371 Willcox, P., and Rattray, S., Secretion and uptake of β-N-acetylglucosaminidase by fibroblasts. Biochim. biophys. Acta *586* (1979) 442–452.
372 Williams, M. A., and Fukuda, M., Accumulation of membrane glycoproteins in lysosomes requires a tyrosine residue at a particular position in the cytoplasmic tail. J. Cell Biol. *111* (1990) 955–966.
373 Wilson, P. J., Morris, C. P., Anson, D. S., Occhiodoro, T., Bielicki, J., Clements, P. R., and Hopwood, J. J., Hunter syndrome: isolation of an iduronate-2-sulfatase cDNA clone and analysis of patient DNA. Proc. natl Acad. Sci. USA *87* (1990) 8531–8535.
374 Woolford, C. A., Daniels, L. B., Park, F. J., Jones, E. W., van Arsdell, J. N., and Innis, M. A., The PEP4 gene encodes an aspartyl protease implicated in the posttranslational regulation of *Saccharomyces cerevisiae* vacuolar hydrolases. Molec. cell. Biol. *6* (1986) 2500–2510.
375 Yamada, M., Myeloperoxidase precursors in human myeloid leukemia HL-60 cells. J. biol. Chem. *257* (1982) 5980–5982.
376 Yen, P. H., Allen, E., Marsh, B., Mohandas, T., Wang, N., Taggart, R. T., and Shapiro, L. J., Cloning and expression of steroid sulfatase cDNA and the frequent occurrence of deletions in STS deficiency: implications for X-Y interchange. Cell *49* (1987) 443–454.
377 Yonezawa, S., Takahashi, T., Ichinose, M., Miki, K., Tanaka, J., and Gasa, S., Structural studies of rat cathepsin E: Amino-terminal structure and carbohydrate units of mature enzyme. Biochem. biophys. Res. Commun. *166* (1990) 1032–1038.
378 Yonezawa, S., Takahashi, T., Wang, X-j., Wong, R. N. S., Hartsuck, J. A., and Tang, J., Structures at the proteolytic processing region of cathepsin D. J. biol. Chem. *263* (1988) 16504–16511.
379 Yoshihisa, T., and Anraku, Y., A novel pathway of import of α-mannosidase, a marker enzyme of vacuolar membrane, in *Saccharomyces cerevisiae*. J. biol. Chem. *265* (1990) 22418–22425.
380 Youngdhal-Turner, P., Rosenberg, L. E., and Allen, R. H., Binding and uptake of transcobalamin II by human fibroblasts. J. clin. Invest. *61* (1978) 133–141.
381 Zubenko, G. S., Park, F. J., and Jones, E. W., Mutations in PEP4 locus of *Saccharomyces cerevisiae* block final step in maturation of two vacuolar hydrolases. Proc. natl Acad. Sci. USA *80* (1983) 510–514.
382 Zühlsdorf, M., Imort, M., Hasilik, A., and von Figura, K., Molecular forms of β-hexosaminidase and cathepsin D in serum and urine of healthy subjects and patients with elevated activity of lysosomal enzymes. Biochem. J. *213* (1983) 733–740.

0014-4754/92/020130-22$1.50 + 0.20/0

Proteases and proteolysis in the lysosome

P. Bohley[a] and P. O. Seglen[b]

[a] *Institute for Physiological Chemistry, University of Tübingen, D-7400 Tübingen (Germany), and* [b] *Department of Tissue Culture, Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, N-0310 Oslo 3 (Norway)*

Abstract. Proteins sequestered by a non-selective bulk process within the lysosomes turn over with an apparent half-life of about 8 minutes and this rapid lysosomal proteolysis is initiated by endopeptidases, in particular by the cathepsins D and L. We describe also the cathepsins B and H which show mainly exopeptidase and only low endopeptidase activity. Especially cathepsin H is most probably the only lysosomal aminopeptidase in many cell types. Additionally, the properties of other mammalian lysosomal endo- and exopeptidases are compared.
Finally, we discuss some of the conditions for the action of lysosomal proteases as the low intralysosomal pH, the high part of lysosomal thiol groups and the absence of intralysosomal proteinase inhibitors.
Key words. Lysosome; protein degradation; proteinase; cathepsin.

Introduction

The autophagic-lysosomal pathway of intracellular protein degradation is a non-selective bulk process[44] that accounts for the main part of total protein breakdown. It has been estimated that more than 90% of all long-lived protein and a large fraction of the short-lived protein are degraded in lysosomes[2]. Protein sequestered within the lysosomes turns over with an apparent half-life of about 8 minutes[54], a value in excellent agreement with data on the functional morphology of lysosomes[58].

Such rapid proteolysis needs a highly effective proteolytic environment, and it is the aim of this review to describe briefly some of the lysosomal proteases. For earlier reviews and more detailed reports compare[1,3–6,11–17,19,35,41,42,51,52,62,68].

We assume that as a rule lysosomal proteolysis is initiated by endopeptidases (proteinases), which are rate-limiting, and that this process is immediately continued by the lysosomal exopeptidases. The free amino acids, and also some dipeptides, can diffuse through the lysosomal membrane. Cytosolic exopeptidases are able to split the remaining peptides rapidly. The resulting free amino acids can be used as substrates for the synthesis of new proteins or for degradation as energy sources.

