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THE SYNTHESIS AND PROCESSING OF β-GLUCURONIDASE IN NORMAL AND EGASYN DEFICIENT MOUSE KIDNEY

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SUMMARY

The presence of a precursor form of β-glucuronidase, with a subunit molecular weight of 75,000 was demonstrated in mouse kidney. This was later processed to the mature form, with subunit molecular weight of 71,500. Tissue fractionation revealed that the precursor was associated with the microsomes whereas the mature form was associated with the lysosomes. In mice lacking egasyn both forms of β-glucuronidase were present, but the rate of processing was elevated compared to normal.

INTRODUCTION

Recent findings from this and other laboratories show that several lysosomal enzymes, in cultured macrophages (1), fibroblasts (2) in cultured kidney cells and an *in vitro* protein synthesizing system (3), are synthesized as precursors with a higher molecular weight than the final mature form. In that all lysosomal enzymes examined to date are synthesized as precursor forms, this appears to be a frequent, if not an obligatory, step in all lysosomal enzyme processing.

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Abbreviations used: PBS = phosphate buffered saline
SDS = sodium dodecyl sulphate
EDTA = ethylene diaminetetraacetic acid

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It is believed that lysosomal enzymes are synthesized on ribosomes bound to the rough endoplasmic reticulum, pass into the lumen of the endoplasmic reticulum, and are processed in a manner similar but distinct to secretory proteins (4).

In this report β-glucuronidase from kidney is shown to exist in a precursor form, and that this precursor form is associated with the microsomal fraction, whereas the mature form is associated with the lysosomal fraction.

MATERIALS AND METHODS

Three weeks prior to use, female mice were induced by implantation of a testosterone pellet subcutaneously (5).

For whole kidney studies, 10% homogenates were prepared in 0.1M Tris, 0.15M NaCl pH 7.5 and homogenized in a Brinkman Polytron homogenizer, then Triton was added to give a final concentration of 5%. The homogenates were subsequently centrifuged for 6 million G minutes. The resulting supernatant contained most of the glucuronidase activity (99%).

For subcellular fractionation studies, 10% homogenates were prepared in 0.3M sucrose in 20 mM imidazole pH 7.4 with 1.0 mM EDTA by six passages of a motor driven teflon pestle in a Potter Elvehjem homogenizer followed by filtration through four layers of cheesecloth. The homogenate was then fractionated, by osmotic shock, into microsomal and lysosomal fractions as described by Paigen (6). Basically, a 3 million G-minute pellet was produced from the homogenate. This was resuspended in 20 mM imidazole pH 7.4 of equal volume to the supernatant removed, incubated for 30' at 4°C, then recentrifuged for 3 million G minutes. The pellet was washed in an equal volume of imidazole and the three resulting supernatants were pooled and referred to as the lysosomal fraction. The pellet was solubilized in 1% Triton and any insoluble material removed by centrifugation. The resulting supernatant was referred to as the microsomal fraction and again contained most of the glucuronidase activity.

β-Glucuronidase was purified from each supernatant by precipitation with monospecific antibody, then electrophoresed on SDS acrylamide gels as previously described (7). The gels were then impregnated with PPO, dried, and exposed to photographic plates as described by Bonner and Laskey (8).

β-Glucuronidase (EC 3.2.1.31) was measured by the method of Brandt using 4-methylumbelliferyl-β-D-glucuronide as substrate (9).

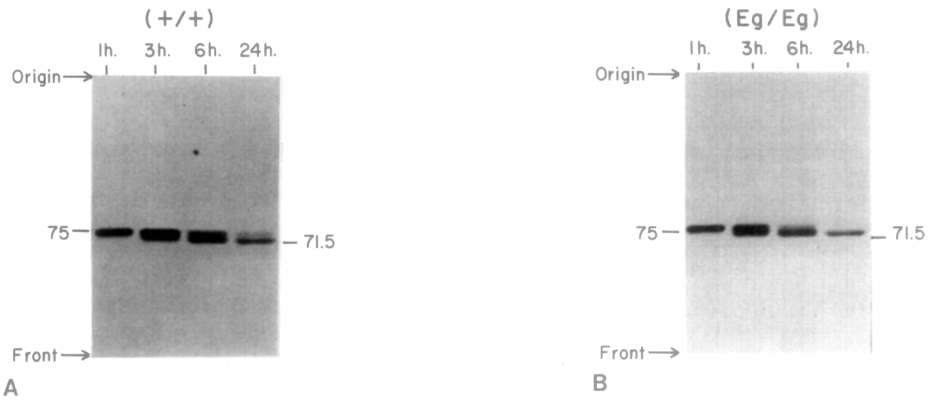


Fig. 1.

