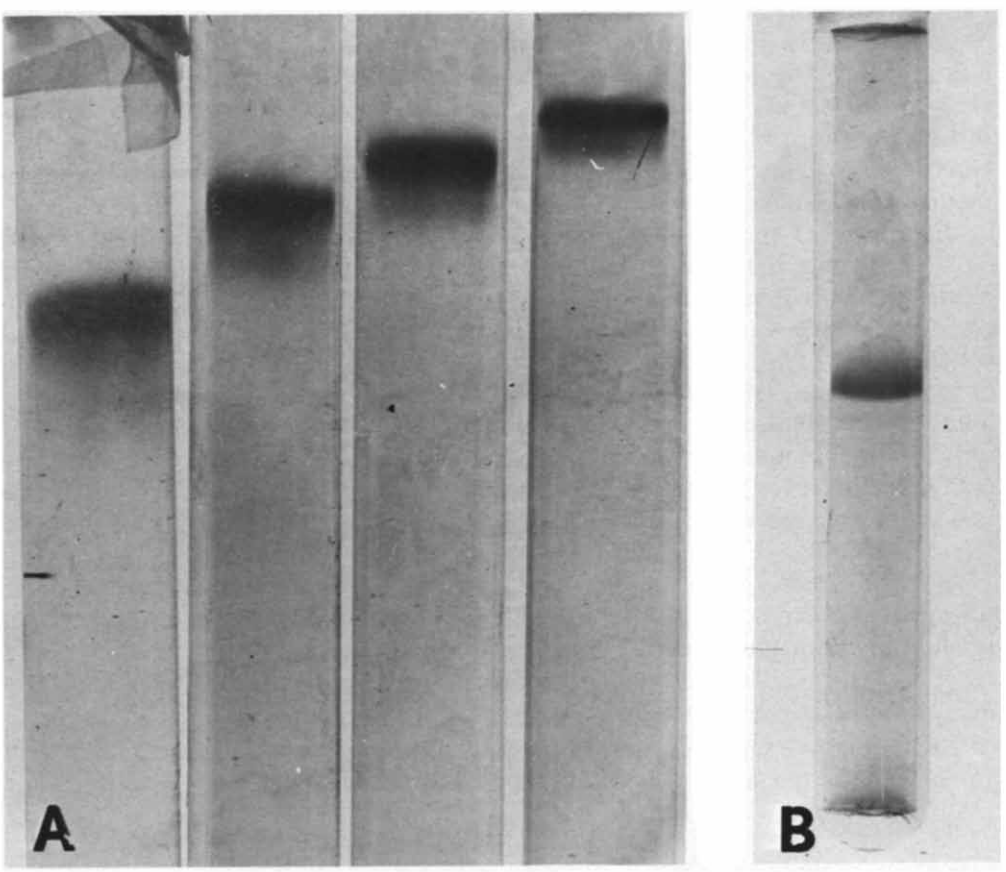

Fig. 1.(A) Polyacrylamide gel electrophoresis of 30 μg purified rat liver microsomal β-glucuronidase in the pH 9.5 system of Davis [12]. Gel concentrations are left to right: a, 4%; b, 5%; c, 6%; d, 7%. Electrophoresis and staining for protein were according to Methods and Materials. (B) Polyacrylamide gel electrophoresis of 25 μg purified rat liver microsomal β-glucuronidase in the sodium dodecyl sulphate system of Weber and Osborn [13]. The sample was preincubated in 1% sodium dodecyl sulphate, 1% mercaptoethanol, and 0.1 M sodium phosphate buffer, pH 7.1, for 30 min at 37°C. Electrophoresis was according to Methods and Materials; staining by the technique of Fairbanks et al. [14].