Lysosomal proteases

An old name for cellular proteases is cathepsin. This name was introduced in 1929 and is derived from the Greek term meaning 'to digest'[74].

Some of the lysosomal cathepsins are now recognized as exopeptidases, such as cathepsin A, which is a carboxypeptidase, or cathepsin C, which is a dipeptidylpeptidase[16,52]. Other cathepsins act as endo- as well as exo-

Table 1. Mammalian lysosomal endopeptidases

Name	Molec.wt	Isoelectric point	Catalytic group	EC number
Cathepsin B	27000	5.4	Cys	3.4.22.1
" D	42000	5.5–6.5	Asp	3.4.23.5
" E	100000	4.1–4.4	Asp	3.4.23.-
" G	30000	10	Ser	3.4.21.20
" H	28000	7.1	Cys	3.4.22.16
" L	29000	5.8–6.1	Cys	3.4.22.15
" N	34000	6.2	Cys	3.4.22.-
" S	24000	6.3–6.9	Cys	3.4.22.-
" T	34000	?	Cys	3.4.22.-
Prolyl endopeptidase	115000	4.8	Ser	3.4.21.16

Cathepsins B and H also show exopeptidase activity (see table 2).

Table 2. Mammalian lysosomal exopeptidases

Name	Molec.wt	Isoelectric point	Catalytic group	EC number
Cathepsin A	100000	5.1	Ser	3.4.16.1
" B	27000	5.4	Cys	3.4.22.1
" C	200000	5.0	Cys	3.4.14.1
" H	28000	7.1	Cys	3.4.22.16
Dipeptidylpeptidase II	130000	4.9	Ser	3.4.14.2
Tripeptidylpeptidase I	57000	?	Ser?	-
Carboxypeptidase B	2× 25000	5.0	Cys	3.4.18.1
Prolylcarboxypeptidase	115000	?	Ser	3.4.16.2
Tyr-carboxypeptidase	?	?	Ser?	3.4.16.3
Peptidyldipeptidase A	145000	4.0	?	3.4.15.1
Dipeptidase I	180000	5.4	Me^{2+}	3.4.13.-
Dipeptidase II	120000	?	Cys	3.4.13.-
-Glu-carboxypeptidase	108000	8.2	Me^{2+}	3.4.22.12

Group Cys = Cysteine as essential catalytic group (+His+Asp).
" Ser = Serine as essential catalytic group (+His+Asp).
" Me^{2+} = Metal ions essential in the catalytic active site.
References can be found in refs 3–5, 11–17, 25, 41, 52 and also in the 'Enzyme nomenclature' of the International Union of Biochemistry, Academic Press, London 1984.
A more detailed table of all cathepsins including some molecular properties and references on the cathepsins F, G, I, J, K, M and R can be found in Bohley[16]. Cathepsin I has been shown to be simply rabbit lung cathepsin H[41]. Cathepsin A is a lysosomal carboxypeptidase A. Cathepsin B is a lysosomal peptidyldipeptidase B. Cathepsin C is a lysosomal dipeptidylpeptidase I. Cathepsin H is a lysosomal aminopeptidase.
Only cathepsin B and cathepsin H also show some endopeptidase activity (compare table 1).

peptidases: cathepsin B shows weak endopeptidase activity and acts also as a peptidyldipeptidase[4, 5, 41] and cathepsin H seems to be the only lysosomal aminopeptidase and acts in some cases also as an endopeptidase[4, 5, 39, 41, 42]. It should be noted that the endopeptidase activities of cathepsin B and cathepsin H are very low compared to the activities of the two main lysosomal endopeptidases: cathepsins D and L[4, 5, 12–17, 40–42, 60]. Nevertheless it is necessary to include cathepsin B and cathepsin H among both the endopeptidase and the exopeptidase, because in these two cases the different activities have been shown unequivocally[4, 5, 16, 41, 52].

Table 1 is a list of selected lysosomal endopeptidases. It does not contain some more specialized proteinases which occur only in a few cell types as for instance acrosin, tryptase and the chymases (for these and also for the cathepsins F, G, I, J, K, M, P, R and S see refs 5, 16 and below). This table shows that lysosomal endopeptidases are mainly cysteine- or aspartic-proteinases with low isoelectric points. The most active proteinases will be described in detail below.

Table 2 shows all mammalian lysosomal exopeptidases which have so far been characterized in some detail. It shows that mainly serine- and cysteine-proteinases belong to this group.

It is impossible to describe all lysosomal proteases in detail in this short review. Therefore, we concentrate here on a few more important examples of cathepsins and make only a few remarks on the other lysosomal proteinases (see below).