Mice were injected at zero time via the tail vein with 100 µCi [^{35}S]methionine in 0.1 ml PBS, then at various time intervals sacrificed, their kidneys excised and homogenized, 12 units of β-glucuronidase precipitated with monospecific antibodies, from aliquots of the homogenate and electrophoresed on SDS acrylamide gels. Fig. 1A, samples from normal C57Bl/6J mice. Fig. 1B samples from $\underline{Eg}^{o}$ mice.

Mice (φ C57Bl/6J) were purchased from Jackson Laboratories, Bar Harbor, Maine. The congenic C57Bl/6J YBR $\underline{Es\text{-}1}^{b}$ $\underline{Eg}^{o}$ (N15 F 6) were raised in our own facilities by Dr. V. Chapman. These mice were genetically identical to normal C57Bl/6J mice except for a small segment of chromosome 8 containing the esterase 1 and egasyn genes. The $\underline{Eg}^{o}$ mutant does not produce the membrane binding protein egasyn.

Testosterone pellets were a gift from Dr. K. Pfister, Division of Metabolism, Univ. Pediatric Dept. Kinderspital Zurich, Steinwiesstrasse 75, 8032 Zurich, Switzerland.

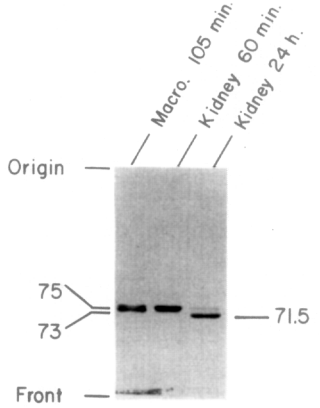
RESULTS & DISCUSSION

To facilitate the study φ mice were treated with testosterone. Testosterone stimulation produces an increase in female mouse kidney size which is accompanied by a 100 fold increase in the rate of β-glucuronidase synthesis and secretion (10). After testosterone stimulation, the average kidney weight was found to be 0.36 gm/two kidneys, which contained 23 units glucuronidase or 66 units/gram of tissue. Two kidneys

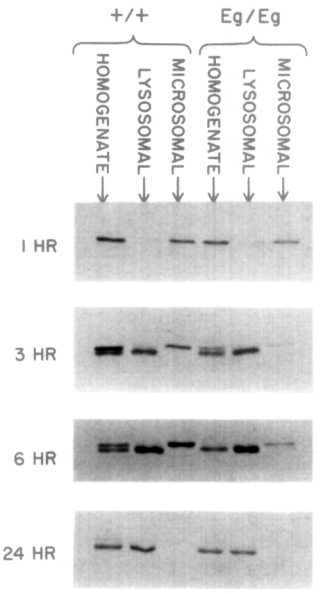
routinely incorporated 5.5 x 10^6 CPM from 100 μCi of injected [^{35}S]methionine with 1500 counts incorporated into glucuronidase.

Purifying samples of glucuronidase and electrophoresing the purified material on SDS gels revealed that at early times the label appeared in a polypeptide of molecular weight 75,000 (Fig. 1). With increasing time, this species lost label with a concomitant appearance of label in a second polypeptide with a slightly decreased molecular weight (71,500). The molecular weight change observed was much less than that reported for other lysosomal enzyme processing (1-3), in which the decrease in molecular weight was in the order of 20,000 daltons. However, the change in kidney glucuronidase was comparable to that we have recently observed in macrophage β-glucuronidase (7). In that system β-glucuronidase decreased in molecular weight from 75,000 to 73,000. The initial molecular weights of each species were the same (Fig. 2), indicating that β-glucuronidase in both tissues were similar but that in kidney the enzyme underwent some further processing compared to macrophages.

Separation, by osmotic shock in hypoosmolar buffer, of the soluble (lysosomal) and membrane-associated (microsomal) enzyme, followed by purification with antibodies, revealed that the microsomal fraction contained exclusively the precursor form, whereas the lysosomal fraction contained predominantly the mature form. A trace of the precursor form was associated with the lysosomal fraction at intermediate time points (Fig. 3). From quantitation of the autoradiograms by visualization, it would appear that after synthesis, the precursor form of β-glucuronidase resides 1 hour in a pool



(2)



(3)

Fig. 2.

Radiolabelled β-glucuronidase was isolated from C57Bl/6J mouse kidney and from C57Bl/6J cultured peritoneal macrophages and electrophoresed in adjacent wells. M_r is indicated to the left and right of the bands.

Fig. 3.

