Egasyn, a Protein Which Determines the Subcellular Distribution of β-Glucuronidase, Has Esterase Activity*

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The glycoprotein egasyn complexes with and stabilizes precursor β-glucuronidase in microsomes of several mouse organs. Several observations indicate egasyn is, in addition, an esterase. Liver homogenates of egasyn-positive strains have specific electrophoretically separable esterases which are absent in egasyn-negative mice. These esterases react with anti-egasyn serum. A specific esterase was likewise complexed with immunopurified microsomal β-glucuronidase. The esterases were, like egasyn and microsomal β-glucuronidase, concentrated in the microsomal subcellular fraction. Egasyn which is not bound to β-glucuronidase, which represents 80–90% of total liver egasyn, is not complexed with other liver proteins. Egasyn, therefore, specifically stabilizes β-glucuronidase in microsomes. The esterase activity is inhibited by bis-p-nitrophenyl phosphate indicating it is a carboxyl esterase. Several possible functions of egasyn-esterase activity are discussed.

Mouse β-glucuronidase (EC 3.2.1.31) is an unusual acid hydrolase in that it has a dual intracellular localization, being present in both lysosomes and microsomes of several organs including liver and kidney (reviewed in Lusis and Paigen, 1977). In microsomes, but not in lysosomes, an accessory 64-kDa glycoprotein, egasyn, is complexed with β-glucuronidase and serves to stabilize from 40 to 60%, depending on the inbred strain examined, of total liver β-glucuronidase in microsomes. All biochemical and genetic criteria indicate the same β-glucuronidase polypeptide subunit is present at each intracellular site. From 1 to 4 molecules of egasyn complex with microsomal β-glucuronidase to form components of progressively increasing molecular weight termed M1–M4 (Swank and Paigen, 1973). Inbred mouse strains that lack egasyn are unable to maintain β-glucuronidase in microsomes (Ganschow and Paigen, 1967). Thus, the egasyn-β-glucuronidase system serves as a model system for the targeting of an enzyme to a specific subcellular location by complexation with an accessory protein.

The facts that the β-glucuronidase gene is located within a cluster of esterases on chromosome 8 (Karl and Chapman, 1974; Peters, 1982) and that egasyn has structural similarities to rat microsomal esterases (Heymann, 1980; Robbi and Beaufay, 1983) encouraged us to test if egasyn has esterase activity. In the present report, we conclude that egasyn not only complexes with and stabilizes β-glucuronidase in microsomes but is also a carboxyl esterase with multiple electrophoretic forms. Also, egasyn not complexed with β-glucuronidase, which represents 90% of total liver egasyn (Lusis et al., 1976), exists as a free protein rather than being complexed with other proteins.

EXPERIMENTAL PROCEDURES

Mouse Strains—Mouse strain C57BL/6J (Eg+/Eg+) was obtained from the Jackson Laboratory (Bar Harbor, ME). A congenic mouse strain, deficient in egasyn (Eg+/Eg−), was produced by Dr. Verne Chapman of this department. The congenic strain was produced by 14 generations of backcrossing the Eg− gene from the YBR ES/Eg− strain into the C57BL/6J strain with selection for the Eg− gene, which is closely linked to the Eg− allele on chromosome 8. These mice are, thus, genetically identical to wild type mice except for a small segment of chromosome 8 which carries the egasyn mutation. The congenic strain C57BL/6J [Gus+], also developed by Dr. Chapman, was produced by 12 generations of backcrossing to C57BL/6J and selecting for the [Gus+] allele of the β-glucuronidase gene on chromosome 8. This allele of β-glucuronidase has a more basic isoelectric point than normal [Gus0] alleles. LTS/A and STS/A inbred strains, also deficient in egasyn, were obtained from Dr. Nikkels and Dr. Hilgers of the Netherlands Cancer Institute, Amsterdam. Other mouse strains were obtained either from the Jackson Laboratory or from our breeding colony at Roswell Park. Unless otherwise stated, 4–8-week-old mice were used throughout the experiments.