Cathepsins are synthesized on membrane-bound ribosomes as precursors, and are transferred co-translationally into the endoplasmic reticulum and later into the Golgi regions. After post-translational proteolytic processing and modification of carbohydrate moieties, they are transported to the lysosomes. This transport is mediated by a receptor that recognizes mannose-6-phosphate residues present on the precursor cathepsins. The process involves signal peptide cleavage, propeptide cleavage and in some cases finally a carboxy-terminal processing[21, 27]. Cathepsin D[60] is one of the most abundant lysosomal enzymes, at least in rat liver, comprising about 10% of the lysosomal protein in this organ[22]. Different cell types vary enormously in their content of cathepsin D, Kupffer cells of rat liver being especially rich[17, 41]. Cathepsin D is an aspartic proteinase with pepsin-like specificity which cleaves preferentially between hydrophobic amino-acid residues. The pH-optima with different substrates have been determined to be in the range 3–5.

The simplest synthetic substrates are peptides with at least five amino acids, for instance GlyPhe – Leu-Gly-Phe[41]. Cathepsin D splits hemoglobin, myosin, angiotensinogen, cytosol proteins, proteoglycans, myelin proteins and endorphins, but there is little action on serum albumin and very little action on collagen or gelatin[41]. Pepstatin is a tight-binding reversible inhibitor of cathepsin D and can be used for affinity purification[8,41]. Cathepsin D contains two oligosaccharides which, like the carbohydrates in other lysosomal enzymes, are involved in the targeting of this proteinase to lysosomes. It is predominantly a two-chain protein, whereas most other aspartic proteinases are single-chain proteins. The proteolytic conversion of a single-chain to a multi-chain enzyme is apparently a universal phenomenon in lysosomal enzymes[77]. Cathepsin D is membrane-associated in macrophage endosomes, but much less in lysosomes[23]. The membrane-associated form is a biosynthetic precursor for the soluble form found in endosomes and in lysosomes[23].

Cathepsin L[40–42] is by far the most powerful lysosomal cysteine proteinase against protein substrates. It degrades proteins at least ten times faster than the other cellular cysteine proteinases, including the cathepsins B and H[12–17]. Some substrate proteins are: myosin, actin, troponins, calmodulin, parvalbumins, tubulin, vimentin, collagen, elastin, hemoglobin, albumin, histones, insulin, glucagon, many cytosolic enzymes, except lactate dehydrogenase[17], and also proteoglycans from bovine nasal septum cartilage[66]. A well-suited peptide substrate is benzyloxycarbonyl-phenylalanyl-arginyl-4-methyl-7-coumarylamide[4,17,41] and a potent, and under very special conditions specific inhibitor is benzyloxycarbonyl-phenylalanyl-phenylalanyl-diazomethyl ketone[4–6,17,41]. This is in good agreement with the substrate specificity of cathepsin L: hydrophobic amino acids at the positions P3 and P2 are necessary, and a further apolar amino acid at P1 may be helpful for rapid hydrolysis[41]. Cathepsin L has been found in many species such as man, rat, pig, pigeon, frog, carp, and *Euglena gracilis* (but not in yeast and not in *E. coli*), and in many organs such as liver (where it occurs mainly in parenchymal cells), kidney, spleen, lung, brain, muscle, stomach, pancreas and in many tumor cells[4–6,12–17,40–42]. The amino acid sequence of cathepsin L shows a high homology to the cathepsins B and H (see below) and to the plant cysteine proteinase papain[59]. The cloned cDNA of cathepsin L encodes a 334-residue protein containing both a 17-amino acid preregion and a 96-amino acid proregion[59]. A 36-kDa precursor was converted intracellularly into a 28-kDa protein and subsequently into a 21-kDa protein[59]. This is in agreement with earlier work which showed a molecular weight of 28 kDa for the unreduced proteinase and the existence of two chains of 22 and 6 kDa, respectively, after reduction of cathepsin L[4]. For a summary of recently reported in vivo studies on the conversion of lysosomal cysteine proteinase precursors and for new results in this field see refs 55 and 73. A comparison of the different chain structures of cathepsin B, H and L is given in table 3.

New inhibitor studies indicate that active cathepsin L is probably essential to its own processing in cultured fibroblasts[64].

Cathepsin L contains carbohydrate and is partially adsorbed to concanavalin A-sepharose[41]. Multiple forms with different isoelectric points (see table 1) may be due to variations in the carbohydrate part. Cathepsin L degrades proteins in the pH range 3–7 in vitro. The specificity for the bonds cleaved seems to be broad in the acidic range, but becomes narrow at pH 7[41].

The great number of different proteinases in mammalian cells makes it necessary to compare the activities against natural substrates to calculate the share of each cathepsin to the total activity. Such comparisons showed that cathepsin L is by far the most active proteinase in hepatocytes and contributes more than 40% of their total proteolytic activity[14–17].

The major excreted protein of transformed fibroblasts is cathepsin L, and human cancer cell lines secrete a thermostable complex of the cathepsin-L-proenzyme (37 kDa) and the mature cathepsin L (31 kDa) which is very sensitive toward the diazomethane inhibitors[75]. The results indicate that cathepsin L secreted from cancer cell lines may play a role in the destruction of basal lamina, invasion of tissue, and metastasis[75]. In both murine and human melanomas, plasma membrane fractions were found to be enriched in cathepsin L[63].

