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Egasyn affects the processing of β-glucuronidase in mouse liver

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Three differently modified forms of β-glucuronidase are known to exist: a microsomal enzyme form (M) existing in tissues where egasyn, a second microsomal protein, is present; and an acidic (L_a; complex-type oligosaccharide) and a basic (L_b; non-complex type oligosaccharide) lysosomal form which occur in all mouse tissues. L_b predominates in tissues containing microsomal β-glucuronidase, L_a in those lacking it. In pulse-labelling experiments using mouse strain C57BL/6 liver containing egasyn (Eg^+/Eg^+) and microsomal enzyme, about half of the newly synthesized β-glucuronidase was processed to the microsomal enzyme form, which was evidently further processed to L_b, and about half directly to L_a. In contrast, in liver of the congenic line C57BL/6.YBR Es-1^b Eg^0 that lacks egasyn (Eg^0/Eg^0) and microsomal enzyme, most of the labelled β-glucuronidase was processed to L_a, and only a minor portion to L_b. Newly synthesized enzyme appeared first in microsomal, then in light and heavy lysosomal fractions of Eg^+/Eg^+ liver. In Eg^0/Eg^0 liver, no labelled enzyme was measurable in the microsomes, but it appeared rapidly in both types of lysosomes. Taken together these findings indicate that the microsomal enzyme form serves as a precursor of L_b, and that L_a is synthesized independently. The apparent half-life of L_a is only two-thirds that of L_b; this fact accounts for the reduced β-glucuronidase activity in Eg^0/Eg^0 liver, which contains L_a as the predominant form.

INTRODUCTION

β-Glucuronidase (EC 3.2.1.31) occurs not only in lysosomes, but is also present in substantial amounts in the endoplasmic reticulum of several organs of mice (Paigen, 1961; Fishman *et al.*, 1967) and rats (Strawser & Touster, 1980) where, perhaps, it has an enzymic function (Gigon & Bickel, 1979; Belinsky *et al.*, 1984). In mice, the enzymes are known to differ in molecular mass, isoelectric point and oligosaccharide structure (Lusis & Paigen, 1978; Strawser & Touster, 1980; Beltramini-Guarini *et al.*, 1984; Swank *et al.*, 1986). We recently discovered that there are two distinct lysosomal forms of β-glucuronidase which can be separated by isoelectric focusing; one is an acidic form with a mean pI of 5.4, designated L_a, the other a basic form with a mean pI of 5.9, designated L_b (Beltramini-Guarini *et al.*, 1984). Several lines of evidence have established that microsomal and lysosomal glucuronidase forms are derived from the same gene and that their differences arise during post-translational precessing (Paigen, 1979).

Although all tissues contain the L_a and L_b forms, albeit in varying proportions (Beltramini-Guarini *et al.*, 1984; Swank *et al.*, 1986), only some tissues contain the microsomal enzyme form (Lusis & Paigen, 1977). It has been shown that to be located in microsomes, β-glucuronidase requires the presence of a second microsomal enzyme, egasyn (Tomino & Paigen, 1975), to which it is complexed. Egasyn is only present in some tissues and has recently been identified as esterase-22 (Medda & Swank, 1985; Medda *et al.*, 1986). Egasyn is missing from mouse strain YBR, a mutant at the *Eg* gene. This strain also lacks microsomal β-glucuronidase (Ganschow & Paigen, 1967; Tomino & Paigen, 1975). Studies of the *Eg* gene and its function have been facilitated by the construction, by Dr V. Chapman (Roswell Park Memorial Institute, Buffalo, NY., U.S.A.), of a congenic strain C57BL/6.YBR Es-1^b Eg^0 carrying the Eg^0 mutation of strain YBR on the genetic background of strain C57BL/6.

The metabolic relationships between the microsomal and lysosomal β-glucuronidases have been uncertain. Smith & Ganschow (1978) suggested that microsomal and lysosomal β-glucuronidases are synthesized and degraded independently and that neither is a precursor of the other. Conversely, Tsuji & Kato (1977) considered microsomal β-glucuronidase to be a transition form in the biosynthetic processing pathway that leads to lysosomal β-glucuronidase. The latter view received support when Brown *et al.* (1981) obtained evidence indicating that in mouse kidney a portion of a microsomal precursor was processed to a lower-molecular-mass mature lysosomal β-glucuronidase after a 1 h lag, which was shorter in egasyn-free animals. They speculated that egasyn diverted a fraction of the microsomal precursor and stabilized it as the microsomal enzyme.

That the situation must be more complex became clear when we identified two lysosomal enzyme forms (Beltramini-Guarini *et al.* 1984). The L_a glucuronidase was endoglycosidase H-insensitive and contained both galactose and sialic acid (complex-type oligosaccharide), indicating that this enzyme form traversed the Golgi apparatus *en route* to the lysosomes (Swank *et al.*, 1986). In contrast, the L_b glucuronidase lacked these features (non-complex type oligosaccharide), indicating either that this form was routed along a different pathway to the lysosomes, or that it did follow the same pathway but was somehow protected from further processing. (Differences in oligosaccharide structure are, at least in part, responsible for the different isoelectric points of the enzyme forms.) A role for egasyn in the formation of L_b was suggested by the finding that in most tissues

Abbreviations used: Eg, egasyn; M, microsomal form; L_b, basic lysosomal form; L_a, acidic lysosomal form.