Liver Homogenates—Liver tissues from various mouse strains were homogenized at 0 °C in 9 volumes of 0.05 M Tris-HCl pH 7.4, using a Polytron (Brinkmann) for 2 min at setting 5.5. 1.5 ml of homogenized sample was sonicated (Heat System Model-220) for 45 s at 0 °C at output setting 3. In some experiments, a portion of each sample was heated at 56 °C for 6 min. This treatment served to intensify the esterase activity of heat-stable egasyn esterase components in comparison to other relatively heat-labile esterases. Both heated and unheated samples were centrifuged at 100,000 g for 30 min using the Beckman Ti-50 rotor. Supernatants between pellet and floating lipid layers were collected. Ten μl of supernatant were used for polyacrylamide gel electrophoresis. For recognition of egasyn by anti-egasyn antibody, 10–20 μl samples were left overnight at 4 °C with 10 μl of anti-egasyn antibody, and the total mixture was loaded on the gel. Egasyn antibody was developed and provided by Lusis et al. (1976). It has been demonstrated by several criteria (Lusis et al., 1976) that this antibody does not react with β-glucuronidase.

Electrophoresis—Multiple forms of esterases and glucuronidase were separated on nondenaturing polyacrylamide slab gels at pH 8.1 as described by Swank and Paigen (1973). The 10-cm gel contained 6% polyacrylamide with a 1.5-cm layer of 3% polyacrylamide at the gel origin. Electrophoresis was at 4 °C for 2 h at constant voltage (300 V).

Enzyme Activity Staining—Gels were stained for β-glucuronidase activity using naphthol AS-BI-β-D-glucuronide substrate as described by Swank and Paigen (1975). The 10-cm gel contained 6% polyacrylamide with a 1.5-cm layer of 3% polyacrylamide at the gel origin. Electrophoresis was at 4 °C for 2 h at constant voltage (300 V).

Gels were stained for β-glucuronidase activity using naphthol AS-BI-β-D-glucuronide substrate as described by Swank and Paigen (1973) except that Fast Garnet GBC salt was used in place of paraarsonialine. The final staining solution contained 40 mg of naphthol AS-BI-β-D-glucuronide and 40 mg of Fast Garnet in 100 ml of 0.2 M sodium acetate, pH 5.2. Esterase activity was visualized with α-naphthyl acetate/Fast Blue BB (Komm, 1963). Forty mg of α-naphthyl acetate and 200 mg of Fast Blue BB salt in 100 ml of 0.1 M phosphate buffer, pH 7.0, were used for esterase staining.

Isolation of Egasyn from the Microsomal Glucuronidase-Egasyn Complex—Egasyn was isolated as described by Lusis et al. (1976) with

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some modifications. Unless otherwise stated, all operations were at 2-4 °C. Livers were immediately transferred into an ice-cold beaker and suspended at 10% (w/v) in 0.02 M imidazole-HCl, pH 7.4, containing 0.25 M sucrose. The suspended liver tissues were homogenized in a Polytron (Brinkman) setting 5,5, for 2-4 min depending upon the tissue weight. Homogenates were centrifuged at 35,000 rpm for 45 min using a Beckman 35 rotor. The pellet was collected and suspended in the same buffer containing 0.25 M sucrose in its original volume. After recentrifugation at 35,000 rpm for 45 min, the pellet was suspended at its original volume in 0.15 M NaCl, 0.05 M Tris-HCl, pH 7.4, and sonicated in 8-ml portions for 1.5 min at 0 °C in a Heat Systems Model 220 sonifier at setting 3.0. We noticed that some lipid or lipoprotein-like material which interferes in the later stages of egasyn purification is extracted by Triton X-100, whereas sonication minimizes this problem. The supernatant was collected by centrifugation at 35,000 rpm for 45 min, the β-glucuronidase activity of this supernatant (90% of total) was measured.

An equivalent amount of antiguaronidase antibody (Skudlarek and Swank, 1979) was added to the above glucuronidase preparation, and the mixture was allowed to stand overnight at 4 °C. The immunoprecipitate was collected by centrifugation and washed five times with 0.05 M Tris-HCl, pH 7.4, containing 1% Triton X-100 and 0.15 M NaCl. Finally, the complex was suspended in 0.05 M Tris-HCl, pH 7.4, and egasyn was released from the immunocomplex by heating at 56 °C for 6 min and obtained by collecting the supernatant after centrifugation.

Subcellular Fractionation—Microsomal, lysosomal, and soluble fractions were prepared by the osmotic shock method (Brandt et al., 1975) except that a Dounce homogenizer (4 loose strokes followed by 1 tight stroke) was used in place of a Potter-Elvehjem homogenizer to minimize breakage of microsomal vesicles.