A differentiated myelomonocytic cell line has a novel pathway that is responsible for the uptake and processing of extracellular cathepsin L[61]. The activity of this in-

Table 3. Comparison of chain structures of the cathepsins B, H, and L.

Cathepsin B	1 ———	29 ———	43 – 44 ———————————		252
	L	C*	N V		F
Cathepsin H	1 ———	26 ————————————	169 – 170 ———————		220
	Y	C*	N G		V
Cathepsin L	1 ———	25 ————————————————	178 – 179 ————		221
	I	C*	N K		N

C* marks the catalytically active cysteine residues.
Cathepsin B chains: 1–43 and 44–252.
Cathepsin H chains: 1–169 and 170–220.
Cathepsin L chains: 1–178 and 179–221.
During the heavy-light chain cleavage, cathepsin L loses three or at least two amino acids, cathepsin B loses two amino acids, and cathepsin D even loses amino acids. However, these processing steps are species- and cell-type-specific[41].

ducible pathway is a major determinant of levels of intracellular cathepsin L[61]. Cathepsin L is a potent collagenase and a potent elastase, and the regulation of its processing, secretion and uptake may play an important role in diseases such as cancer and emphysema.

Cathepsin B appears to be ubiquitous in mammals, having been detected in nearly all organs, and tissues and enzymes with similar properties occur in many lower organisms[41]. The detailed study of pure cathepsin B became possible once the enzyme had been separated from lysosomal carboxypeptidase B (formerly cathepsin B_2) by Otto[56]. Nevertheless, the exact specificity of the endopeptidase activity of cathepsin B is still unclear[41].

The activity of cathepsin B against protein substrates is low in comparison to the activity of cathepsin L (less than 10%). An excellent composite alignment of the amino acid sequences of rat and human cathepsin B with mouse cathepsin L, rat cathepsin H, aleurain, actinidin, papain and a cysteine proteinase from *Dictyostelium discoideum* can be found in Portnoy et al.[59]. Rat cathepsin B consists of 252 amino acids, including 14 cysteine residues, 12 of which form disulphide bonds, leaving the active site Cys-25 and an additional free thiol[41]. The arrangement of the seven disulphide bridges of bovine spleen cathepsin B has been described in detail[7]. Cathepsin B shows a proteolytic cleavage, but in this special case in the amino-terminal part between Asn-43 and Val-44[41]. Obviously, the catalytic cysteine is in the light chain (see table 3 for a comparison of cathepsin chain structures). There are indications that precursors of cathepsin B may be secreted by some tumor cells in culture. Human and mouse breast tumors secreted a catalytically active cathepsin-B-like protease that was recognized by an antiserum against cathepsin B[69]. Also the subcellular distribution of cathepsin B in metastatic tumors is bimodal, being distributed in both a lysosomal and a plasma membrane fraction[69].

Of potential relevance to metastasis is that the percentage of cathepsin B associated with the plasma membrane fraction correlates with the metastatic ability of the tumor line, and cysteine proteinase inhibitors show a striking negative correlation with metastatic capability[69].

Cathepsin H[39] was detected in all organs and tissues of the rat that were tested with antibodies. It has been purified from rat liver, rat skin, human liver, rabbit lung, beef lymph node and rat spleen (for references see Kirschke and Barrett[41]). Cathepsin H acts optimally at pH 6–7 as an aminopeptidase on N-terminal unblocked peptides, and presumably on the N-termini of proteins and of peptide products of its (very weak) endopeptidase action. The peptide bond specificity of the endopeptidase action of cathepsin H has not yet been determined, but it cleaves the insulin B-chain even when the N-terminus is blocked, and it attacks vimentin and tubulin[41]. The activity against proteins is, however, very limited and less than 5% compared to that of cathepsin L (see above).

No precursor of cathepsin H has been identified yet, but cathepsin H is also a subject of limited proteolysis (see table 3), and the heavy chain does not show catalytic activity after separation of the light chain (although the essential Cys and His residues are located on the heavy chain). The three-dimensional structure of cathepsin H is undoubtedly analogous to that of papain, and on this basis one would expect Asn-Ser-Trp[175–177]to form part of the active-site cleft. The fact that these residues are contained in the light chain seems to provide sufficient explanation for the inactivity of cathepsin H molecules without it[41]. Earlier names for cathepsin H are: BANA-hydrolase, leucyl naphthylamidase, amino acid naphthylamidase, lysosomal aminopeptidase, lysosomal arginyl aminopeptidase, cathepsin III, cathepsin B_3, cathepsin B_1A and hydrolase H[5,52]. It should be noted that, except for cystinyl aminopeptidase (which occurs in the lysosomes of placenta as oxytocinase) no other lysosomal aminopeptidase has been described as yet. Therefore, the main function of cathepsin H in lysosomes is most probably to work as an aminopeptidase on peptides liberated by cathepsin L and, in some more specialized cells, by cathepsin D.

Cathepsin E is a lysosomal aspartic proteinase[47,76] found in macrophages, blood platelets, leucocytes and bone marrow[5]. In contrast to cathepsin D, it shows a higher activity against serum albumin relative to hemoglobin[47].

