The Lysosome and its Membrane

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Proteolytic and other metabolic pathways in lysosomes

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It is obvious that the maintenance of the differentiated structure of an organism must require approximately as much breakdown of complex molecules as synthesis of them. Moreover, the selectivity of the breakdown processes must precisely complement that of biosynthesis. Discovering the ways in which this is achieved represents an important and challenging aspect of biochemistry and cell biology.

At one time, the lysosomal system tended to be thought of primarily in connection with cell pathology. There was the idea that the lysosomes represent a self-destroy system for the cell, and much emphasis was put on the dire consequences of release of the lysosomal enzymes into the bulk of the cytoplasm, or into extracellular space. Direct experiments cast doubt on this view, however, and today most of us would tend to put more emphasis on the role of the lysosomal system as an integral part of the normal biochemical machinery of the cell.

It is now clear that both lysosomal and non-lysosomal pathways make appreciable contributions to the intracellular degradation of complex molecules, including proteins, polysaccharides, nucleic acids and lipids. The relative importance of the lysosomal and non-lysosomal routes undoubtedly varies between cell types, and different metabolic conditions. Information about the size of the lysosomal contribution can be obtained by determining the effect of treating cultured cells with ammonium chloride, or the salts of other weak bases that accumulate in the lysosomes and interfere with their function. In particular, the bases interfere with the acidification of the lysosomal space, which is necessary for the action of the lysosomal hydrolases, essentially all of which have acid pH optima. The acidification depends on ATP-dependent proton transport, so lysosomal metabolic pathways are at least partially ATP dependent. More specific ways of probing lysosomal metabolism depend on the use of inhibitors of particular enzymes, or the study of the effects of the many known genetic deficiencies of individual lysosomal enzymes.

About 50 lysosomal enzymes are known, and their properties are reviewed by Barrett & Heath (1977). Table I is intended to indicate how these enzymes can be seen as contributing to the pathways for catabolism of four major classes of complex molecules.

I have called the four pathways the ‘proteolytic’, ‘nuclease’ and ‘lipolytic’ pathways. The proteolytic and nuclease pathways start with enzymes specialized for action on the inner regions of the intact linear polymers that are their substrates, the endopeptidases and endonucleases, respectively. There are definite sequences of reactions in the other two pathways, too, although not arranged in quite the same way. It is possible that some of the enzyme molecules may be physically organized in the lysosomes in the order in which they act, as has been found in some other organelles, so that substrate can be passed from one to another in an efficient process. There is little direct evidence for this, and yet, although it has sometimes been found that intact lysosomes provide more efficient catalysis than simple mixtures of the appropriate enzymes in solution. Also, the existence of the proton-transport system that acidifies the lysosomal contents is a clear demonstration that vectoral processes do occur in lysosomes.

The proteolytic pathway

The proteolytic system of the lysosome is one in which no hereditary deficiency has yet been discovered. This may be because most of the deficiencies that occur are lethal in utero. Certainly, the lysosomal proteases must be extremely active recycling proteins in the embryo, where remodelling and growth of tissues are so rapid. Even in the adult, there is no doubt that lysosomal proteolysis is a quantitatively important aspect of metabolism. One tends to think of the major site of proteolysis in the body being the digestive system, but in fact far more protein is broken down in the tissues in protein turnover, and half or more of this in the lysosomal system. Correspondingly, the lysosomes are a more important source of amino acids for new protein synthesis than is the gut.

Mego et al. (1972) isolated lysosomes from cells that had been allowed to endocytose radiolabelled protein, and showed that intracellular degradation of this exogenous substrate proceeded in vitro. The stimulation of the process by ATP and Mg2+ suggested that an active system for acidification of the lysosomal contents made an important contribution. Early evidence of the role of lysosomes in the intracellular degradation of endogenous proteins came from the work of Dean (1975) with liver cells, in which it was shown that pepstatin partially inhibited the process. Since pepstatin is a rather specific inhibitor of lysosomal cathepsin D in cells, this result showed that there must be a lysosomal pathway for degradation of endogenous proteins too. Further work with inhibitors of the lysosomal cysteine proteinases (Shaw & Dean, 1980) and with weak bases, has
more precisely defined the size of the lysosomal contribution. Often, it is in the order of 50% of the total intracellular proteolysis.

