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The Transport of Lysosomal Enzymes

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This paper reviews the experimental evidence for the proposal that hydrolytic enzymes are introduced into lysosomes of cultured fibroblasts only after secretion and receptor-mediated recapture.

Key words: lysosomes, lysosomal enzymes, pinocytosis, secretion, α-L-iduronidase

How are hydrolytic enzymes transferred from the site of their synthesis to lysosomes? In the usual concept of lysosome formation, the hydrolases proceed from rough to smooth endoplasmic reticulum, then either to the Golgi apparatus or to a specialized region of the smooth endoplasmic reticulum known as GERL. There they are concentrated into small vesicles (primary lysosomes) which detach from the Golgi or from GERL. Eventually these vesicles fuse with phagocytic, pinocytic, or autophagic vacuoles which contain macromolecules to be hydrolyzed but not the necessary enzymes. The organelles that result from the fusion – i.e., the secondary lysosomes – contain enzymes, substrates, and the appropriately acid environment for hydrolysis to take place (for reviews, see Refs. 1–4). The best evidence for the transport of the hydrolytic enzymes by way of primary lysosomes has been obtained in polymorphonuclear leukocytes. Particles which are rich in acid hydrolases and which correspond to primary lysosomes – the azurophilic granules – have been isolated by centrifugation and have been seen to fuse with phagocytic vacuoles after ingestion of bacteria (2). However, the evidence is weaker for nonphagocytic mammalian cells, since primary lysosomes have not been isolated and their role in enzyme transport has been deduced from static morphological studies by electron microscopy.

THE SECRETION-RECAPTURE HYPOTHESIS

An alternative hypothesis would have hydrolytic enzymes secreted to the cell exterior (perhaps through secretory vesicles), recaptured by a receptor-mediated endocytosis, and only then packaged into lysosomes (Fig. 1). Prior to secretion, the enzymes would be equipped with a structural feature (a "recognition marker") to insure binding to the

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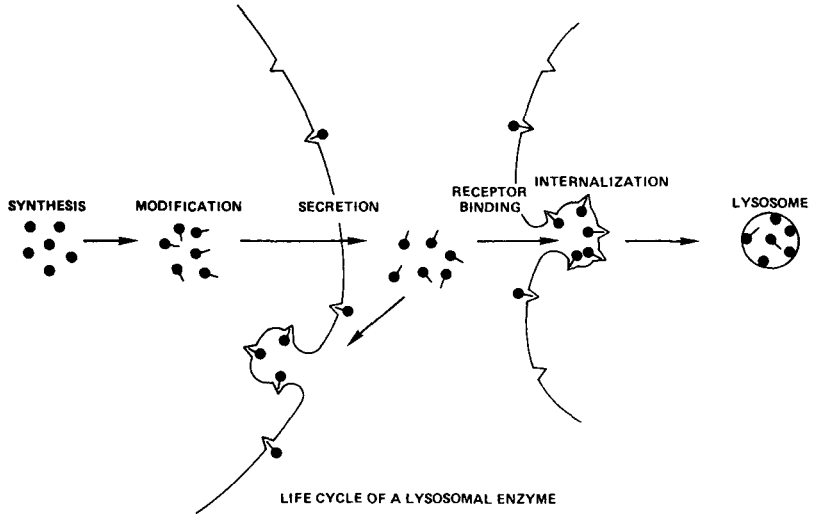

Fig. 1. The secretion-recapture hypothesis for introducing hydrolytic enzymes into lysosomes.

receptors on the cell surface. In this hypothesis, the pinocytic vacuoles could be considered either as primary lysosomes (if no substrate were taken in at the same time) or as secondary lysosomes (if substrate were taken in with the enzymes, or if the internalized membrane were itself the substrate to be hydrolyzed).

This proposal provides a unifying theory for the following observations made on cultured human skin fibroblasts: a) some hydrolytic enzymes, when introduced into the culture medium, are taken into the fibroblasts by an efficient and selective mechanism which depends on a recognition marker on the enzyme and a receptor on the fibroblasts; and b) in fibroblasts from certain human mutants, hydrolytic enzymes appear to lack a recognition marker and are inappropriately located in the extracellular fluid rather than within the lysosomes (5).