glucuronidase (pI = 5.6—6.0) [3]. To test whether microsomal β-glucuronidase, isolated by the above procedure, had not been altered during its isolation, purified microsomal β-glucuronidase was subjected to isoelectric focusing in both a LKB column containing 6 M urea and in urea/polyacrylamide gels stained for enzymatic activity. β-Glucuronidase is very protease-sensitive and protease-modified microsomal β-glucuronidase has electrofocusing properties very similar to lysosomal β-glucuronidase [3]. 250 units of microsomal β-glucuronidase were focused on an LKB 110 column as described previously. The enzyme was eluted as a single peak with a pI of 6.7. Moreover, purified enzyme was electrofocused on polyacrylamide gels by the method of Owens et al. [3]. When the gels were stained for enzymatic activity, the enzyme was resolved into three closely related bands very similar, if not identical to, the pattern observed in crude microsomal enzyme preparations. These results indicate that microsomal β-glucuronidase was not modified during purification. It is noteworthy that when urea eluates from antibody-Sepharose are dialyzed against Tris · HCl, pH 7.5, concentrated and stored at 4°C for periods up to several days, one can observe changes in the electrofocusing properties of microsomal β-glucuronidase. Since the microsomal β-glucuronidase is protease-sensitive, it is likely that proteases are co-purified on the antibody-Sepharose columns. We have found that alterations in the electrofocusing properties of microsomal β-glucuronidase can be avoided by gel filtration in the presence of urea immediately following elution of the enzyme from antibody-Sepharose.

(iii) Effect of substrate concentration on activity. Microsomal and lysosomal β-glucuronidase, prepared as described in Methods and Materials, were employed in a comparative study using substrates phenolphthalein glucuronide, *p*-nitrophenyl glucuronide and 4-methylumbelliferyl glucuronide. With substrate concentrations which ranged from 0.025 to 2 mM under standard assay conditions, both lysosomal and microsomal β-glucuronidase displayed normal Michaelis-Menten kinetics for all substrates tested. The calculated K_m values (Table II) indicate identical values for each substrate studied. Moreover, at high substrate concentrations, the formation of product was linear with respect to time (0—15 min) and enzyme concentration (0.05—1.25 μg), respectively, for all the substrates tested.

(iv) The effect of pH on activity. The effect of pH on the catalytic activity of purified microsomal and lysosomal β-glucuronidase was determined using phenolphthalein glucuronide, *p*-nitrophenyl glucuronide and 4-methylumbelliferyl glucuronide. Using phenolphthalein glucuronide, both lysosomal and microsomal β-glucuronidase display dual pH optima of 4.6 and 5.0, respectively (Fig. 2). On the contrary, *p*-nitrophenyl glucuronide and 4-methylumbelliferyl glucuronide show a single optimum for both enzymes. While the optima for

TABLE II

	P	L
Phenolphthalein-β-glucuronide	$7.14 \cdot 10^{-5}$ M	$7.05 \cdot 10^{-5}$ M
p-Nitrophenyl-β-glucuronide	$1.82 \cdot 10^{-4}$ M	$2.32 \cdot 10^{-4}$ M
4-Methylumbelliferyl-β-glucuronide	$1.42 \cdot 10^{-4}$ M	$1.53 \cdot 10^{-4}$ M

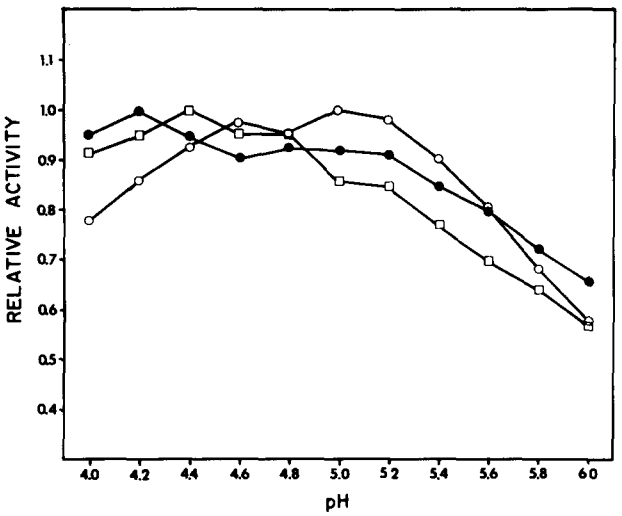

Fig. 2. Lysosomal (•) and microsomal (■) β-glucuronidase were assayed in 1 mM phenolphthalein glucuronide and 50 mM acetate buffer at the appropriate pH. Values for μmol phenolphthalein released/h are normalized at corresponding pH values for comparison.

these two substrates is somewhat lower than that for phenolphthalein glucuronide, the pH curve is quite broad over the pH range 4.0—5.5.