Materials—Naphthyl acetate, Fast Blue BB, Fast Garnet GBC salt, α-naphthol AS-β-d-glucuronide, EDTA, phenylmethylsulfonyl fluoride, 1,10-phenanthroline, apoprotein (bovine lung), peptatin A, trypsin inhibitor (soybean), and bis-p-nitrophenyl phosphate were from Sigma. Reagents for polyacrylamide gel electrophoresis were from Bio-Rad. All other reagents were of analytical grade.

RESULTS

Glucuronidase Electrophoretic Components of Egasyn-Positive and -Negative Strains—Three egasyn-positive (Eg+/Eg+) (C57BL/6J, C57BL/6J [GusN] and BALB/cJ) and three egasyn-negative (Eg+/Eg+) (C57BL/6J E$/E$, STS/A and LTS/A) inbred mouse strains were examined for β-glucuronidase and esterase electrophoretic components. The congenic mouse strain C57BL/6J E$/E$ is genetically identical to C57BL/6J [GusN] except for the region of chromosome 8 containing the egasyn gene. The mutations in inbred strains LTS/A and STS/A are allelic to the egasyn gene. The mutations in inbred strain E$/E$ and absent in nonheated extracts, because a structural gene alteration in egasyn shifts the isoelectric point to a more negative strain, YBR. They are thus also homozygous Eg+/Eg+ strains, although they are independent of origin.

Eg+/Eg+ mouse strains possess two distinct groups of β-glucuronidase activity bands when whole liver homogenates are electrophoresed on pH 8.1 nondenaturing gels (Fig. 1). The first group, designated M1-M5 (M6 requires additional staining to visualize), represents the series of microsomal β-glucuronidase-egasyn complexes and has lower mobility than the second group, lysosomal β-glucuronidase, which is not complexed with egasyn (Swank and Paigen, 1973). Eg+/Eg+ mouse strains have only lysosomal glucuronidase. The lack of microsomal β-glucuronidase in Eg+/Eg+ mice is due to the lack of egasyn, which normally remains complexed with β-glucuronidase (Lusis and Paigen, 1977). These three mouse strains were used as controls which lack egasyn in the following experiments.

Electrophoresis of Egasyn-positive and Egasyn-negative Strains—A large number (Fig. 2) of liver esterases are separable by acrylamide gel electrophoresis.

The two faintly staining esterases of low mobility designated M2 and M3 are present in all nonheated samples of Eg+/Eg+ and absent in nonheated Eg+/Eg- extracts. Several lines of evidence suggest these two esterase isozymes represent the previously described (Swank and Paigen, 1973) high molecular weight M forms of the β-glucuronidase-egasyn complex. First, they were found to co-migrate with microsomal β-glucuronidase components M4 and M5 (Fig. 4) in the pH 8.1 gel system. Second, in Eg+/Eg- mice, which contain no microsomal β-glucuronidase, there are no detectable esterase components in the M2 and M5 region (Fig. 2). Third, esterase components M2 and M3 are lost in heated extracts (Figs. 3 and 5) of Eg+/Eg+ strains in agreement with the known rapid dissociation of the β-glucuronidase-egasyn complex at 56 °C (Swank and Paigen, 1973). Finally, treatment of nonheated Eg+/Eg+ extracts with anti-egasyn sera removed components M2 and M3 (Fig. 8).

Another group of esterases designated E1-E4 is present in Eg+/Eg+ and absent in Eg+/Eg- extracts. This group is not easily discerned in nonheated extracts because of other co-migrating esterases (Fig. 2). Their resolution is somewhat improved when extracts are heated at 56 °C prior to electrophoresis (Fig. 3), since E1-E4 is relatively heat-stable compared to other esterases. In heated extracts, at least two of these esterases, E1 and E4, are present in all Eg+/Eg+ and absent in all Eg+/Eg- strains. That the entire group of esterases, E1-E4, is present in C57BL/6J (Eg+/Eg+) and absent in congenic Eg+/Eg+ mice is clear when extracts of younger (10-day-old) mice are examined (Fig. 5). In 10-day-old mice, the liver esterase pattern is greatly simplified, as noted by Eisenhardt and von Deimling (1982).

Finally, it should be noted that there are Eg+/Eg+ mouse strains such as Mus musculus molossinus (Fig. 8) in which both the M (M2 + M3) and E (E1-E4) esterases are clearly discernible even in nonheated extracts, because a structural gene alteration in egasyn shifts the isoelectric point to a more

\[ V. M. Chapman, unpublished data. \]
Egasyn Has Esterase Activity

Fig. 2. Esterases of nonheated extracts of Eg⁺/Eg⁺ and Eg⁺/Eg⁻ mouse strains. 10% liver homogenates were sonicated for 45 s at 0 °C and centrifuged at 100,000 × g for 35 min. 10 µl of the supernatant between the pellet and the floating lipid layer was applied to nondenaturing gels and esterase activity was detected by staining with α-naphthyl acetate/Fast blue BB.