Selectivity and efficiency of uptake of some hydrolytic enzymes were first noted in the case of α-L-iduronidase (6) and later for a number of other enzymes which participate in the hydrolysis of mucopolysaccharides, glycoproteins, and glycolipids (Table I). Each of the hydrolases listed also exists in low uptake form, the distinction between "high" and "low" uptake being in part one of degree. Since fibroblasts can probably take in almost any macromolecular substrate introduced into the culture medium (e.g., dextran, ^{125}I-albumin, or horseradish peroxidase) to a slight extent, uptake is considered "low" if it represents internalization of about 1% of the amount present in the medium (over a period of 1–2 days) and "high" if it substantially exceeds that value. Uptakes of 25% or greater have been reported.

More important than the extent of uptake is its saturability, which must be expected of a receptor-mediated mechanism. Saturability of uptake has been shown for urinary α-N-acetylglucosaminidase (14), testicular β-galactosidase (17), platelet β-glucuronidase (19), and urinary α-L-iduronidase (Fig. 2). An apparent K_m of 10^{-9}M has been calculated for the uptake of iduronidase (20).

It is apparent from Table I that there is no correlation between the source of a hydrolytic enzyme or the reaction catalyzed, and its occurrence in high uptake form. The

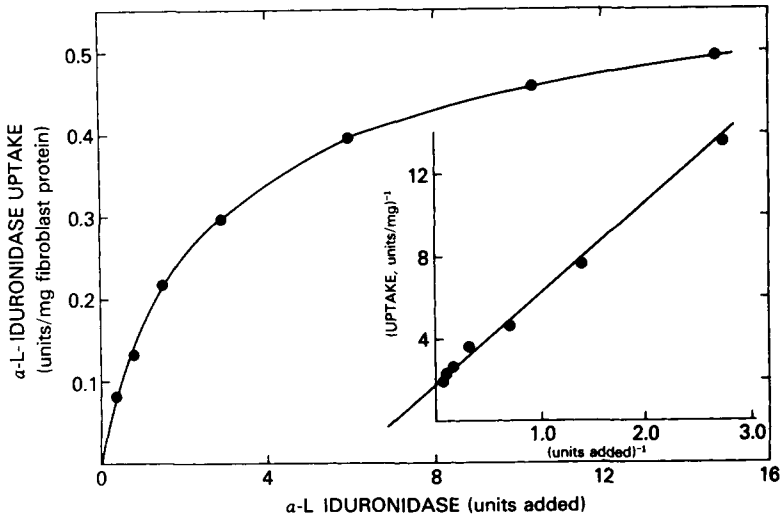

Fig. 2. Dependence of the rate of α-L-iduronidase uptake by cultured fibroblasts on the concentration of enzyme in the medium. Hurler fibroblasts were incubated with increasing levels of iduronidase activity in serum-free medium, at 35°C, for 4 hr. Internalized enzyme activity was assayed in homogenates of the trypsinized cells after 3 cycles of freezing and thawing (21). The assumptions implicit in treating pinocytosis by Michaelis-Menten kinetics are discussed in Ref. 20.

TABLE I. Hydrolytic Enzymes Known to Exist in High or Low Uptake Form

Enzyme	Sources of high uptake form[a]	Sources of low uptake form[a]
α-L-Iduronidase	Urine (6, 7)	Urine (7), kidney (8)
β-Glucuronidase	Platelets (9), spleen (10), liver (11), placenta (11), fibroblasts (11)	Same tissues as high uptake form (10, 11)
β-Hexosaminidase	Fibroblasts (12)	Placenta (12), liver (13)
α-N-Acetylglucosaminidase	Urine (14)	Placenta (15)
Arylsulfatase A	Urine (16)	
β-Galactosidase	Bovine testes (17)	
Iduronate sulfatase	Urine (18)	Serum (18)

[a]All sources are of human origin unless otherwise indicated.

high uptake form of β-hexosaminidase has been converted to the low uptake form (i.e., the recognition marker was destroyed) without affecting the catalytic activity of the enzyme, by treatment with dilute $NaIO_4$ (12). The recognition marker of β-galactosidase was destroyed by digestion with a partially purified preparation of mannosidase from Aspergillus niger (17). These experiments led to the suggestion that a carbohydrate residue is the recognition marker for uptake into fibroblasts, perhaps mannose or some sequence of sugars containing mannose.