(v) Stability of purified β-glucuronidase. Purified microsomal β-glucuronidase (8 units/ml) was dialyzed overnight against 50 mM Tris · HCl (pH 8.0), 50 mM sodium phosphate (pH 6.5) or 50 mM sodium acetate (pH 5.0). The dialyzed samples were stored at 4°C for assay at appropriate times. Microsomal β-glucuronidase, like lysosomal β-glucuronidase, is relatively stable at pH 8.0 (Fig. 3) loosing only 10% of its activity over 2 weeks. However, at pH 6.5 and 5.0 the loss of activity was 37 and 82%, respectively. While storage in pH 8.0 solutions at 4°C results in small losses of activity, freezing temperatures (—20°C) slowly inactivate the enzyme. However, if the enzyme is frozen in 50% glycerol, only negligible amounts of activity are lost over periods up to 9 months (Fig. 4). A similar observation has been made with rabbit β-glucuronidase [5].

(vi) Molecular weight estimation. Oligomeric molecular weight was estimated by the electrophoretic method of Hendrick and Smith [15] and by gel filtration on 8% agarose. Lysosomal β-glucuronidase (0.5 unit), microsomal β-glucuronidase (0.5 unit), bovine serum albumin (25 μg), ferritin (25 μg), and ovalbumin (25 μg) were electrophoresed on separate gels adjusted to 5, 6, 7, 8 and 9% acrylamide. The gels containing enzyme were stained for β-glucuronidase activity as described by Stahl and Touster [2]. All other gels were fixed and stained for protein [14]. The results in Fig. 5A suggest that microsomal and lysosomal β-glucuronidase are charge isomers with similar molecular weights. Using standards (Fig. 5B), a molecular weight of approx. 290 000 is estimated for microsomal and lysosomal β-glucuronidase. In addition, purified microsomal (20 units) and lysosomal (20 units) β-glucuronidase were co-chromatographed on 8% Agarose (2.5 × 85 cm) equilibrated with 50 mM NaCl and 20 mM Tris · HCl, pH 7.5, containing 0.2% NaN_3. 1-ml fractions were

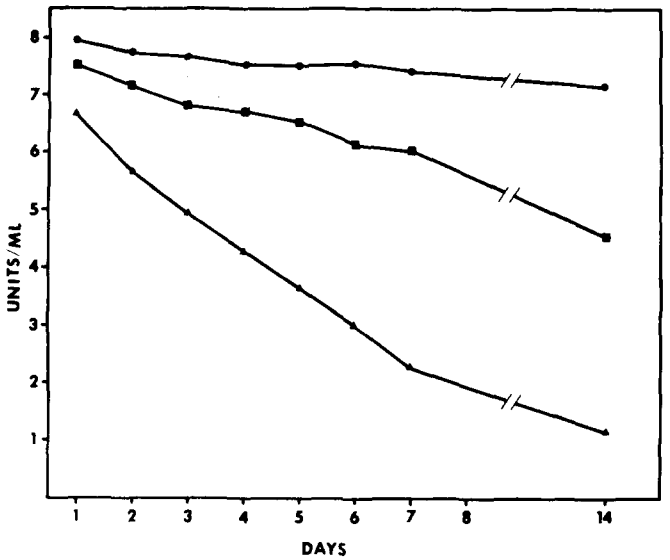

Fig. 3. Samples of 20 units of microsomal β-glucuronidase in 2.5 ml were dialyzed overnight against 50 mM Tris · HCl, pH 8.0 (●), 50 mM sodium phosphate, pH 6.5 (■), and 50 mM sodium acetate, pH 5.0 (▲). After dialysis the samples were kept at 4°C and assayed at appropriate times.

collected. The enzyme activity was eluted as a single peak. Fractions across the peak were further subjected to isoelectric focusing in polyacrylamide gels containing 6 M urea and pH 6—8 Ampholine. The results, not shown here, indicate that both the leading and trailing edge of the β-glucuronidase elution peak contained both lysosomal and microsomal β-glucuronidase. Therefore, β-glucuronidase from these two subcellular sites have very similar molecular dimensions.