Cathepsin F occurs in cartilage and splits proteoglycans[9]. The catalytic mechanism is unknown, because cathepsin F resists inhibitors of all classes of proteinases[5,9].

Cathepsin G is a serine proteinase localized in azurophil granules of neutrophil leucocytes[5,10], and acts extracellularly in inflammatory processes.

Cathepsin I has been shown to be simply rabbit lung cathepsin H (see above and ref. 41).

Cathepsin J[48] is a particulate cysteine proteinase found in human, rat and mouse liver with a molecular weight of 230,000 and maximal activity in splitting Cbz-Phe-Arg-aminomethylcoumarin at pH 6.2–6.8. The major isoenzyme has a pI of 5.8[48]. Protein substrates have not been tested with cathepsin J.

Cathepsin K[48] is a particulate cysteine proteinase found in human kidney, spleen, lung, liver and pancreas, but not in brain, erythrocytes, serum, placenta and skeletal muscle[48]. It shows a molecular weight of 650,000 and an isoelectric point of 5.3 for the major isoenzyme. The activity against protein substrates is not known yet.

Cathepsin M[50] is a lysosomal cysteine proteinase found in brain[50] and in liver[41,53]. Approximately 50% of the total cathepsin M activity is associated with membranes from disrupted lysosomes. In the membrane-bound form the enzyme is active at neutral pH, but the soluble enzyme and the activity eluted from the membranes are maximally active at pH 5[41,53]. It has been shown to be located on the outside of lysosomes[12,53].

Cathepsin N[26] is a lysosomal cysteine proteinase that has been detected in bovine spleen, human placenta, leucocytes and in rat granulomata[5,26,41]. It shows high collagenolytic activity at pH 3.5 and also splits hemoglobin, azocasein and histones[5,26,41].

Cathepsin P[24] is a cysteine proteinase in rat pancreatic islet lysosomes. It converts proinsulin to insulin at pH 5–6 and still shows activity at pH 7.5[24]. The molecular weight is 31,500. A general role for this protease in propolypeptide processing has been proposed[24].

Cathepsin R[46] is a ribosomal serine proteinase occurring in rat liver ribosomes in a cryptic form on the small ribosomal subunit[46]. There are no known connections with lysosomal proteolysis.

Cathepsin S[71] is a lysosomal cysteine proteinase isolated from calf lymph nodes[71] and bovine spleen. It digests hemoglobin, azocasein and collagen[20,41,43] and still shows high activity at pH 7[20,43]. It is clearly different from cathepsin L[20,41,43].

Cathepsin T[29,30,33] is a lysosomal cysteine proteinase occurring in kidney, spleen, liver, intestine and lung. It splits azocasein, ribonuclease and hemoglobin, and it converts tyrosine aminotransferase from a 53,000-dalton monomer to a 49,000-dalton monomer[33]. Even under conditions specially selected for cathepsin T – pH 6.5, glycerol, dithiothreitol – the activity of cathepsin T in rat liver lysosomal extracts is very low (less than 8%) in comparison with the activity of cathepsin L[14].

Acrosin[3,5,65,70] is a serine proteinase in the very specialized lysosome of spermatozoa, the acrosome. It is located on the exterior of the head of the capacitated spermatozoan. The molecular weight is 38,000 and the isoelectric point 10.5. There is an inactive precursor zymogen, proacrosin[5]. For more detailed information see EC 3.4.21.10 and refs 3, 5, 65, 70.

Chymase I and chymase II[32,37,45,72] are chymotrypsin-like serine proteinases in granules of human, bovine, dog, cat and rat mast cells which degrade chymotrypsin substrates[32,37,45,72].

Tryptase[45,72] is a trypsin-like serine proteinase in granules of human, dog and mouse mast cells which splits trypsin substrates[5,45,72].

Conditions for the action of lysosomal proteases

The intralysosomal concentration of proteases is comparatively high – in some cases more than 1 mmol[22] – which is undoubtedly one of the main reasons for the rapid hydrolysis of proteins immediately after the rate-limiting entry into these organelles. Autophagic sequestration of cytosolic proteins does not exceed 4% per h[44,68], but the proteins sequestered within the lysosomes turn over with a half-life of less than 10 min[54], which means that the proteolytic capacity of these organelles is much more than 20 times higher than the highest uptake of substrate proteins. One of the conditions for such a rapid proteolysis is the low intralysosomal pH[34,49,57,68] which has been reported to be between pH 4 and 6. Heterogeneity of these values was recognized using a video image-processing technique[57]; the values change with pH-changes in the medium.

A simple Donnan equilibrium cannot account alone for the low pH-values in lysosomes[34,57]. It is now generally accepted that the acidity of lysosomes in vivo is maintained by an ATP-dependent proton pump located in the lysosomal membrane[57]. It is still not clear how the lysosomal pH is kept at around pH 5 and whether there are other controlling factors besides the H^+-ATPase molecules[57]. The function of the lysosomal proton pump may be to provide a favorable environment for lysosomal hydrolysis and extrusion of hydrolysis products from lysosomes; the proton pump may thus serve to create polarity in intracellular traffic[57]. After entry of substrate proteins into the lysosomal compartment, the acidic intralysosomal pH value favors the exposure of formerly buried hydrophobic areas, making these proteins much more sensitive substrates of cathepsin D and L (see above). Because of the specificity of these cathepsins against hydrophobic amino acids[11–17,41] on the surface of the substrate proteins, it is evident that proteins with more apolar surface amino acids must be degraded faster[11–17].