Modification of the recognition marker of the enzyme is but one of the approaches to discover its structure; another is the use of inhibitors to compete for the

receptor site. Inhibition experiments have ruled out a terminal β-galactose and β-N-acetylglucosamine residue as the recognition marker for α-L-iduronidase (20), thereby differentiating the recognition of hydrolases by fibroblasts from recognition of circulatory glycoproteins by hepatocytes (22–24). D-Mannose, L-fucose, and some mannosides inhibit the uptake of several enzymes (17, 19, 20). A potent glycoprotein inhibitor of iduronidase uptake, of $K_i \sim 10^{-8}$ M, has been purified from normal human urine; it is thought to be a mixture of hydrolases and denatured hydrolases that use the same receptor. An exciting and unexpected development has been the finding that mannose-6-phosphate and some phosphomannans inhibit the uptake of β-glucuronidase, and that the enzyme itself can be converted to the low uptake form by treatment with alkaline phosphatase (19). The uptake of α-L-iduronidase is likewise competitively inhibited by mannose-6-phosphate and diminished by pretreatment with phosphatase (20). Sly and co-workers (19) have proposed that the recognition marker is a phosphorylated carbohydrate residue, probably phosphomannose, on the high uptake form of hydrolases.

Although most of the uptake systems studied have involved enzymes and fibroblasts of human origin, there appears to be considerable cross-species interaction; bovine and rat fibroblasts recognize the marker on human β-glucuronidase (25) whereas human fibroblasts recognize bovine β-galactosidase (17).

Lysosomal enzymes injected intravenously into rats are rapidly cleared from the circulation, primarily into the liver and spleen (26–28). The clearance of circulating lysosomal enzymes, which has been studied most thoroughly with β-glucuronidase, is mediated by a recognition system different from that of fibroblasts, since enzymes which are of the low uptake form with respect to fibroblasts can be rapidly taken out of the plasma. Periodate reduces the rate of clearance, and from competition experiments it is thought that the recognition is through N-acetylglucosamine residues (29, 30) or through mannose (30a).

GENETIC DISEASES ASSOCIATED WITH INAPPROPRIATE LOCALIZATION OF HYDROLYTIC ENZYMES

As first noted by Wiesmann and colleagues (31, 32), fibroblasts of patients with mucolipidosis II (I-cell disease) are deficient in several lysosomal glycosidases and sulfatases. These intracellular deficiencies are accompanied by an excess of the same enzymes in the patient's body fluids. These observations have been extended to patients with mucolipidosis III (pseudo-Hurler polydystrophy), a clinically milder condition (for reviews of the clinical and biochemical findings in the two disorders see Refs. 5 and 33).

Fibroblasts from such patients are not "leaky" and appear to have normal receptors for hydrolases, for when presented with high uptake α-L-iduronidase, they internalize it with the same velocity and kinetic constant as do other fibroblasts and retain it with the same 9-day half-life (20, 34). On the other hand, the enzymes secreted by fibroblasts of patients with mucolipidoses II or III are of the low uptake form (34). As seen from Fig. 1, the secretion-recapture hypothesis provides a simple explanation for these diverse findings: a mutation in the synthesis of the marker would cause secretion of low uptake enzymes, which would fail to be recaptured and, if stable, would accumulate outside the cell. The many other abnormalities of these mutant fibroblasts [e.g., presence of unusual isozymes (35), excessive sialic acid content of some hydrolases (36), and increased fragility of the fibroblast membranes to freezing and detergent (37)], may be viewed as additional effects

of the primary enzyme defect, or as secondary effects of the many enzyme deficiencies, particularly of the recently discovered deficiency of sialidase (38).

Determination of lysosomal deficiencies, whether by direct measurement of enzyme activity or by observation of storage vacuoles by electron microscopy, shows that the mutation of mucolipidoses II and III is manifested primarily in cells of connective tissue (as well as in certain kidney cells and in Schwann cells) but is not shown by leucocytes, hepatocytes, and neurons. Even in cultured fibroblasts, acid phosphatase and β-glucosidase are not depressed. Thus the effects of the mutation appear limited to some cells and to some hydrolytic enzymes. The secretion-recapture hypothesis must likewise be limited until it is known whether these variations are caused by the existence of more than one recognition system or more than one mechanism for transporting hydrolytic enzymes into lysosomes.

PHYSIOLOGICAL IMPLICATIONS

Although a mechanism involving secreted enzyme as a transport form has not been proposed for any other group of intracellular enzymes, it has an analogy in the secretion and subsequent endocytosis of thyroglobulin by epithelial cells of the thyroid gland (39).