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containing egasyn, and hence microsomal glucuronidase, lysosomal glucuronidase was present mainly in the L_b form whilst in tissues lacking egasyn it was present mainly in the L_a form (Beltramini-Guarini *et al.*, 1984). This led us to speculate that microsomal glucuronidase might be the precursor of the L_b, but not the L_a, form of glucuronidase, and that in effect, Smith & Ganschow (1978), Tsuji & Kato (1977) and Brown *et al.* (1981) were both partially correct, microsomal glucuronidase being, perhaps, the precursor of one, but not of both, lysosomal forms of the enzyme.

The aim of the present study was to test this hypothesis. We further addressed the question of whether modification and cellular transport of the enzyme forms were correlated. We measured the proportions of the three intracellular glucuronidase forms in liver, and followed the time course of label incorporation into each, both in mouse strain C57BL/6 (Eg^+/Eg^+), which expresses egasyn and contains microsomal glucuronidase, and in the congenic line C57BL/6.YBR Es-1^b Eg^0 lacking both. The results indicate that microsomal glucuronidase is, indeed, a precursor of the basic, L_b, lysosomal glucuronidase, but is not a precursor of the acidic, L_a, form. Additionally, we found that the newly synthesized L_a form appeared earlier in the lysosomal compartment than did the L_b form, indicating a correlation between modification and transport rate.

EXPERIMENTAL

Animals

The mouse strain C57BL/6Zur (Eg^+/Eg^+) was purchased from Kantonales Tierspital (Zürich, Switzerland). The congenic strain C57BL/6J YBR Es-1^b Eg^0 (Eg^0/Eg^0) was kindly provided by Dr. V. Chapman (Roswell Park Memorial Institute, Buffalo, NY, U.S.A.). It was constructed from an F_1 between C57BL/6 and YBR Eg^0 by repetitive back-crossing to C57BL/6 with retention of the Eg^0 allele, followed by interbreeding. Female mice were used for the labelling studies of the β-glucuronidase enzyme forms, male mice for the labelling studies of the cell fractions; the mice used were 3–4 months old.

Enzyme Assay

β-Glucuronidase was assayed using a fluorimetric procedure with 4-methylumbelliferyl-β-glucuronide as substrate (Owerbach & Lusis, 1976). One unit is the amount of enzyme forming 1 μmol of product/h at 37 °C.

Molecular activities of β-glucuronidase forms

To compare the catalytic activities of the microsomal and the two lysosomal enzyme forms, three Eg^+/Eg^+ and three Eg^0/Eg^0 livers were homogenized [10%(w/v)] in hypo-osmotic 20 mM-Tris/HCl, pH 7.4, at 4 °C and centrifuged (100000 *g* for 60 min). This 'osmotic shock' procedure (Paigen, 1961) resulted in the release of lysosomal β-glucuronidase and its separation (supernatant) from the microsomal enzyme (pellet). The microsomal pellets were washed once with the same buffer and the supernatants were combined. The microsomal pellet from the Eg^0/Eg^0 livers (lacking β-glucuronidase) was discarded; that from the Eg^+/Eg^+ livers was resuspended in 150 mM-NaCl/20 mM-Tris/HCl/0.5% (v/v) Triton X-100, pH 7.4, centrifuged (100000 *g* for 60 min) and the supernatant containing microsomal β-glucuronidase was then treated further. The lysosomal supernatants were supplemented by the addition of NaCl to a final concentration of 150 mM and Triton X-100 to 0.5% (v/v). All supernatants were further purified by heat treatment (56 °C, 20 min), followed by centrifugation (100000 *g* for 30 min) and concanavalin A-column chromatography (see below). Increasing amounts of enzyme activity (0.5–3.0 units) were precipitated quantitatively with rabbit anti-(β-glucuronidase) antibody, and the immunoprecipitates were subjected to SDS/polyacrylamide-gel electrophoresis (see below). Gels were stained for protein in 0.025% (w/v) Coomassie Brillant Blue R-250/25% (v/v) isopropanol/10% (v/v) acetic acid/5% (w/v) trichloracetic acid, destained in 7% (v/v) acetic acid/10% (v/v) methanol, and the β-glucuronidase band was quantified by densitometry at 570 nm. The peak area of stained, purified β-glucuronidase protein was plotted against the enzyme activity applied to SDS/polyacrylamide gels. Specific activity was expressed as enzyme activity per unit of protein staining intensity. β-Glucuronidase purified from the Eg^+/Eg^+ microsomal supernatant was in the microsomal form; β-glucuronidase from the lysosomal supernatants of both strains contained acidic (L_a) and basic (L_b) lysosomal forms.

Radioactive labelling

Mice were injected intraperitoneally with 0.6–1.0 mCi of L-[4,5-^{3}H]leucine (190 Ci/mmol, Amersham). After a specific time (between 2 and 96 h), livers were excised and either immediately subjected to the cell fractionation procedure or stored at −20 °C for later purification of the various β-glucuronidase forms.