Radiolabelled β-glucuronidase was isolated from homogenate samples, and lysosomal and microsomal fractions of C57Bl/6J, and $\underline{Eg}^o$ mouse kidneys at various times after injection, electrophoresed, and developed as described. The time of sacrifice after injection is shown on the left hand side. The homogenate contained an undefined fraction of the total homogenate, but the microsomal and lysosomal fractions were purified from equal volumes of homogenate with similar yields and thus are directly comparable.

in the microsomes, from which it randomly passes into the lysosomal system, with a half-life of approximately six hours.

In certain murine tissues, including kidney, there exists a microsomal protein, egasyn, which is capable of binding β-glucuronidase (11). A mutant ($\underline{Eg}^o$) does exist in

which egasyn is missing. This mouse contains normal levels of β-glucuronidase, but has an altered distribution, with decreased levels of enzyme associated with the microsomes. In these experiments 7.7 ± 0.88% of total β-glucuronidase was associated with the microsomes in $\underline{Eg}^{o}$ mice, as opposed to 16 ± 1.8% in C57Bl/6J. The route of processing of β-glucuronidase appeared to be the same in C57Bl/6J and $\underline{Eg}^{o}$ mice, but the rate was elevated in $\underline{Eg}^{o}$ mice to about twice that in the wild type. It would appear that egasyn decreases the rate of precursor processing, probably by competing with the lysosomes for a pool of newly synthesized enzyme. Once withdrawn from this pool, the egasyn-bound material, while remaining microsomal and in the precursor form, is unable, or only slowly able to return to this pool for further processing. Smith and Ganschow have reported that liver microsomal glucuronidase of normal and $\underline{Eg}^{o}$ mice had similar half-lives. However, their ^{3}H-leucine measurements were at much later time points than our experiments (12).

Twelve units of β-glucuronidase were immuniprecipitated from each fraction, without the use of carrier enzyme, electrophoresed and stained for protein (Fig. 4). The major protein band in each fraction had the same molecular weight as the radiolabelled band associated with that fraction, indicating most microsomal enzyme had the same molecular weight as the precursor form, whereas all the lysosomal form had undergone a covalent modification to the mature form. Some mature form could be seen in the microsomal fraction, but was not observed in autoradiograms. The quantity of this form corresponded to about 2% of the total β-glucuronidase of the original homogenate.

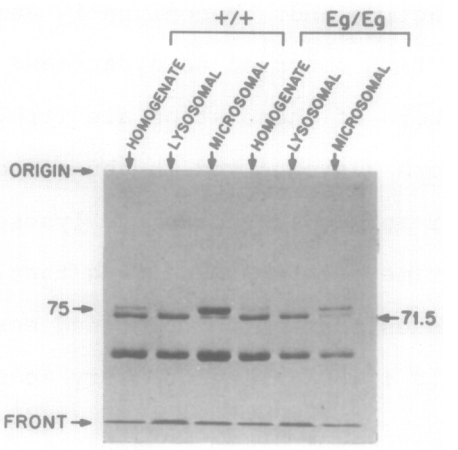


Fig. 4.

Glucuronidase was purified from the homogenate, lysosomal, and microsomal fractions of C57Bl/6J and Eg^{o} mouse kidney and electrophroesed as described, then stained with Coomassie Blue for total protein. Twelve units of enzyme activity were precipitated in each sample except for Eg^{o} microsomal fraction, where only four units were precipitated. The two intense bands in each well below the glucuronidase bands represent the lgG heavy and light chains.

Since no mature labelled enzyme was seen in the microsomal fraction but a trace of precursor form was visible in the lysosomal fraction, it could be concluded that the conversion from precursor to mature form occurred in the lysosomes. A possible second explanation is that the conversion to the mature form occurred as a very late event in the enzyme's association with the microsomes followed by a rapid transfer to the lysosomal system with the small amount of precursor form present in the lysosomal fraction being contamination released from the microsomal fraction. These experiments do not distinguish between these two possibilities.

Apparent from these findings is that newly synthesized enzyme is associated with elements of the microsomal system for some time after synthesis. The enzyme then passes to

the lysosomal fraction and, concomitantly undergoes some covalent modification observed as a decrease in molecular weight. One or both of these steps are irreversible since neither mature form was observed in the microsomal fraction, nor was precursor form observed in the lysosomal fraction except at early time points, indicating that, once committed, the precursor passes through an undefined series of events culminating in its irreversible delivery to the lysosomal system.

These findings are in agreement with the results reported by Tsuji et al. (13, 14) who studied β-glucuronidase in rat liver, reporting the specific radioactivity was higher in microsomal β-glucuronidase than lysosomal β-glucuronidase after labelling with leucine.

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