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Carbohydrate mediated recognition of lysosomal enzymes by cell surface receptors

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Abstract. The recognition of lysosomal enzymes by various carbohydrate specific cell surface receptors is reviewed. In particular the biosynthesis of mannose 6-phosphate residues in lysosomal enzymes and their role for targeting of lysosomal enzymes to lysosomes are discussed.

Keywords. Mannose 6-phosphate; carbohydrate specific receptors; lysosomal enzyme transport.

Most of the lysosomal enzymes that have been assayed for are glycoproteins (Strawser and Touster, 1980). Among the physiological functions ascribed to the carbohydrate moiety in lysosomal enzymes, were a possible contribution to the catalytic activity and stability towards lysosomal proteases. At present no example is kown in which the catalytic activity of a lysosomal enzyme was shown to depend on its carbohydrate moiety. In contrast, removal or modification of the carbohydrate, had no effect on the catalytic properties of the lysosomal enzymes so far studied. Lysosomal enzymes display a considerable stability towards various proteinases. It is, however, not known to what extent the carbohydrate moiety contributes to the stability of lysosomal enzymes in their physiological environment. Several examples of non lysosomal glycoproteins are known in which oligosaccharides seem to function in stabilizing the polypeptides (Gibson *et al.*, 1980).

In the past ten years it became evident that the carbohydrate moiety in lysosomal enzymes is of importance for the transfer of lysosomal enzymes into lysosomes. A variety of cells expose on their surface receptors that recognize the carbohydrate part of lysosomal enzyme and transfer the receptor bound lysosomal enzymes into lysosomes or related structures. Most of the receptors have been isolated and characterized (Neufeld and Ashwell, 1980).

In table 1 the carbohydrate specific receptors mediating endocytosis of lysosomal enzymes are listed up along with the cell types where they have been identified. The receptors specific for galactose/N-acetylgalactosamine, mannose/ N-acetylglucosamine or L-fucose residues in glycoproteins are not specific for lysosomal enzymes, and it is not clear at present whether they mediate the transfer of lysosomal enzymes under normal conditions.

The recognition of lysosomal enzyme *via* mannose 6-phosphate specific recaptors has gained special interest. Hickman and Neufeld (1972) had shown that genetic deficiency of a recognition marker in lysosomal enzymes from I-cell (mucolipidosis II) fibroblasts is accompanied by loss of newly synthesized lysosomal enzymes into the medium and intracellular deficiency of thse enzymes (II).

Table 1. Carbohydrate specificity of cell surface receptors for lysosomal enzymes

Carbohydrate recognized	Receptors expressed in
Mannose 6-phosphate	Fibroblasts (Neufeld and Ashwell, 1980) Smooth muscle cells (Hasilik *et al.*, 1981) Hepatocytes (Ullrich *et al.*, 1979a)
Mannose/N-acetylglucosamine	Macrophages (Stahl *et al.*, 1978; Ullrich *et al.*, 1979b) Hepatocytes (Ullrich *et al.*, 1979a)
Galactose/N-acetylgalactosamine	Hepatocytes (Ullrich *et al.*, 1979a; Furbish *et al.*, 1978)
L-Fucose	Hepatocytes (Furbish *et al.*, 1980) Macrophages (Shepherd *et al.*, 1981)

This indicated that the physiological function of the recognition marker in lysosomal enzyme, which was later identified as that containing mannose 6-phosphate residues (Kaplan *et al.*, 1977; Sando and Neufeld, 1977; Ullrich *et al.*, 1978; von Figura and Klein, 1979; Distler *et al.*, 1979; Natowicz *et al.*, 1979; von Figura *et al.*, 1979;Hasilik and Neufeld,1980) may be that of a signal,allowing the transfer of newly synthesized lysosomal enzymes into lysosomes. Further evi dence for the requirement of a phosphorylated recognition marker for the proper transfer of lysosomal enzymes was provided by experiments with tunicamycin (Varki and Kornfeld, 1980; von Figura *et al.*, 1981). Tunicamycin interferes with the formation of asparagine linked oligosaccharides, including the mannose 6-phosphate containing ones. The lysosomal enzymes formed in the presence of tunicamycin which are smaller by size are mainly secreted. Intracellular enzyme activities decrease and lysosomal enzymes bound to receptors at the cell surface are absent as in I-cell fibroblasts.