Chromatofocusing of β-glucuronidase

Livers were homogenized in 0.02 M-Tris/0.15 M-NaCl, pH 7.4, at 4 °C with a Polytron homogenizer (Kinematica, Luzern, Switzerland), supplemented with 0.5% (v/v) Triton X-100, incubated at 56 °C for 20 min and centrifuged (100000 *g* for 30 min). The supernatant was filtered through a 0.22 μm filter before chromatofocusing. The microsomal and the two lysosomal β-glucuronidase forms were separated by chromatofocusing on a Mono-P column HR 5/20 (Pharmacia, Uppsala, Sweden). The column was equilibrated with starting buffer (0.025 M-Tris/HCl, pH 6.7), and after loading the sample, was washed with 5 ml of starting buffer, and focusing and elution were started by switching to a 1:20 diluted Polybuffer 74 (Pharmacia) adjusted to pH 5.0 with HCl. The flow rate was 0.8 ml/min throughout.

Fractions (1 ml) were collected and assayed for β-glucuronidase activity. As shown in Fig. 1, three peaks contained β-glucuronidase. The form present in each peak was identified by isoelectric focusing (Lusis & Paigen, 1978): peak I contained the microsomal (pI 6.4), peak II the L_b (pI 5.9), and peak III the L_a (pI 5.4) lysosomal enzyme form (Beltramini-Guarini *et al.*, 1984). Peak fractions were further purified on a column of concanavalin A–Sepharose (Pharmacia; 0.5 ml bed volume) maximally loaded with 3 units of β-glucuronidase activity. The column was equilibrated with 0.02 M-Tris/HCl/0.15 M-NaCl, pH 7.4, and after the loading the sample, washed with 1.5 ml of the same buffer. Bound protein was eluted with 2 × 0.75 ml and 1 × 1 ml of

0.02 M-Tris/HCl/0.5 M-methylmannoside, pH 8.5, at 45 °C. The recovered enzyme (yield 95 %) was subjected to immunoprecipitation.

Cell fractionation

All steps were carried out at 4 °C. Freshly excised livers (1.5–2 g) were minced with scissors and homogenized with a teflon Potter homogenizer in 0.25 M-sucrose to yield a 10 % (w/v) homogenate. Cell debris and nuclei were removed by centrifugation (100 *g* for 10 min) in a swingout rotor (TST 28.38/17; Kontron, Switzerland), and the postnuclear supernatant was centrifuged (8000 *g* for 15 min) in the same rotor to yield a lysosome-containing pellet (P2) and a microsome-containing supernatant (SN2). SN2 was layered in two portions of 7–9 ml each on a Percoll solution (Pharmacia) in 0.25 M-sucrose (density 1.07 g/ml) and centrifuged (50000 *g* for 1 h) in a fixed-angle rotor (TFT 65.38, Kontron). The gradient was fractionated into 1.4 ml portions from the top, by displacement with a heavy medium (Fluorinert FC-40; ISCO, Lincoln, NB, U.S.A.) in a model 185 fractionator (ISCO). This procedure yielded two fractions containing β-glucuronidase activity, a microsomal [identified by NADH-cytochrome *c* reductase activity (Hodges & Leonard, 1974)] and a light lysosomal [identified by acid mannosidase activity (Farriaux & Fontaine, 1976)]. Pellet P2 was resuspended in 3 ml of 0.25 M-sucrose and layered onto 30 ml of Percoll in 0.25 M-sucrose (density 1.10 g/ml). Gradient centrifugation was performed as described above. Two fractions contained β-glucuronidase activity. The heavier fraction contained heavy lysosomes, whilst the lighter fraction consisted of a mixture of light lysosomes and microsomes as judged by marker enzymes. This mixture was pooled and subjected to a second gradient centrifugation (9 ml of the pooled sample on 24 ml of Percoll/0.25 mM-sucrose, density 1.06 g/ml) as described above, and yielded a microsomal and a light-lysosomal fraction with the same densities as seen in SN2. Therefore, microsomes and light lysosomes from SN2 and P2 were pooled for further purification. Peak fractions containing β-glucuronidase were supplemented with Tris/HCl buffer, pH 7.4 (20 mM), and Triton X-100 (0.5 %, v/v). Percoll was removed by centrifugation (100000 *g* for 90 min). The supernatant was heated for 20 min at 56 °C, cooled and the denatured protein removed by centrifugation (100000 *g* for 30 min). Finally, β-glucuronidase was purified by immunoprecipitation.

Immunoprecipitation of β-glucuronidase and determination of incorporated radioactivity

After chromatofocusing or cell fractionation, β-glucuronidase was incubated overnight at 4 °C with a monospecific rabbit anti-(β-glucuronidase) antibody, raised in our own laboratory. Precipitates were collected by centrifugation (10000 *g* for 30 min), washed twice in 0.02 M-Tris/HCl/0.15 M-NaCl, pH 7.4, containing 4 % (v/v) Triton X-100 (at 4 °C), and then resuspended in the same buffer containing 0.1 % SDS (at 18 °C). The air-dried precipitates were dissolved in sample buffer (Laemmli, 1970) supplemented with 4 M-urea and subjected to SDS/polyacrylamide-gel electrophoresis in glass tubes (6 mm × 130 mm) at 3 mA per tube. Gels were cut into 2 mm slices, which were dissolved in a Solutron/Lipotron mixture (Kontron, Switzerland) and counted in a liquid-scintillation counter.

RESULTS

Synthesis and degradation of β-glucuronidase

For the study of the synthetic relationship between the three β-glucuronidase forms by pulse labelling, the separation of the microsomal and the two lysosomal enzyme forms was a prerequisite. The differences in molecular masses were too small to allow separation based on this parameter (Beltramini-Guarini *et al.*, 1984). However, the differences in isoelectric points allowed the separation of three distinct peaks by chromatofocusing, one microsomal (M), one basic lysosomal (L_b), and one acidic lysosomal (L_a) (Fig. 1).