The protein substrates for the lysosomal proteases may originate mainly within the cell, or mainly outside, depending upon the cell type. Proteolysis is initiated by the proteinases (endopeptidases), which act in the middle part of the polypeptide chains. The major lysosomal proteinases are cathepsin D (an aspartic proteinase), and cathepsins B, H and L (all cysteine proteinases homologous with papain). They all have acid pH optima, but the acid pH of the interior of the lysosomes facilitates proteolysis not only by providing ideal conditions for the enzymes, but also by their being degraded. The mechanisms by which this occurs are partially unfolded. This is very important for collagen, providing an environment in which many substrate proteins would be expected to have evolved resistance to the proteolytic processing of a number of the lysosomal enzymes, but it is obviously essential that this proteolysis is strictly limited. Naturally, the other lysosomal proteins would be expected to have evolved resistance to the proteinases with which they have to co-exist. One way in which deficiencies of lysosomal enzymes may arise is by a breakdown of this resistance. There are also instances of proteins being transported in the lysosomal system without being degraded. The mechanisms by which this occurs are somewhat mysterious, but their existence is another indication of the sophistication of the lysosomal proteolytic system.

One reason for the effectiveness of the lysosomal system in proteolysis is the very high concentration of some of the enzymes. We have been able to calculate that both cathepsin D and cathepsin B exist at about 1 mmol concentration in the lysosomes of human liver (Dean & Barrett, 1976), this represents 25–40 mg/ml, about 10 000 times more concentrated than they would be in conventional test-tube assays of proteolytic activity. For this reason, the real-life specificities of the enzymes may be much broader than conventional tests with dilute systems would indicate. Such breakdown of specificity suggests another reason why deficiencies of individual proteinases have not been detected: most of the enzymes are not indispensable. This situation could have arisen in evolution to make the important system of lysosomal proteolysis more robust in the face of genetic variation. It may explain why the three homologous cysteine proteinases exist, since copying of the original gene would protect it against deletion. The enzymes have now diverged so that each has its own specificity, but if necessary they could perhaps substitute for one another quite well.

Proteinases are very dangerous enzymes to have at large in the body, and effective systems always exist for keeping them localized. The lysosomal proteinases are, of course, confined by the lysosomal membrane, but additionally, cathepsin D undergoes a conformational change at neutral pH that immediately inactivates it completely although reversibly outside the lysosome. All three of the cysteine proteinases are irreversibly denatured at neutral pH. This process takes a few minutes, but there are also protein inhibitors to regulate the cysteine proteinases. These are the cystatins in the cytosol, and cystatins, α-cysteine proteinase inhibitor and α2-macroglobulin in the plasma (Starkey & Barrett, 1973; Barrett et al., 1984; Gounaris et al., 1984).

It is natural to wonder what interactions exist between the lysosomal proteinases and the other lysosomal enzymes. There is evidence that the lysosomal proteinases bring about proteolytic processing of a number of the lysosomal enzymes, but it is obviously essential that this proteolysis is strictly limited. Naturally, the other lysosomal proteins would be expected to have evolved resistance to the proteinases with which they have to co-exist. One way in which deficiencies of lysosomal enzymes may arise is by a breakdown of this resistance. There are also instances of proteins being transported in the lysosomal system without their being degraded. The mechanisms by which this occurs are somewhat mysterious, but their existence is another indication of the sophistication of the lysosomal proteolytic system.

Intact proteins can be subject to the action of two other types of lysosomal enzyme, in addition to the endopeptidases. These are the phosphoprotein phosphatases, which remove phosphate ester groups from proteins such as casein containing serine phosphate, and the glycosidases which whittle down the carbohydrate prosthetic groups of glycoproteins by stepwise attack from the non-reducing termini. These enzymes form part of the glycanolytic pathway of the lysosomes, of course.

The oligopeptides produced by the action of the lysosomal endopeptidases are further degraded by exopeptidases. The activity of these enzymes should not be underestimated. It is difficult to detect intermediates in lysosomal proteolysis, and this indicates that the endopeptidases are rate limiting, and that once proteolysis has been initiated, it is rapidly taken to completion. Many of the lysosomal exopeptidases are very efficient catalysts. For example, dipeptidyl peptidase I cleaves more bonds in the B-chain of insulin than trypsin, chymotrypsin and pepsin.
together. Evidence is beginning to appear that the sequential action of exopeptidases may become important at an earlier stage in the degradation of many proteins than has previously been thought. Thus, McDonald & Hoisington (1983) has recently shown the existence of a lysosomal tripeptidyl peptidase that liberates N-terminal Gly-Pro-X tripeptides. This is likely to be very effective in breaking up the tripeptide repeating sequence of denatured collagen chains. The Gly-Pro-X tripeptides would be favoured has previously been thought. Thus, McDonald & No/enst ton (1983) has recently shown the existence of a lysosomal tripeptidyl peptidase that liberates N-terminal Gly-Pro-X tripeptides. This is likely to be very effective in breaking up the tripeptide repeating sequence of denatured collagen chains. The Gly-Pro-X tripeptides would be favoured has previously been thought. 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**Metabolic consequences of genetic defects in lysosomes**

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The lysosomal apparatus functions as an intracellular digestive system, in which hydrolytic enzymes with an acid pH optimum bring about the breakdown of biological macromolecules to their low-molecular-weight building blocks. The digestion of macromolecules in the lysosomes is, in general, a stepwise process: each step is catalysed by a specific enzyme, and the product of one reaction forms the substrate for the next reaction in the catabolic pathway.