In the acidic environment within the lysosome, oligomeric proteins dissociate into their monomers, proteins dissociate away from protecting membranes, and stabilizing ligands (substrates, cofactors) dissociate away from proteins. All these processes can increase the surface hydrophobicity of the substrate proteins. This can also occur after the splitting of stabilizing disulfide bonds, but it is still not known to what extent this actually occurs in lysosomes. Most of the lysosomal thiol groups are found in the lysosomal proteins[67], which is in good agreement with the high concentration of cysteine proteinases in lysosomes. A remarkable group of lysosomal disulfides are the mixed disulfides formed from proteins and low-molecular-weight thiols[67]. It seems possible that lysosomal proteolytic activity may be regulated by changes in the lysosomal thiol-disulfide-status, which would also be influenced by lysosomal cystine. Lysosomal cystine transport is a carrier-mediated process[36,49], and polyamines were found to stimulate it[36]. Because lysosomes may be the main intracellular location of cystine it has been speculated that during times of cell growth and active protein turnover lysosomal cystine can be utilized by alterations in the species and concentrations of polyamines[36].

Compared to the very short half-life of substrate proteins (about 8 min, see above) the half-life of lysosomal proteases can be very long, from a few days up to several weeks[16,41]. This might be due to the unusually high content of acidic oligosaccharides found on many lysosomal proteins[31,34], which somehow protects the lysosomal membrane and the lysosomal matrix proteins against degradation. Consistent with this are findings that sever-

al lysosomal membrane proteins exhibit reduced life spans in cells treated with tunicamycin, an inhibitor of glycosylation [34, 73].

Intralysosomal proteinase inhibitors have not been found yet, but the cytosol is very well protected against escaping proteases by a highly effective set of proteinase inhibitors [6].

Peptides liberated by lysosomal proteolysis cannot be reutilized. After rapid peptide degradation, the resulting free amino acids can be reutilized for protein synthesis or finally degraded to yield energy. Lysosomal proteolysis is a necessary precondition for both processes.

The main part of cellular proteins appear to be degraded in lysosomes by the enzymes and mechanisms discussed [3–5, 11–17, 19, 22, 25, 28, 34, 35, 40–42, 51, 54, 58, 62, 68].

However, some short-lived proteins, and especially very-short-lived proteins from the cytosol, must be degraded by selective extralysosomal pathways [18, 44]. These pathways involve proteolytic mechanisms which are totally different from lysosomal proteolysis [16–18], and which cannot be discussed in this short review on lysosomal proteases.