The proportions of the enzyme forms in individual livers of Eg^+/Eg^+ and Eg^0/Eg^0 mice were determined. They varied by only 2–3 % between animals of the same strain (results not shown), but differed considerably between the strains (Table 1). As expected, microsomal enzyme was present only in Eg^+/Eg^+ tissue, and the proportion of the two lysosomal enzymes forms was inverted between the two strains. The specific activity was determined (see the Experimental section) for the Eg^+/Eg^+ microsomal enzyme form, and for the lysosomal enzyme preparations of both strains. The specific activities were almost the same for the three preparations (Table 1). Thus, the β-glucuronidase assay provided a valid measure of the amount of enzyme protein in all β-glucuronidase forms. We therefore based our measurements of the synthesis and degradation of β-glucuronidase protein on activity assays. The total β-glucuronidase activity per gram of Eg^+/Eg^+ livers was twice that of Eg^0/Eg^0 livers. Since all forms of the

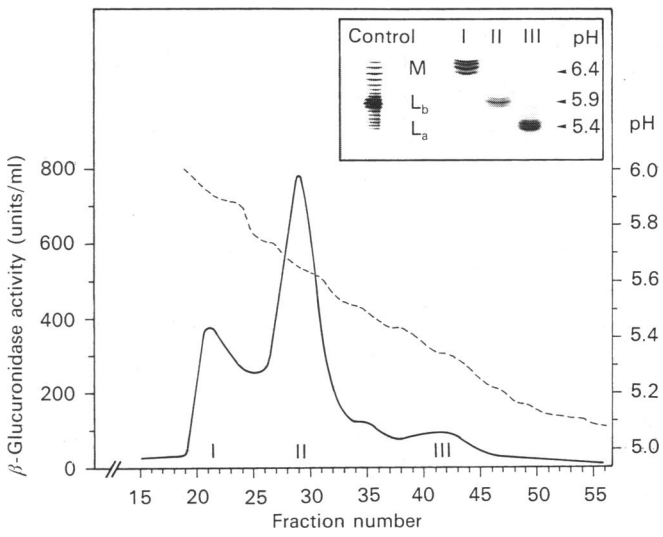

Fig. 1. Separation of β-glucuronidase by chromatofocusing

Elution profile of β-glucuronidase from one Eg^+/Eg^+ mouse liver after chromatofocusing. Enzyme activity, solid line; pH, dotted line. Peaks I–III: microsomal (M), basic lysosomal (L_b), acidic lysosomal (L_a) β-glucuronidase, respectively. Inset: isoelectric-focusing pattern of the peak fractions; control was a Triton X-100 extract of whole liver homogenate. Bands were visualized by activity staining.

Table 1. Specific activity of β-glucuronidase and the distribution and specific activity of its forms in livers of Eg^+/Eg^+ and Eg^0/Eg^0 mice

		Eg^+/Eg^+	Eg^0/Eg^0
Specific activity (units per g wet weight of liver)	Mean	30.2	14.3
	Range	24.8–34.2	12.6–16.9
	(*n*)	12	12
Distribution*			
Microsomal form (M)		29	
Basic lysosomal form (L_b)		52	17
Acidic lysosomal form (L_a)		19	83
Specific activity (units per arbitrary protein staining unit)			
Microsomal form	Mean (S.D.)	84.9 (8.3)	
	(*n*)	(5)	
Basic + acidic lysosomal form	Mean (S.D.)	88.6 (9.8)	88.6 (6.7)
	(*n*)	(7)	(7)

* Percent distribution, calculated from the peak areas after chromatofocusing (Fig. 1).