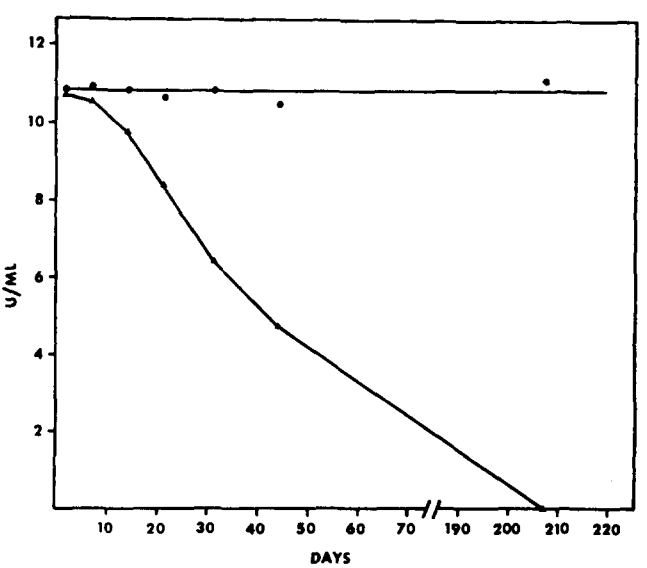

Fig. 4. Purified microsomal β-glucuronidase was diluted to 12 units/ml in samples of 20 mM Tris · HCl. pH 8.0 (▲) and 50% glycerol in 20 mM Tris · HCl, pH 8.0 (●); both were stored at —20°C. At appropriate intervals samples were withdrawn for assay.

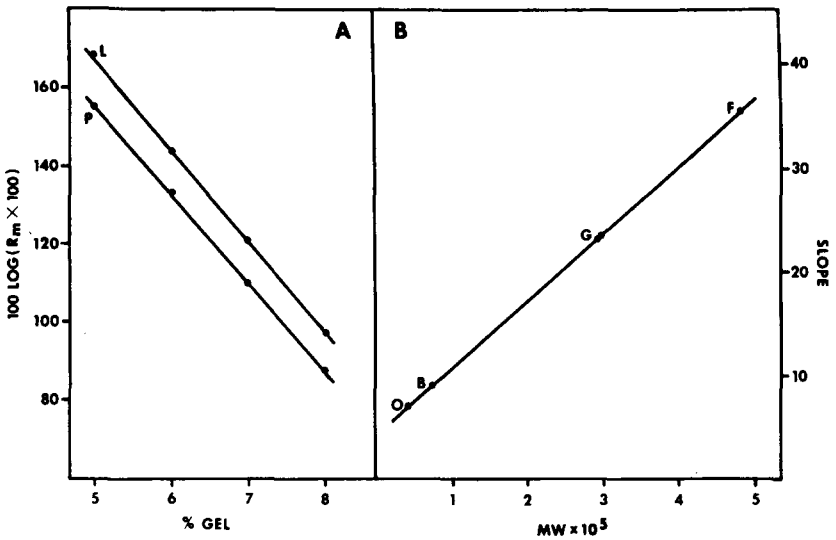

Fig. 5. Polyacrylamide gel electrophoresis in the pH 9.5 system of Davis [12] using gel concentrations of 5, 6, 7 and 8%. (A) Plot of relative mobility for microsomal and lysosomal β-glucuronidase by the method of Hendrick and Smith [15]. Gels were stained for enzyme according to Fairbanks et al. [14]. (B) Plot of slope of the relative mobility vs. molecular weight according to Hendrick and Smith [15]. Markers were run simultaneously with enzyme; approx. 25 μg of marker was run on an individual gel and stained for protein according to Fairbanks et al. [14]. In A, P is microsomal and L is lysosomal β-glucuronidase. In B, O, is ovalbumin, B, bovine serum albumin; G, β-glucuronidase; and F, ferritin.