1 Agarwal, S. K., Proteases cathepsins – A view. Biochem. Education *18* (1990) 67–72.
2 Ahlberg, J., Berkenstam, A., Henell, F., and Glaumann, H., Degradation of short and long lived proteins in isolated liver lysosomes. J. biol. Chem. *260* (1985) 5847–5854.
3 Barrett, A. J., Proteinases in mammalian tissues and cells. North Holland, Amsterdam 1977.
4 Barrett, A. J., and Kirschke, H., Cathepsin, B., cathepsin, H., and cathepsin, L., Meth. Enzymol. *80* (1981) 535–561.
5 Barrett, A. J., and McDonald, J. K., Mammalian Proteases, vol. 1, Endopeptidases. Academic Press, London 1980.
6 Barrett, A. J., and Salvesen, G., (Eds), Proteinase Inhibitors. Elsevier, Amsterdam 1985.
7 Baudys, M., Meloun, B., Gan-Erdene, T., Pohl, J., and Kostka, V., Disulfide bridges of bovine spleen cathepsin B. Biol. Chem. Hoppe-Seyler *371* (1990) 485–491.
8 Beynon, R. J., and Bond, J. S., Proteolytic Enzymes. A Practical Approach, pp. 1–259. IRL Press, Oxford 1989.
9 Blow, A. M. J., The detection and characterization of cathepin F, a cartilage enzyme that degrades proteoglycan. It. J. Biochem. *24* (1975) 13–14.
10 Blow, A. J. J., and Barrett, A. J., The action of human cathepsin G on the oxidized insulin B chain. Biochem. J. *161* (1977) 17–19.
11 Bohley, P., Intrazelluläre Proteolyse. Naturwissenschaften *55* (1968) 211–217.
12 Bohley, P., Kirschke, H., Langner, J., Ansorge, S., Wiederanders, B., and Hanson, H., Intracellular protein breakdown, in: Tissue Proteinases, pp. 187–219. Eds A. J. Barrett, J. T. Dingle. North Holland Publ., Amsterdam 1971.
13 Bohley, P., Kirschke, H., Langner, J., Miehe, M., Riemann, S., Salama, Z., Schön, E., Wiederanders, B., and Ansorge, S., Intracellular protein turnover, in: Biological functions of Proteinases, pp. 17–34. Eds H. Holzer and H. Tscheche. 30th Mosbach Coll., Springer, Berlin 1979.
14 Bohley, P., Kirschke, H., Schaper, S., and Wiederanders, B., Principles of the regulation of intracellular proteolysis. Symp. Biol. Hung. *25* (1984) 101–117.
15 Bohley, P., Hieke, C., Kirschke, H., and Schaper, S., Protein degradation in rat liver cells. Progr. clin. biol. Res. *180* (1985) 447–454.
16 Bohley, P., Intracellular Proteolysis in: Hydrolytic Enzymes; New Comprehenseive Biochemistry, vol. 16, p. 307–332. Eds A. Neuberger and K. Brocklehurst. Elsevier, Amsterdam 1987.
17 Bohley, P., Proteolysis in hepatocytes. Adv. clin. Enzym. *6* (1988) 203–208.
18 Bohley, P., Kopitz, J., Adam, G., Rist, B., von Appen, F., and Urban, S., Post-translational arginylation and intracellular proteolysis Biomed. biochim. Acta *50* (1991) 343–346.
19 Bond, J. S., and Butler, P. E., Intracellular proteases. A. Rev. Biochem. *56* (1987) 333–364.
20 Brömme, D., Steinert, A., Friebe, S., Fittkau, S., Wiederanders, B., and Kirschke, H., The specificity of bovine spleen cathepsin S – a comparison with rat liver cathepsin L and cathepsin B. Bioch. J. *264* (1989) 475–481.
21 Conner, E. G., Blobel, G., and Erickson, A. H., Biogenesis of lysosomal proteinases: polypeptide chain in: Lysosomes – their role in protein breakdown, p. 151–161. Eds H. Glaumann and F. J. Ballard. Academic Press, London 1987.
22 Dean, R., and Barrett, A. J., Lysosomes. Essays Biochem. *12* (1976) 1–40.
23 Diment, S., Leech, M. S., and Stahl, P. D., Cathepsin D is membrane-associated in macrophage endosomes. J. biol. Chem. *263* (1988) 6901–6907.
24 Docherty, K., Carroll, R. J., and Steiner, D. F., Conversion of proinsulin to insulin: Involvement of a 31500 molecular weight thiol protease. Proc. natl Acad. Sci. USA *79* (1982) 4613–4617.
25 Dubin, A., (Ed.) Proteinases and Their Inhibitors: Regulation of Cellular Metabolism. Nakl. Univ. Jagiellonskiego, Warszawa–Krakow 1990.
26 Evans, P., and Etherington, D. J., Action of cathepsin N on the oxidized B-chain of bovine insulin. FEBS Lett. *99* (1979) 55–58.
27 Erickson, A. H., Biosynthetic forms of cathepsin, L, in: Intracellular Proteolysis–Mechanisms and Regulations, pp. 74–81. Ed. N. Katunuma. Japan Scientific Societies Press, Tokyo 1989.
28 Glaumann, H., and Ballard, J., (Eds), Lysosomes: Their Role in Protein Breakdown. Academic Press, London 1987.
29 Gohda, E., and Pitot, H. C., A new thiol proteinase from rat liver. J. biol. Chem. *256* (1981) 2567–2572.
30 Gohda, E., and Pitot, H. C., Purification and characterization of a new thiol proteinase from rat kidney Biochem. biophys. Acta *659* (1981) 114–122.
31 Goldberg, D. E., Biogenesis of lysosomal enzymes: Oligosaccharide chains, in: Lysosomes: Their Role in Protein Breakdown. pp. 163–191. Eds H. Glanmann and J. Ballard. Academic Press, London 1987.
32 Haas, R., Heinrich, P. C., and Sasse, D., Proteolytic enzymes of rat liver mitochondria. Evidence for a mast cell origin. FEBS Letters *103* (1979) 168–171.
33 Hargrove, J. L., Gohda, E., Pitot, H. C., and Granner, D. K., Cathepsin T (Convertase) generates the multiple forms of tyrosine aminotransferase by limited proteolyis. Biochemistry *21* (1982) 283–289.
34 Holtzman, E., Lysosomes. Plenum Press, New York 1989.
35 Holzer, H., and Heinrich, P., Control of proteolysis. A. Rev. Biochem. *49* (1980) 63–91.
36 Jonas, A. J., Symons, L. J., and Speller, R. J., Polyamines stimulate lysosomal cystine transport. J. biol. Chem. *262* (1987) 16391–16393.
37 Katunuma, N., Kominami, E., Kobayashi, K., Banno, Y., Suzuki, K., Chichibu, K., Hamaguchi, Y., and Katsunuma, T., Studies on new intracellular proteases in various organs of rat. 1. Purification and comparison of their properties. Eur. J. Biochem. *52* (1975) 37–50.
38 Katunuma, N., and Kominami, E. (Eds), Intracellular Proteolysis Mechanisms and Regulations. Japan Scientific Societies Press, Tokyo 1989.
39 Kirschke, H., Langner, J., Wiederanders, B., Ansorge, S., Bohley, P., and Hanson, H., Cathepsin H: an Endoaminopeptidase from Rat Liver Lysosomes. Acta biol. med. germ. *36* (1977) 185–199.
40 Kirschke, H., Langner, J., Wiederanders, B., Ansorge, S., and Bohley, P., Cathepsin L: A new proteinase from rat liver lysosomes. Eur. J. Biochem. *74* (1977) 293–301.
41 Kirschke, H., and Barrett, A. J., Chemistry of lysosomal proteases, in: Lysosomes: Their Role in Protein Breakdown. pp. 193–238. Academic Press, London 1987.
42 Kirschke, H., Langner, J., Riemann, S., Wiederanders, B., Ansorge, S., and Bohley, P., Lysosomal cysteine proteinases, in: Protein Degradation in Health and Disease, p. 15–35. CIBA Foundation Symposium 75 (new series). Excerpta Medica, Amsterdam 1980.
43 Kirschke, H., Wiederanders, B., Brömme, D., and Rinne, A., Cathepsin S from bovine spleen – Purification, distribution, intracellular localization and action on proteins. Biochem. J. *264* (1989) 467–473.
44 Kopitz, J., Kisen, G. O., Gordon, P., Bohley, P., and Seglen, P., Nonselective autophagy of cytosolic enzymes by isolated rat hepatocytes. J. Cell Biol. *111* (1990) 941–953.
45 Lagunoff, D., The properties of mast cell proteases. Biochem. Pharmac. suppl. (1968) 221–227.
46 Langner, J., Kirschke, H., Bohley, P., Wiederanders, B., and Korant, B. D., The ribosomal serine proteinase, cathepsin R occurrence in rat liver ribosomes in a cryptic form. Eur. J. Biochem. *125* (1982) 21–26.