If the function of mannose 6-phosphate containing recognition marker is that of a signal for the segregation of lysosomal enzymes from other glycoproteins and their transport into lysosomes, the recognition signal should be specific for lysosomal enzymes (and non-enzymic lysosomal glycoproteins), and operate in many cell types. So far lysosomal enzymes (von Figura and Klein, 1979; Distler *et al.*, 1979; Natowicz *et al.*, 1979; Hasilik and Neufeld, 1980; Varki and Kornfeld 1980; Hasilik *et al.*, 1980) and their equivalent in yeast, the vacuolar enzymes (Hashimoto *et al.*, 1981) are the only class of glycoproteins in which mannose 6-phosphate containing structures have been detected. Mannose 6-phosphate specific receptors are present at the cell surface and intracellular membranes (Gonzalez-Noriega *et al.*, 1980; Fischer *et al.*, 1980a). They have been found in very organ thus far examined (Fischer *et al.*, 1980b) and cell surface associated and intracellular receptors may operate at two different levels. Mannose 6-phosphate specific receptors at the cell surface transfer lysosomal enzymes form the extracellular compartment into lysosomes. This pathway operates in fibro blasts, hepatocytes, smooth muscle cells and glial cells, whereas endothelial cells and chondrocytes are not capable of internalizing lysosomal enzymes *via* endo cytosis (von Figura *et al.*, 1980). The uptake of lysosomal enzymes from the extracellular compartment seems to be of minor importance for the equipment of the cell with lysosomal enzymes. Selective inhibition of the endocytotic pathway has prac-

tically no effect on cellular levels of lysosomal enzymes (von Figura and Weber, 1978; Sly and Stahl, 1978; Vladutiu, and Rattazzi, 1979). Of major importance for the transfer of newly synthesized lysosomal enzymes into lysosomes, seems their binding to intracellular (Golgi associated) mannose 6-phosphate specific recaptors. It has been postulated that receptor bound lysosomal enzymes are either segregated from other glycoproteins into specific vesciles (primary lysosomes) which fuse with secondary substrate containing lysosomes (intracellular transfer) (Sly and Stahl, 1978), or that the receptor-enzyme complex is not segregated from other glycoproteins at an intracellular level, but is transported with secretory glycoproteins to the cell surface, where they remain largely bound to the cell sur face and enter lysosomes only after endocytosis (transfer *via* cell surface) (von Figura and Weber, 1978). Both pathways do not exclude each other, and have so far not been discriminated experimentally.

Recently some progress was made in elucidating the pathway by which mannose 6-phosphate containing recognition markers are synthesized. High-mannose oligosaccharides in lysosomal enzymes are phosphorylate by transfer of N-acetylglucosamine 1-phosphate to C-6 hydroxyl of mannose residues. UDP-N-acetylglueosamine serves as donor for the N-acetylglucosamine 1 -phosphate (Hasilik *et al.,* 1981; Reitman *et al.,* 1981). The enzyme is located in membranes fractionating with the Golgi marker galactosyltransferase (Waheed *et al.,* 1981). Up to two N-acetylglucosamine 1-phosphate residues appear to become transferred per oligo saccharide. It is unknown whether a single enzyme is responsible for the transfer of both N-acetylglucosamine 1-phosphate residues. The transferase has been solubilized and partially purified from rat liver Golgi membranes. The solubilized transferase requires Mg^{2+} and transfer N-acetylglucosamine 1-phosphate only onto lysosomal enzymes, such as β-hexosaminidase and arylsulphatase *A* from various human and non-human sources, whereas glycoproteins such as ovalbumin, horse radish peroxidase and thyroglobulin did not serve as acceptors (Waheed. *et al.,* 1981 unpublished). The deficiency of the transferase in I-cell fibroblasts explains the formation of lysosomal enzymes that lack the mannose 6-phosphate containing recognition marker (Hasilik *et al.,* 1981). Fibroblasts from patients with mucolipidosis III, a disease closely related to I-cell disease, show residual activities of the transferase of less than 10% of controls (Reitman *et al.,* 1981; Waheed, A., Hasilik, A., Cantz, M. and von Figura K,unpublished).