(vii) Subunit molecular weight by sodium dodecyl sulphate electrophoresis. *Purified microsomal* β-glucuronidase, bovine serum albumin, ovalbumin, and chymotrypsinogen were subjected to electrophoresis by the method of Weber and Osborn [13] in 5% gels containing 0.1% sodium dodecyl sulphate. After electrophoresis, the gels were stained for protein, and the migration distance of each protein measured and expressed as a ratio (R_c) to the migration of chymotrypsinogen. The results, shown graphically in Fig. 7, indicate a subunit molecular weight of 80 000 for microsomal β-glucuronidase. As reported for lysosomal β-glucuronidase [2], the inclusion of 6 M urea in the incubation mixture results in the appearance of two bands. The molecular weight of the second band suggests the formation of a subunit dimer.

(viii) Immunological studies. The observation that microsomal β-glucuronidase binds avidly to antibody-Sepharose prepared with rabbit antibody to rat preputial gland β-glucuronidase, strongly suggests that the enzyme from these two sources are very similar. This notion was examined further by comparing the cross-reactivity of lysosomal and microsomal β-glucuronidase, using immunodiffusion in agar and the precipitin reaction, against rabbit anti-rat preputial gland β-glucuronidase antibody. The β-glucuronidase used for these experiments was prepared by chromatography on concanavalin A-Sepharose rather than by the antibody-Sepharose method. The precipitin curve shows that partially purified enzyme from both sources, reacted in 1.0-ml test mixtures with 10 units of enzyme activity and serial dilution of antisera, precipitated with the same dilution of antibody. The immunodiffusion test showed β-glucuronidase from lysosomes and microsomes forming a continuous line, with-

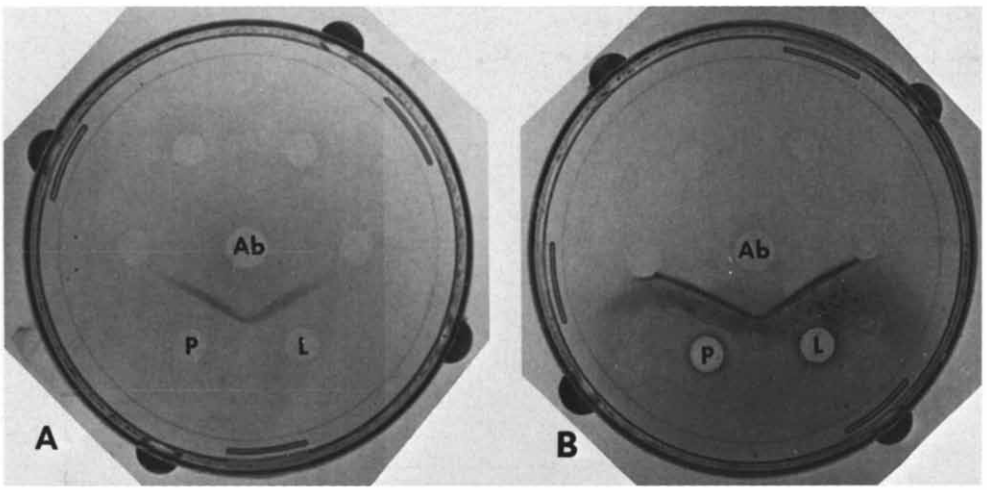

Fig. 6. Lysosomal (L) and microsomal (P) β-glucuronidase were individually purified on concanavalin A-Sepharose, further purified on 8% agarose, and concentrated to 100 units/ml. Two units of each enzyme was applied to each of the outer wells and 10 λ of antibody (Ab) to the center well. After 48 h at 4°C the plates were washed three times for 8 h each in 50 mM sodium phosphate, pH 7.0. (A) Stained for protein according to Fairbanks et al. [14]. (B) Stained for β-glucuronidase according to Stahl and Touster [2].

out spurs, indicating immunologic identity against rabbit anti-rat preputial β-glucuronidase antisera (Fig. 6). The results do not rule out the possibility that lysosomal and microsomal β-glucuronidase have other different antigenic determinants which are absent from preputial gland β-glucuronidase. The results do,