Acidic form so that there are no esterases with overlapping isoelectric point.

A few other esterases in addition to the M and E forms are either missing or altered in mobility in individual Eg⁺/Eg⁻ strains (Figs. 2 and 3). However, only the M and E form esterases are present in all Eg⁺/Eg⁺ and absent in all Eg⁺/Eg⁻ strains. The fact that other esterases, in addition to E₁ and E₂, are either missing or altered in mobility in the congenic mutant (as compared with the C57BL/6J strain) is expected. A short segment of chromosome 8 of strain YBR, the strain of origin of the Eg⁻ mutant, is retained in the congenic after 16 generations of backcrossing the Eg⁻ gene into strain C57BL/6J. This segment would likely include the chromosomal location of eight closely linked esterases (Peters, 1982), several of which differ between C57BL/6J and YBR.

Recognition of Esterases E₁-E₄ by Anti-egasyn Serum—Liver homogenates of Eg⁺/Eg⁺ and Eg⁺/Eg⁻ mice were treated with anti-egasyn serum prior to electrophoresis to determine if egasyn antibody interacted with specific esterases. That all four esterase components E₁-E₄ are recognized or altered in mobility with specific esterases. That all four esterase components E₁-E₄ are recognized by egasyn antibody became apparent after analysis of the simplified patterns of 10-day-old mice (Fig. 5). Antibody recognition was judged by the loss of esterase activity bands and by the concomitant appearance of bands at the gel origin of esterase activity arising from the formation of high molecular weight egasyn-antibody complexes. Similar analyses of unheated liver extracts of the M. m. molossinus strain (Fig. 8) showed that both M and E esterases were recognized by egasyn antibody. In other experiments there were no interactions of esterases with normal serum. After treatment of extracts of Eg⁺/Eg⁻ mice with anti-egasyn, there were traces of activity at the 3–6% gel boundary, but no activity in high molecular weight complexes at the gel origin. Similar results were obtained when extracts of other Eg⁺/Eg⁻ strains LTS/A and STS/A were treated with egasyn antibody (not shown). The trace activity at the 3–6% gel boundary may arise from small amounts (less than 1%) of egasyn in Eg⁺/Eg⁻ (Lusis et al., 1976) mice or from minor interaction of the egasyn antiserum with other esterases. Eisenhardt and von Deimling (1982) have reported the mouse esterases of chromosome 8 have some immunological relatedness.

Copurification of Esterase and β-Glucuronidase by Anti-β-Glucuronidase Serum—An independent method of determining if egasyn associated with β-glucuronidase in the microsomal complex contains esterase activity is to purify the complex with anti-β-glucuronidase antibody (Lusis et al., 1976) and analyze the washed antibody precipitate for esterase activity. When the immune complex isolated from Eg⁺/Eg⁻ mice was heated at 56 °C to dissociate the egasyn-β-glucuronidase complex and the supernatant electrophoresed, there was considerable esterase activity with mobilities identical to E₁-E₄ (Fig. 5). These purified components E₁-E₄ were recognized...
Egasyn Has Esterase Activity

FIG. 4. Co-migration of microsomal β-glucuronidase and esterase components of Eg⁺/Eg⁺ mice. Liver homogenates of C57BL/6J Eg⁺/Eg⁺ mice were prepared as in Fig. 2. After electrophoresis of 10-μl nonheated samples in adjacent lanes, the lanes were separated. One lane was stained for β-glucuronidase using α-naphthol AS-BI-P-D-glucuronide/Fast Garnet and the other for esterase with α-naphthyl acetate/Fast Blue BB.

by egasyn antiserum but were unaffected by normal serum (not shown). Mouse strain C57BL/6J-Es₁Egᵦ which lacks egasyn had negligible esterase activity associated with the β-glucuronidase immunoprecipitate (not shown). Thus, esterase components E₁-E₄ represent egasyn. Also, free egasyn and egasyn which was associated with microsomal β-glucuronidase have similar physical properties since they have identical mobility in the pH 8.1 gel system. In other experiments, we have shown that immunopurified β-glucuronidase not complexed with egasyn has no detectable esterase activity.² Thus, it is the egasyn portion of the microsomal β-glucuronidase-egasyn complex which contains the esterase activity.