The product of the transferase are lysosomal enzymes with phosphodiester groups, that have been termed "blocked" phosphate groups, because of their inaccessibility to alkaline phosphatase. The blocking N-acetylglucosamine residues are removed by an α-N-acetylglucosaminyl phosphodiesterase, which is most enriched in the Golgi apparatus, but also present in other membranes of the smooth and rough endoplasmic reticulum (Varki and Kornfeld, 1980; Waheed *et al.,* 1981a, b). The phosphodiesterase is distinguishable by several criteria from the lysosomal α-N-acetylglucosaminidase, which also cleaves the blocking N-acetylglucosamine groups (Varki and Kornfeld, 1980; Waheed *et al.,* 1981). Apparently part of the blocking N-acetylglucosamine residues escapes from the action of the phosphodiesterase (Varki and Kornfeld, 1980; Hasilik *et al.,* 1980; Tabas and Kornfeld, 1980). In β-hexosaminidase and cathepsin D, secreted by human skin fibroblasts, about 80% of the phosphate residues in high-mannose oligosaccharides are blocked (Hasilik *et al.,* 1980). The structural features of the

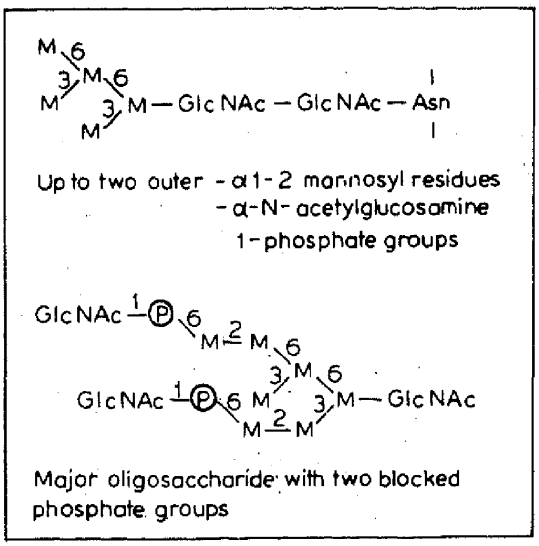

Table 2. Biosynthetic Intermediates of phosphorylated recognition markers in β-hexozaminidase and cathepsin D.

various oligosaccharides with blocked phosphate groups found in β-hexosaminidase and cathepsin D are summarized in table 2. In β-glucuronidase, and total glycoproteins, from mouse lymphoma cells, more than two dozens of different phosphorylated high-mannose oligosaccharides with either blocked, or unblocked phosphate groups, or both, were found (Varki and Kornfeld, 1980).

The elucidation of the pathways for the formation of the phosphorylated recognition marker allowed the identification of two new reactions involved in the processing of the carbohydrate in glycoproteins and of the primary defect in two genetic disorders. Among the interesting questions that came up by these studies are: What kind of signal in lysosomal enzymes directs the N-acetylglucosamine 1-phosphate transferase and confers to the required specificity of the acceptor in that reaction ? Which of the various phosphorylated oligosaccharides in lysosomal enzymes mediate binding to receptors and transfer into lysosomes ?

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