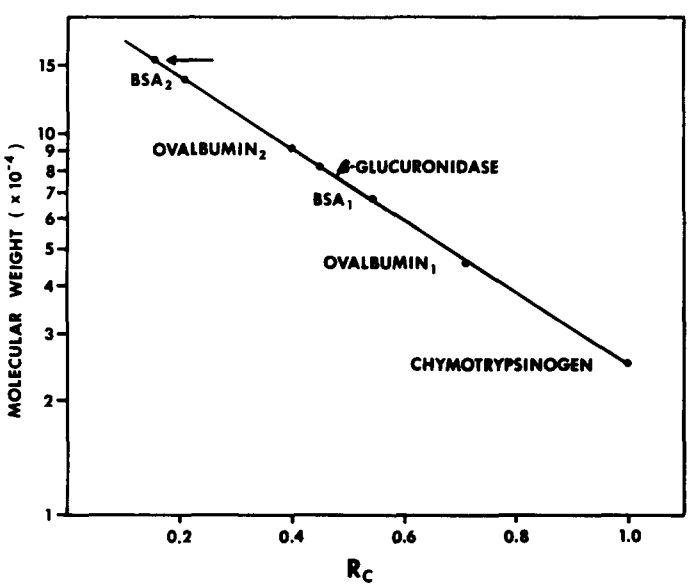

Fig. 7. 25 μg each of bovine serum albumin, ovalbumin, chymotrypsinogen, and purified microsomal β-glucuronidase were electrophoresed in 5% polyacrylamide and 0.1% sodium dodecyl sulphate gels according to Weber and Osborn [13]; 6 M urea was eliminated from the preincubation solution. Gels were stained for protein and the migration distances of the front face of the protein bands from the top gel surface measured. The distances are expressed relative to chymotrypsinogen.

however, point out that lysosomal, microsomal and preputial gland β-glucuronidase have a common set of antigenic determinants.

To compare the subunit sizes of microsomal and lysosomal β-glucuronidase, 50 units of each enzyme were individually precipitated with anti-rat preputial gland β-glucuronidase antibody. The precipitates were centrifuged, washed twice in 1 ml 0.15 M NaCl, washed once in 1 ml of 5 mM Tris · HCl, pH 7.5,

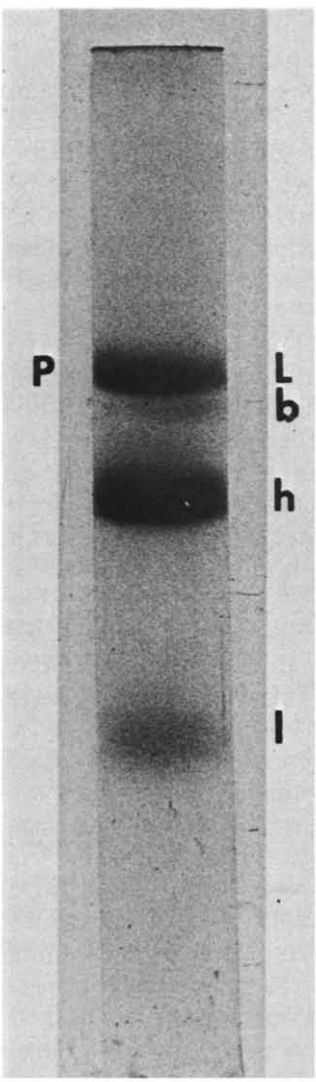

Fig. 8. 50 units each of microsomal and lysosomal β-glucuronidase were individually precipitated with rabbit anti-rat preputial gland β-glucuronidase antibody. The precipitates were individually spun down, washed twice with 1 ml 0.15 M NaCl, and once in 1 ml M Tris · HCl, pH 7.5. The precipitates were dissolved in 0.1 ml 1% sodium dodecyl sulphate and 1% mercaptoethanol and incubated for 30 min at 37°C. The samples were then applied to a split sodium dodecyl sulphate gel and electrophoresed according to Weber and Osborn [13]; stained according to Fairbanks et al. [14]. P, microsomal β-glucuronidase; L, lysosomal β-glucuronidase; b, 5 μg bovine serum albumin to mark L side of split gel; h, heavy antibody chain; l, light antibody chain.

and then solubilized in 0.1 ml of a solution containing 1% sodium dodecyl sulphate and 1% mercaptoethanol. The samples were incubated at 37°C for 30 min, and applied to a split gel for sodium dodecyl sulphate electrophoresis by the procedure of Weber and Osborn [13]. The results, shown in Fig. 8, indicate no differences between the migrations of lysosomal and microsomal β-glucuronidase.