From Fig. 1, it is clear that enzymes may be synthesized in one cell and packaged into the lysosomes of its neighbors. Intercellular exchange of lysosomal enzymes has been invoked to explain the cross-correction of defective mucopolysaccharide catabolism by fibroblasts cultured from patients with genetically distinct mucopolysaccharidoses (5). Intercellular transfer of β-hexosaminidase from normal to deficient cells has been demonstrated by direct assay of the enzyme in single fibroblasts, before and after cocultivation (40); however, no transfer of β-galactosidase or of α-glucosidase was demonstrated in similar experiments, and the reason for the apparent difference is not clear. Transfer of β-glucuronidase has been observed to occur in vivo between cells of many tissues of tetraparental mice (41, 42).

In an attempt to test the secretion-recapture hypothesis, we have grown normal human skin fibroblasts in the presence of goat antibody to human α-L-iduronidase (8). This treatment resulted in a drop of up to one-half of the intracellular iduronidase activity, and the effect was completely reversed when the antibody was withdrawn (Fig. 3). Three other lysosomal enzymes, β-galactosidase, β-glucuronidase, and arylsulfatase A were unaffected. The antibody does not act by inhibiting the catalytic activity of α-L-iduronidase. These data may be interpreted as the result of competition between the antibody and the fibroblast receptors for the extracellular iduronidase. In view of the high affinity of the fibroblasts for the enzyme (see above), it is not surprising that the antibody was least effective at high cell density.

Tulkens et al. (43) previously showed that fibroblasts cultured in the presence of antibodies to liver lysosomal enzymes took on the appearance of cells from mucolipidosis II patients. Although the authors attributed this effect to an inhibition of lysosomal enzymes by endocytosed antibodies, they noted that the quantity of ingested antibody seemed insufficient to explain the observed reduction in hydrolytic activity. A plausible explanation for their experiments, as for ours, is an inhibition by the antibodies of the packaging, rather than of the activity, of lysosomal enzymes.

The pathway for hydrolytic enzymes shown in Fig. 1 suggests several ways in which the level of intracellular and extracellular enzyme could be influenced: by the rates of secretion and internalization, as well as by the rates of synthesis and degradation of the

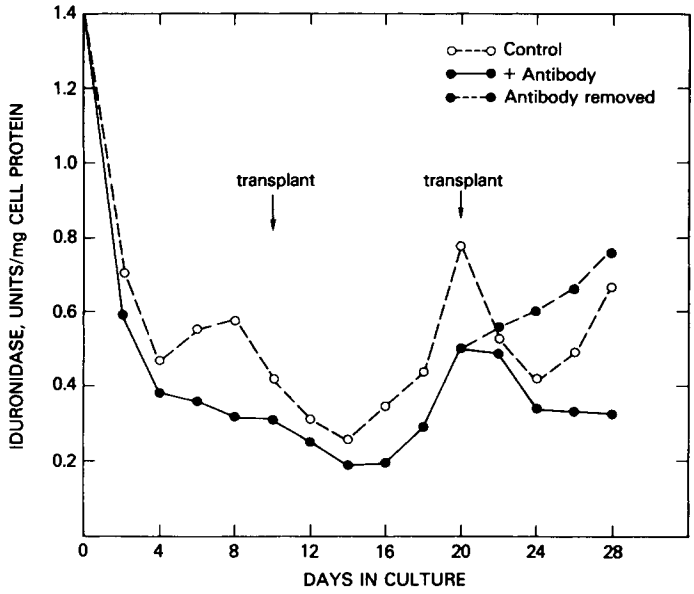

Fig. 3. Effect of anti-iduronidase in the culture medium on the level of intracellular α-L-iduronidase. Normal human skin fibroblasts were grown in 100 mm plastic petri plates, either in modified Eagle's Minimal Essential Medium with 10% fetal calf serum and antibiotics (○--------○) or in the same medium to which goat anti-iduronidase (8) had been added to a concentration of 0.7 mg antibody per ml medium (●——●). Cells were transplanted to low density on days 0, 10, and 20, and medium was changed every other day. On day 20, a set of plates which had been grown in the presence of antibody was transferred to medium without antibody (●---●). Intracellular α-L-iduronidase was measured as previously described (21). Other experimental details will be described elsewhere.

enzyme or receptor. There are physiological and pathological conditions in which the activity of extracellular hydrolases is markedly increased: for instance, in bone exposed to parathyroid hormone (44), in cartilage exposed to Vitamin A (45), and in synovium of patients with rheumatoid arthritis (45). In each case, the increase in extracellular enzyme has been attributed to increased exocytosis from lysosomes. These conditions should be reexamined in the light of the secretion-recapture pathway.

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