Inhibitor Studies—The various esterases of the mouse have been characterized according to their sensitivity to a variety of inhibitors (Peters, 1982). Among seven typical inhibitors tested (Fig. 6), only bis-p-nitrophenyl phosphate, an inhibitor of carboxyl esterase activity (Heymann and Mentlein, 1981), inhibited the esterase activity associated with purified egasyn. Nearly complete inhibition likewise occurred with the lower concentration of bis-p-nitrophenyl phosphate (2 X 10⁻⁴ M), used by Eisenhardt and von Deimling (1982). A much smaller degree of inhibition was caused by phenylmethylsulfonyl fluoride. It is recognized that diffusion of various inhibitors into gels is a variable process, and thus, the results are only an approximation. Nevertheless, clear inhibition with bis-p-nitrophenyl phosphate was observed under these conditions.

Subcellular Location of Esterases E₁-E₄—Previous work has established that both the microsomal β-glucuronidase complex (Swank and Paigen, 1973) and liver egasyn (Lusis et al., 1976) are found almost exclusively in the microsomal fraction of hepatocyte cells (Beltrami et al., 1984). When microsomal, lysosomal, and soluble fractions were prepared by the osmotic shock technique (Fig. 7), both complexed M and free E egasyn pools were nearly exclusively localized in the microsomal fraction (along with glucuronidase-containing M₁, M₂, and M₃ complexes). Almost all liver esterases with the exception of two soluble forms are concentrated in the microsomal fraction. Extracts of M. m. molossinus were used because egasyn from this strain is a variant structural form with faster

² S. Medda, unpublished data.
Egasyn Has Esterase Activity

Combined genetic experiments and immunological experiments with both anti-egasyn and anti-β-glucuronidase sera and electrophoretic data indicate that egasyn is a carboxyl esterase with multiple electrophoretic forms.

Genetically, all three inbred lines which lack egasyn also lack M and at least some of the E esterase components. In the case of the congenic mutant, it was demonstrated by using liver extracts of 10-day-old mice, which have a relatively simplified esterase pattern after gel electrophoresis, that components E₁-E₄ are missing. Additional genetic evidence that egasyn is an esterase was provided by the demonstration that only esterases of Eg⁰/Eg⁰ mice reacted significantly with egasyn antibody. Also, as predicted from the absence of egasyn in Eg⁰/Eg⁰ mice, anti-β-glucuronidase serum specifically purified β-glucuronidase-associated esterases only in Eg⁰/Eg⁰ mice.

These data are consistent with previous studies showing that the egasyn gene is located within a cluster of esterases on chromosome 8 (Karl and Chapman, 1974; Peters, 1982). Genetic evidence suggests that egasyn is in fact identical with the recently characterized Es-22 (Eisenhardt and von Deimling, 1982) on chromosome 8.

The present experiments, which utilize the newly found esterase activity of egasyn as an assay, show that two pools (M forms and E forms) of egasyn with similar physical properties exist. Both the egasyn complexed with β-glucuronidase (in M forms) and that not complexed with β-glucuronidase (in E forms) have esterase activity. That free egasyn-isozymes E₁-E₄, and egasyn purified from the microsomal β-glucuronidase complex have identical mobility on gels strongly suggests that both free and complexed egasyn have identical physical properties. Although complexed M form egasyn-esterase activity is demonstrable on gels, we have noted that preincubation of gels 10 min at 56°C, a condition known to disrupt

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Egasyn Has Esterase Activity

FIG. 8. Egasyn-esterase is either free or complexed with β-glucuronidase. Liver samples of M. m. molossinus mice were prepared as in Fig. 2. 10 μl of nonheated samples were left with or without anti-egasyn antibody overnight at 4 °C. Egasyn was purified from the same mice by coprecipitation with microsomal β-glucuronidase after treatment with β-glucuronidase antiserum as described under “Experimental Procedures.” After electrophoresis at 4 °C, the gel was heated at 56 °C for 10 min and stained for esterase activity. Heating the gel was found to give a 4-fold increase in intensity of staining of the β-glucuronidase complex. Also, Eisenhardt and von Deimling (1982) have shown by Ferguson plot analysis that esterase-22 isozymes, which are identical to egasyn, have a molecular weight of 65,000 as expected for the free egasyn monomer (Lusis and Paigen, 1977). It remains possible that egasyn interacts with other proteins for a much shorter time or much more weakly than it does with β-glucuronidase precursor. It should also be noted that an assay would not have detected the presence of egasyn bound to proteins, other than β-glucuronidase in such a way that all esterase activity is lost. We believe that such inactive complexes, if they exist, could not form a large fraction of the total egasyn pool since our value of 80-90% of total β-glucuronidase in free complexes agrees with the results of Lusis et al. (1976) who found by a radioimmunoassay which does not depend on esterase activity that 90% of egasyn is not complexed with β-glucuronidase.