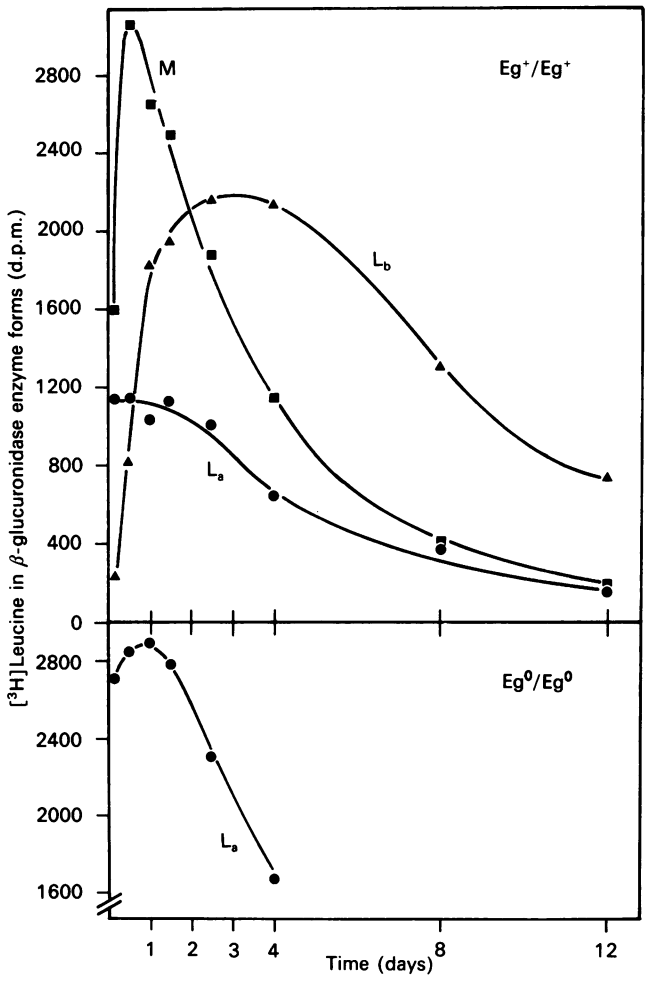

Fig. 2. [³H]Leucine incorporation profiles of β-glucuronidase forms

[³H]Leucine incorporation into microsomal (■), basic (▲) and acidic (●) lysosomal β-glucuronidase forms in Eg^+/Eg^+ liver and of the acidic lysosomal enzyme in Eg^0/Eg^0 liver. Each time point represents the mean of three livers measured. Since the incorporation curve in Eg^0/Eg^0 liver was sufficiently informative up to 4 days, we terminated the experiment at that point.

enzyme have equal molecular activity we concluded that the difference in total β-glucuronidase activity reflected unequal amounts of enzyme protein in the Eg^+/Eg^+ and Eg^0/Eg^0 livers. To determine whether the lower amount of enzyme in Eg^0/Eg^0 livers was due to preferential excretion of the L_a form, we measured the secretion of β-glucuronidase into the medium by cultured hepatocytes of both mouse strains obtained by liver perfusions (procedure described in Beltramini-Guarini *et al.*, 1984). The amount of enzyme activity excreted within 24 h by hepatocytes of both strains was the same [Eg^+/Eg^+ hepatocytes 9.9 ± 0.9 munits/mg of cell protein ($n = 7$); Eg^0/Eg^0 hepatocytes 10.3 ± 1.2 munits/mg of cell protein ($n = 9$) and corresponded to 6.7% (Eg^+/Eg^+) and 12.5% (Eg^0/Eg^0) of the amount of intracellular enzyme activity]. Furthermore, the proportion of the excreted lysosomal forms, as determined by isoelectric focusing, was the same as in hepatocytes and livers of the corresponding mouse strain: the L_a form predominated in Eg^0/Eg^0, L_b in Eg^+/Eg^+ medium. These observations indicated that neither form was preferentially excreted, and that the lower activity in Eg^0/Eg^0 livers was not due to increased excretion of the prevailing L_a form.

The synthesis of β-glucuronidase was observed by measuring the incorporation of [³H]leucine into the microsomal and lysosomal enzyme forms after a single injection of [³H]leucine in both Eg^+/Eg^+ and Eg^0/Eg^0 mice (Fig. 2). At each time point, three livers from each strain were examined individually. Microsomal and lysosomal β-glucuronidase forms were separated by chromatofocusing and were immunoprecipitated before counting radioactivity. In Eg^+/Eg^+ liver, after 4 h, 53% of the label was in the microsomal, 38% in the L_a and only 9% in the L_b enzyme form. After the fourth hour, radioactivity in the L_a form increased only a little, but in the microsomal enzyme form it continued to increase, reaching a maximum at 12 h (Table 2). Labelling of the L_b enzyme form was protracted and reached its maximum between 2.5 and 4 days when labelling of the microsomal enzyme form was already in decline. The maximum amount of label incorporated into L_b was clearly less than that in the microsomal enzyme form. These observations suggest the existence of a precursor–product relationship between the microsomal and the L_b lyso-

Table 2. Radioactivity contained in liver β-glucuronidase at selected times after intraperitoneal injection of [^{3}H]leucine in Eg^+/Eg^+ and Eg^0/Eg^0 mice

Abbreviations: M, microsomal enzyme; L_b, basic lysosomal enzyme; L_a, acidic lysosomal enzyme.

Time after injection (h)	Radioactivity (d.p.m.)*						
	Eg^+/Eg^+				Eg^0/Eg^0		
	M	L_b	L_a	Total	M	L_b	L_a
4	1599	235	1140	2974	—	†	2723
12	3050	815	1146	5011	—	†	2844
24	2648	1819	1031	5498	—	†	2892
60	1870	2164	1003	5037	—	†	2305

* Average of three livers.
† Radioactivity too low for measurement.

somal enzyme forms, although from 12 to 24 h, the loss of label from the microsomal enzyme was less than the increase in label in the L_b enzyme form. The L_a form appeared to be synthesized independently.

In Eg^0/Eg^0 liver no microsomal β-glucuronidase was detected. Labelled leucine was incorporated into the L_a form rapidly, the incorporation following a similar time course as in Eg^+/Eg^+ liver. The maximum label incorporated into L_a was, however, 2.5-fold higher than that seen in Eg^+/Eg^+ mice (Fig. 2). Incorporation into the L_b form was too low to allow accurate measurement. Thus again, the synthesis of the L_a form appeared to be unrelated to that of the microsomal enzyme.

Degradation of β-glucuronidase was calculated from the data shown in Fig. 2 and Table 2. The decline in amount of labelled enzyme of each form was plotted in semi-logarithmic fashion (Fig. 3). The apparent half-life of the microsomal enzyme form was 2.8 days, considerably shorter than the 5.2 days of the L_b lysosomal form. These data are in accord with a product–precursor relationship between the microsomal and the L_b forms. The apparent half-life of the L_a lysosomal form was 4 days in Eg^+/Eg^+ and 3.8 days in Eg^0/Eg^0 liver. The loss of labelled L_a enzyme did not depend on the presence of the microsomal form and appeared to be due to degradation rather than excretion (see above).