A deficiency of a lysosomal enzyme leads to accumulation of the substrates for that enzyme in the lysosomes, and to the specific biochemical changes and characteristic clinical symptoms of a lysosomal storage disease. In man, more than 30 hereditary disorders are at present known in which a lysosomal enzyme, or in some cases more than one enzyme, is deficient. Within each disease entity in the lysosomal storage diseases there is considerable heterogeneity.

All lysosomal enzymes that have been studied so far are synthesized as precursors with molecular masses that are higher than those of the mature products. All are glycoproteins and it is now clear that the oligosaccharide moiety plays an essential role in directing lysosomal enzymes to the primary lysosomes.

The stages in the synthesis and maturation of lysosomal enzymes may be summarized as follows (Hasilik, 1980; Hasilik & Neufeld, 1980a, b; Tager et al., 1981; Sly & Fischer, 1982; Von Figura & Hasilik, 1984). The synthesis of the precursor polypeptides occurs on membrane-bound ribosomes; a signal sequence ( Erickson et al., 1981) and the signal recognition particle (Erickson et al., 1983) provide a mechanism for translocation of the nascent polypeptides across the membrane of the endoplasmic reticulum, where glycosylation of the polypeptides is initiated. Asparagine residues occurring in the tripeptide sequence -Asn-X-Ser- or -Asn-X-Thr- serve as acceptors for a complex oligosaccharide containing more than two carbohydrate units. Nine mannose and three glucose residues which is transferred en bloc from a lipid carrier to the polypeptide chain. In the third stage, processing of the oligosaccharide occurs, which involves stepwise cleavage of glucose and some mannose residues.

So far, the events that occur are common to all N-glycosidically linked glycoproteins. However, in the case of lysosomal enzymes, the processing includes formation of mannose 6-phosphate groups in the glycoprotein so that a precursor molecule is formed with oligosaccharide chains that contain mannose 6-phosphate. This occurs in two steps. Firstly, a specific N-acetylglucosaminylphosphotransferase catalyses the transfer of N-acetylglucosaminylphosphate from UDP-N-acetylglucosamine to specific mannose residues in the oligosaccharide moiety of lysosomal enzymes (Hasilik et al., 1981; Reitman & Kornfeld, 1981). Secondly, a specific N-acetylglucosaminylphosphodiesterase catalyses the hydrolysis of the N-acetylglucosamine groups, so that mannose 6-phosphate groups are exposed (Varki & Kornfeld, 1980; Waheed et al., 1981).

The fourth stage involves binding of the precursor molecule to mannose 6-phosphate-specific receptors in the Golgi apparatus; Brown & Farquar (1984) have recently presented evidence suggesting that this occurs in the cis Golgi cisternae. Finally, the precursor is transferred into vesicles destined to become primary lysosomes; this is accompanied by cleavage of the polypeptide chain. The proteolytic cleavage may occur in several steps, some in prelysosomal compartments. Further processing of the oligosaccharide moieties, including removal of phosphate, may occur.

Thus several genes are involved in the formation of a mature lysosomal enzyme: the structural genes for the polypeptides and the genes coding for enzymes involved in the post-translation modifications of the polypeptides which lead to the formation of precursors that can be transported to the lysosomes.

Our present knowledge of how genetic defects lead to deficiencies of lysosomal enzymes and hence to lysosomal storage diseases is summarized in Table 1.

**Consequences of a deficiency of N-acetylglucosaminylphosphotransferase: studies with cultured fibroblasts**

Mucolipidosis II, also known as I-cell disease, and mucolipidosis III, or pseudo-Hurler leukodystrophy, were the first lysosomal storage diseases to be described in which defective processing occurs. These diseases are due to a deficiency of the enzyme responsible for transferring N-acetylglucosaminylphosphate from UDP-N-acetylglucosamine to mannose residues in the glycoprotein; the deficiency is complete in mucolipidosis II (Hasilik et al., 1981; Reitman et al., 1981) and partial in mucolipidosis III (Reitman et al., 1981; Hasilik et al., 1982). Thus, the precursors of the lysosomal enzymes lack the mannose 6-phosphate recognition marker and cannot be transported to the lysosomes. Instead, they are secreted into the medium. In fibroblasts from patients with these diseases several lysosomal hydrolases are deficient (Leroy et al., 1972; Miller et al., 1981). Different forms of mucolipidosis III can be distinguished biochemically (Varki et al., 1981) and the results of complementation studies indicate that at least three genes are involved in the expression of N-acetyl-