Because of the necessity of treating liver extracts with relatively harsh agents like deoxycholate to obtain successful radioimmunoassays, Lusis et al. (1976) hypothesized that egasyn likely was complexed with other proteins which “masked” its immunogenicity. Both we and Lusis et al. (1976) found, on the other hand, that, by other techniques, antibody to egasyn readily forms high molecular weight complexes with glucuronidase-egasyn in the absence of agents which disrupt protein complexes. Similarly, in the present experiments, we found that antibody to egasyn readily recognizes free egasyn without the use of unmasking agents. Thus, the “masking” effect is an unexplained property of the solution radioimmunoassay which is likely not due to the existence of complexes of egasyn with other proteins.

That both M and E esterase components are localized in the microsomal subcellular fraction is in agreement with previous experiments which have localized both 20-40% of total liver β-glucuronidase and all liver egasyn in this fraction (Lusis and Paigen, 1977). In fact, most liver esterases, like egasyn-esterases, are associated with the microsomal fraction (Heymann, 1980). An interesting feature of several microsomal esterases is that despite being located within the lumen of the endoplasmic reticulum, they are not, like luminal secretory proteins, rapidly secreted but rather are stable (Robbi and Beaufay, 1983). These features also apply to egasyn-esterase as recent experiments have established that it is found in the lumen of microsomal vesicles4 and has a half-life of 4.5–5 days (Smith and Ganschow, 1978).

The physiologic function of the esterase activity of egasyn is uncertain. Carboxyesterases form a major portion (3–7%) of the protein of the microsomal subcellular fraction where they serve to detoxify drugs and pesticides and may serve as lipases (Heymann, 1980). Most characterized esterases of rat liver (Heymann, 1980), such as egasyn (Lusis et al. 1976), have a subunit molecular weight of approximately 60,000. In

the mouse, nine esterases are clustered within a 10 centimorgan region of chromosome 8 (Peters, 1982). Thus, they may have arisen by gene duplication and now have similarities in structure and/or function. Lack of egasyn-esterase in Eg⁰/Eg⁰ mice produces no obvious physiologic consequence under laboratory conditions.

There are several speculative functions and/or observations related to egasyn-esterase activity. First, the esterase may serve in proteolytic processing of the β-glucuronidase precursor. β-Glucuronidase (Skudlarek and Swank, 1981), like all other lysosomal enzymes (Skudlarek et al., 1984; Hasilik and von Figura, 1984), is synthesized as a high molecular weight precursor which is proteolytically trimmed (Erickson and Blobel, 1983) either within or just before reaching the lysosome (Brown and Swank, 1983) to lower molecular weight mature form. The form of β-glucuronidase bound to egasyn is the high molecular weight precursor form (Brown et al. 1981).

Several proteases have been shown to possess esterase activity (Peters, 1982).

Second, it is of interest that newly synthesized lysosomal enzymes contain phosphate groups on oligosaccharide units in diester linkage between the hydroxyl group on C-6 of a mannose residue and C-1 of an outer α-linked N-acetylgalcosamine residue. This linkage is, like the bis-p-nitrophenyl phosphate inhibitor of egasyn-esterase, a phosphodiester (Goldberg et al., 1984). Egasyn may recognize, bind to, and/or hydrolyze this naturally occurring phosphodiester. Additional structural features specific to the β-glucuronidase precursor would presumably explain why egasyn does not form stable complexes with other lysosomal enzyme precursors.

It is of interest, finally, that the egasyn-esterase inhibitor bis-p-nitrophenyl phosphate is a member of a class of organophosphorous insecticides which have been shown to cause rapid release of a portion of microsomal β-glucuronidase into serum (Mandell and Stahl, 1977; Kikuchi et al. 1981; Suzuki et al. 1975; Leng and Nakatugawa, 1983) and to convert another portion to a more acidic lysosomal form (Beltrami, et al. 1984), similar in isoelectric point to that observed in liver of mutant mice which do not contain egasyn. It is possible that organophosphorous compounds specifically disrupt the egasyn-microsomal β-glucuronidase complex to give the above effects.

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