We tried to test mathematically whether the synthesis of the microsomal enzyme, its presumed transformation to the L_b enzyme form, and its degradation, are consecutive steps, by inserting the kinetic parameters from the experimental curves (Figs. 2 and 3) into the equation of a two-step consecutive reaction (Gutfreund, 1972), but found it impossible to describe properly the theoretical incorporation curve of the L_b form. The theoretical maximum of incorporated label in the microsomal enzyme, extrapolated from the degradation curve (Fig. 3), was lower than the maximum amount of label observed in the microsomal and L_b enzyme forms taken together. Two factors may have contributed to this. Firstly, [^{3}H]leucine from degraded protein may have been reutilized; during prolonged experiments *in vivo* this can be substantial (Smith & Ganschow, 1978). Secondly, not all of the L_b form may have originated from the microsomal enzyme; there may also be a pathway

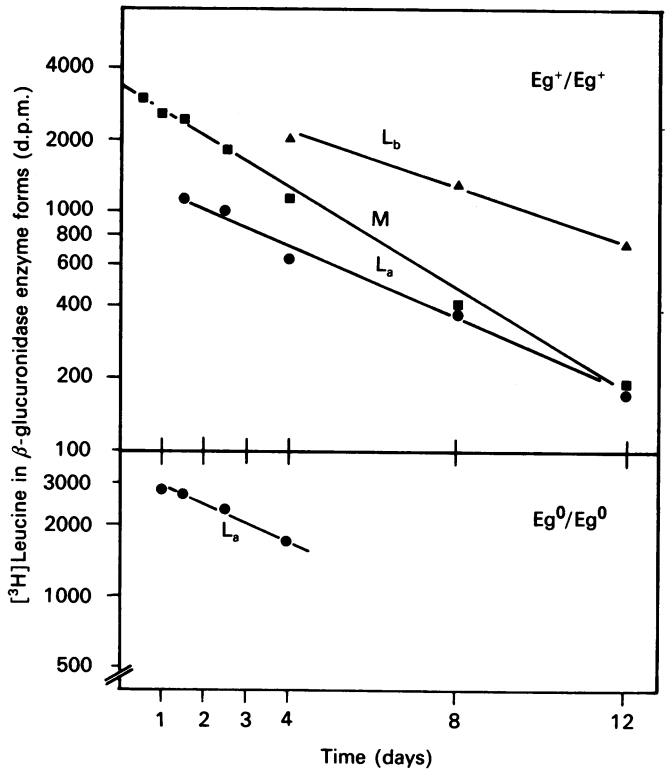

Fig. 3. Semilogarithmic plot of the loss of radioactive enzyme from β-glucuronidase forms

Data from Fig. 2 are plotted in semi-logarithmic fashion. The half-lives of the microsomal (■), basic lysosomal (▲) and acidic lysosomal (●) enzyme forms in Eg^+/Eg^+ liver were 2.8, 5.2 and 4 days, respectively, and that of the acidic form in Eg^0/Eg^0 liver was 3.8 days. The lines were obtained by calculating linear regression.

bypassing the microsomal enzyme form. This is suggested by two observations: synthesis of L_b is more rapid than the loss of labelled M enzyme; and Eg^0/Eg^0 mice do have some L_b form (Table 1).

Intracellular transport

To observe the appearance of newly synthesized M, L_a and L_b enzyme forms in cell fractions, we injected Eg^+/Eg^+ and Eg^0/Eg^0 mice with [^{3}H]leucine. At given times, three livers were excised and cell fractionation on a Percoll gradient was performed with each liver to yield microsomes, light lysosomes and heavy lysosomes. The three cell fractions are thought to represent the sites of synthesis (microsomes), of packing into lysosomal vesicles (light lysosomes) and of exerting function (heavy lysosomes) of the lysosomal enzymes. However, attempts at separating L_a and L_b enzyme forms from the lysosomal fractions by chromatofocusing were unsuccessful, perhaps because contamination with Percoll interfered with the procedure. Furthermore, total enzyme yield was insufficient. Other fractionation procedures using sucrose to form a density gradient resulted in insufficient separation of the cell fractions and/or insufficient yield of labelled enzyme. We therefore measured the appearance of newly synthesized total β-glucuronidase from the cell fractions after Percoll centrifugation, assuming that