Discussion

Antibody-Sepharose chromatography, using rabbit antisera to pure rat preputial gland β-glucuronidase, appears to be an effective method for the isolation β-glucuronidase from rat liver microsomes and lysosomes. β-Glucuronidase from rat serum and other tissues can also be readily isolated using this method (unpublished observations). Furthermore, very high affinity goat anti-rat preputial β-glucuronidase antibody-Sepharose has been successfully applied to the isolation of human β-glucuronidase. In all cases, the stability of β-glucuronidase activity to urea allows for elution of active enzyme from the gel with high yields.

Since the early fractionation work of de Duve [1], it has been known that a considerable portion of rat liver β-glucuronidase, unlike other lysosomal hydrolases, was associated with endoplasmic reticulum. Earlier work from this laboratory [3] and others [16,17] has demonstrated (i) that β-glucuronidase is a true microsomal enzyme; (ii) that β-glucuronidase solubilized from microsomes is chromatographically and electrophoretically distinct from its' lysosomal counterpart and; (iii) that treatment of microsomal but not lysosomal β-glucuronidase with protease (e.g. trypsin) alters its electrophoretic and isoelectric focusing properties.

In the present report, microsomal β-glucuronidase has been purified some 1700-fold from lysosome-free rat liver microsomes, to apparent homogeneity. The purified enzyme, like lysosomal β-glucuronidase, appears to be a tetramer having subunits in the neighborhood of 75 000 daltons. With regard to catalytic properties and stability of the enzyme in solution, microsomal and lysosomal β-glucuronidase appear identical. Microsomal and lysosomal β-glucuronidase are undistinguishable following gel filtration co-chromatography or when electrophoresed into sodium dodecyl sulfate-containing polyacrylamide gels by the split gel technique. The data strongly suggest that the enzyme isolated from lysosomes and microsomes are charge isomers. Controlled proteolysis with trypsin converts microsomal β-glucuronidase multiple forms to those resembling certain lysosomal multiple forms [3]. These observations suggested that microsomal β-glucuronidase may possess a small basic peptide not present, or masked in lysosomal β-glucuronidase and is consistent with the proposal that microsomal and lysosomal β-glucuronidase are charge isomers. Interesting and perhaps of practical value is the observation that while highly purified β-glucuronidase loses activity rapidly in the frozen state, inclusion of 50% glycerol in the buffer allows for long term storage without loss of activity.

Double diffusion antibody-precipitation tests indicate that microsomal and lysosomal β-glucuronidase are immunologically very similar, if not identical. Care was taken to isolate β-glucuronidase without the use of antibody-Sepharose

chromatography to rule out the possibility that a selected population of enzyme molecules were being tested. While these results indicate that the antigenic determinants found on rat preputial gland β-glucuronidase are also found on the liver enzymes, they do not rule out the possibility that microsomal and lysosomal β-glucuronidase have other dissimilar determinants. Moreover, these results are consistent with the suggestion that β-glucuronidases of rat liver lysosomes and microsomes are very similar immunologically [18].

Previous work by Paigen [19] and Kato et al. [20] has focused on comparisons of microsomal and lysosomal β-glucuronidase in the mouse where enzymes from these two subcellular locations are catalytically very similar. Moreover, the innovative experiments of Swank and Paigen [21] have provided a working model for a role for microsomal β-glucuronidase in relation to the biogenesis of lysosomes. A number of oligomeric forms of microsomal β-glucuronidase, of differing molecular weight, have been identified. The basis for the molecular weight differences is the presence of a protein, called egasyn [22], which is bound non-covalently to the enzyme.

There is no evidence that a similar egasyn system exists in rat liver. In fact, using the techniques of Swank and Paigen [21], we have been unable to identify similar oligomeric forms of β-glucuronidase in rat microsomes (Owens and Stahl, unpublished observations). Furthermore, as mentioned above, rat microsomal β-glucuronidase is urea stable whereas the forms of β-glucuronidase found in mouse microsomes are urea sensitive. Conceivably, rat microsomal β-glucuronidase, isolated as described in this report, could be analogous to form X described by Swank and Paigen [21]. However, more precise comparisons between the rat and mouse microsomal β-glucuronidases and their functional importance will have to await biosynthetic and structural evidence.

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