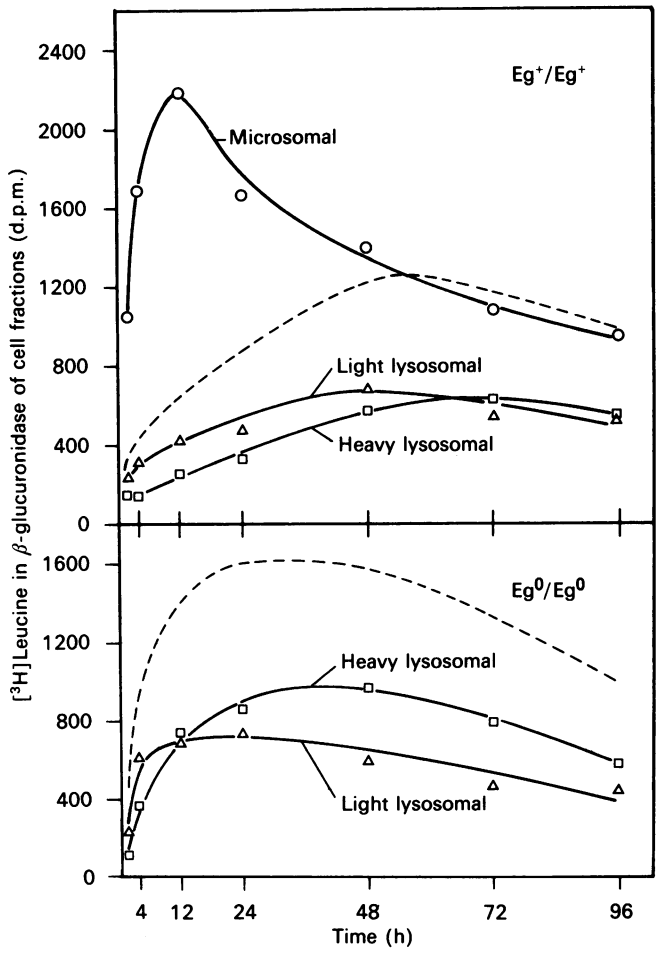

Fig. 4. [^{3}H]Leucine incorporation profiles of β-glucuronidase in cell fractions

[^{3}H]Leucine incorporation into β-glucuronidase in the microsomal (○), light lysosomal (△), and heavy lysosomal (□) fractions in Eg^+/Eg^+ and Eg^0/Eg^0 livers. Each time point is the average of three livers. The dotted line represents the sum of the incorporation values of the two lysosomal fractions together.

the results obtained from Eg^+/Eg^+ livers would represent mainly the appearance of the L_b form and those from Eg^0/Eg^0 livers mainly the L_a form. This assumption was based on the finding, by isoelectric focusing and densitometry, that in both the light and the heavy lysosomal fractions the L_a/L_b ratio was 1:3 in Eg^+/Eg^+ and 4:1 in Eg^0/Eg^0 livers. The microsomal fractions contained only the M form of β-glucuronidase which, in the Eg^0/Eg^0 mice, represented less than 1% of the total enzyme activity.

Labelled β-glucuronidase appeared early in all fractions (Fig. 4), but labelling reached its maximum at different times, i.e. after 12 h in the microsomes and after 50 h in the combined lysosomal fraction. Data suggested an increase of labelled enzyme first in the light and then in the heavy lysosomes, but the difference was small and therefore of questionable significance. In the microsomes of the Eg^0/Eg^0 livers, there was no measurable labelled β-glucuronidase. Here again, labelled β-glucuronidase appeared earlier in the light than in the heavy lysosomes, with a combined maximum at 24 h. Apparent half-lives of β-glucuronidase were 2.9 days in the Eg^+/Eg^+ microsomes, and 3.9 days in the Eg^0/Eg^0 lysosomes (light and heavy lysosomes combined). Since the experiment was not extended beyond 96 h, the data were insufficient to determine the half-life of the enzyme in the Eg^+/Eg^+ lysosomes. Overall, the appearance and disappearance of the labeled β-glucuronidase in the cell fractions mirrored the time course of synthesis and degradation of the β-glucuronidase forms in whole liver. Specifically, the labelling maximum of Eg^+/Eg^+ microsomes (Fig. 4) matched that of the microsomal form (Fig. 2), that of the Eg^+/Eg^+ lysosomes matched that of the L_b form, and the labelling maximum of the Eg^0/Eg^0 lysosomes corresponded to that of the L_a form.

DISCUSSION

Our results appear to confirm the hypothesis that microsomal β-glucuronidase is a precursor of the L_b, but not of the L_a, lysosomal enzyme form (Beltramini-Guarini *et al.*, 1984). Newly synthesized enzyme appeared as the microsomal enzyme form earlier, and more rapidly, than as the L_b form, and the half-life of the former was considerably shorter. The time courses of synthesis of the microsomal and the L_a enzyme forms in Eg^+/Eg^+ liver exclude a precursor–product relationship. Furthermore, the synthesis (Fig. 2) and degradation (Fig. 3) of the L_a form follow the same course whether egasyn is present (Eg^+/Eg^+) or absent (Eg^0/Eg^0).

The synthesis of some L_b enzyme can apparently bypass the microsomal enzyme form. First, in Eg^+/Eg^+ mice, the decline in the amount of labelled microsomal enzyme form was slower than the rise in the amount of labelled L_b form. Secondly, some L_b enzyme (17% of the total) was present in Eg^0/Eg^0 livers, in the absence of the microsomal enzyme. This finding extends that of Swank *et al.* (1986), who observed that L_b was the major enzyme form in Eg^0/Eg^0 kidney. The proposed relationship between the β-glucuronidase forms is shown in Fig. 5.

Our observation that the microsomal enzyme form appears to be transformed to the L_b form contrasts with the view of Smith & Ganschow (1978). In their incorporation studies in the same Eg^+/Eg^+ mouse strain, they followed the increase of labelled β-glucuronidase in lysosomes and microsomes. It proceeded at the same rate for 24 h in microsomes and for 48 h in lysosomes. They concluded that there was independent processing of the β-glucuronidase in the two organelles and that the processing of the lysosomal and the microsomal β-glucuronidases was independent, and that the latter remained stationary in the microsomes. At the time the existence of two lysosomal enzyme forms (Beltramini-Guarini *et al.*, 1984) was unknown. Our present studies have shown that the appearance of the L_a form was independent of the presence or absence of microsomal enzyme, and that, in contrast, the L_b form made a delayed appearance, reaching its maximum labelling 2–3 days after that of the microsomal enzyme, and was prominent only when the microsomal enzyme was also present. (The combined incorporation curves of the lysosomal enzyme forms is almost identical to that published by Smith & Ganschow, 1978.) Therefore, the accumulated evidence indicates that the microsomal enzyme does, in fact, give rise to L_b enzyme. This, of

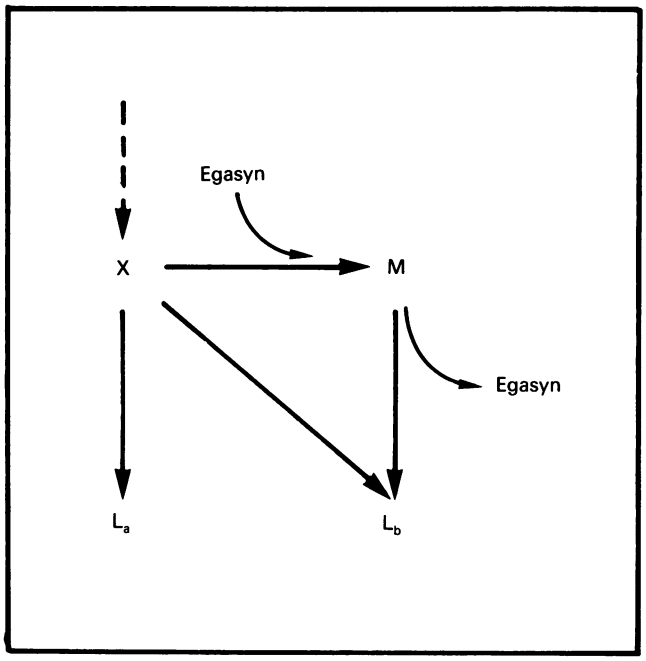

Fig. 5. Hypothetical representation of the biosynthesis of β-glucuronidase forms

Hypothesis of the biosynthesis of mouse β-glucuronidase and the transition of its various forms. X, microsomal precursor form; M, microsomal form complexed to egasyn; L_b, basic lysosomal form; L_a, acidic lysosomal form.

course, does not exclude an enzymic function of the microsomal enzyme while it resides in the microsomal compartment (Gigon & Bickel, 1979; Belinsky *et al.*, 1984).

The microsomal enzyme form occurs in the presence of egasyn, to which it is bound (Swank & Paigen, 1973). The congenic lines of Eg^+/Eg^+ and Eg^0/Eg^0 mice studied here share the same genetic background, including the gene complex for β-glucuronidase on chromosome 5 and, as far as is known, differ only at the egasyn locus on chromosome 8 and the immediate adjacent chromosome region. It is, therefore, reasonable to conclude that differences in β-glucuronidase processing between these lines are related to egasyn. The question arises how egasyn exerts its role. Recently, egasyn has been identified as esterase-22 (Medda & Swank, 1985; Medda *et al.*, 1986), and is presumed to be a carboxypeptidase bound through its active site to the microsomal form of β-glucuronidase. Whether β-glucuronidase is, in fact, enzymically modified by egasyn is unclear. The structural identity (molecular mass, net charge) of the L_b enzyme form in tissues containing or lacking egasyn suggests that no enzymic modification by egasyn occurs. Instead, the delay with which maximal incorporation of label into the microsomal form occurs, suggests that egasyn somehow retains the microsomal enzyme form in the endoplasmic reticulum allowing it to be further modified to the L_b form. Nevertheless, both lysosomal forms do occur, regardless of whether egasyn is present or not, albeit in different proportions and concentrations. Thus, the proportions of L_a and L_b may depend on the residence time of the enzyme in the endoplasmic reticulum. If it is short the molecules become L_a, if long, they become L_b.

It is not known what physiological needs, if any, are met by the formation of two different lysosomal enzyme forms. Eg^0/Eg^0 mice which in liver synthesize less L_b form, and have less total lysosomal β-glucuronidase than the Eg^+/Eg^+ animals, thrive equally well in a laboratory environment. There is no prevalence of one or the other form in the heavy or the light lysosomes, although the two forms do differ in their oligosaccharide moiety (Swank *et al.*, 1986). The L_a form, in contrast to the L_b form, carries complex type oligosaccharides, and, therefore, at least this form must have traversed the trans-Golgi apparatus *en route* to the lysosomes. Our results show that the L_a form arrives earlier in the lysosomal compartment than does the L_b form. The two forms probably travel to the lysosomes along different routes, although firm evidence for this is still lacking. The two forms also differ in that the half-life of the L_a form is shorter. Since the specific activity is identical in all forms of β-glucuronidase, and assuming equal rates of β-glucuronidase synthesis in Eg^+/Eg^+ and Eg^0/Eg^0 mice, which is the case after 4 h of labelling, the shorter survival of the predominant L_a form accounts for the lower total activity of β-glucuronidase/g of Eg^0/Eg^0 liver. To our knowledge, this is the first demonstration that the survival time of an enzyme changes with variation in its post-translational modification.

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