47 Lapresle, C., and Webb, T., The purification and properties of a proteolytic enzyme, rabbit cathepsin E, and further studies on rabbit cathepsin D. Biochem. J. *84* (1962) 455–462.
48 Liao, J. C. R., and Lenney, J. F., Cathepsins J and K: High molecular weight cysteine proteinases from human tissues. Bioch. biophys. Res. Commun. *124* (1984) 909–916.
49 Lloyd, J. B., and Forster, S., The lysosome membrane. Trends biochem. Sci. *11* (1986) 365–368.
50 Marks, N., Specificity of breakdown based on the inactivation of active proteins and peptides by brain proteolytic enzymes, in: Intracellular Protein Catabolism II, pp. 85–102. Eds V. Turk and N. Marks. Plenum Press, New York/London 1977.
51 Marzella, L., and Glaumann, H., Autophagy, microautophagy and crinophagy as mechanisms for protein degradation, in: Lysosomes: Their Role in Protein Breakdown, p. 319–367. Eds H. Glaumann and J. Ballard. Academic Press, London 1987.
52 McDonald, J. K., and Barrett, A. J., Mammalian Proteases, vol. 2: Exopeptidases. Academic Press, London 1986.
53 Pontremoli, S., Melloni, E., Michetti, M., Salamino, F., Sparatore, B., and Horecker, B. C., Localization of two lysosomal proteinases on the external surface of the lysosomal membrane. Bioch. biophys. Res. Commun. *106* (1982) 903–909.
54 Mortimore, G. E., Lardeux, B. R., and Adams, C. E., Regulation of microautophagy and basal protein turnover in rat liver. Effects of short-term starvation. J. biol. Chem. *263* (1988) 2505–2512.
55 Nishimura, Y., Furuno, K., and Kato, K., Biosynthesis and processing of lysosomal cathepsin L in primary cultures of rat hepatocytes. Archs Biochem. Biophys. *263* (1988) 107–116.
56 Otto, K., Cathepsins B1 and B2 Purification from bovine spleen, and properties (with a survey of their occurrence in various organs of the rat) in: Tissue Proteinases, pp. 1–28. Eds A. J. Barrett and J. T. Dingle. North-Holland Publ. Co., Amsterdam/London 1971.
57 Ohkuma, S., The lysosomal proton pump and its effect on protein breakdown, in: Lysosomes: Their Role in Protein Breakdown, pp. 115–148. Eds H. Glaumann and J. Ballard. Academic Press, London 1987.
58 Pfeifer, U., Functional morphology of the lysosomal apparatus, in: Lysosomes: Their Role in Protein Breakdown, pp. 3–59. Eds H. Glaumann and J. Ballard. Academic Press, London 1987.
59 Portnoy, D. A., Erickson, A. H., Kochan, J., Ravetch, J. V., and Unkeless, J. C., Cloning and characterization of a mouse cysteine proteinase. J. biol. Chem. *261* (1986) 14697–14703.
60 Press, E. M., Porter, R. R., and Cebra, J., The isolation and properties of a proteolytic enzyme, cathepsin D, from bovine spleen. Biochem. J. *74* (1960) 501–514.
61 Reilly, J. J. Jr, Chen, P., Sailor, L. Z., Mason, R. W., and Chapman, H. A. Jr, Uptake of extracellular enzyme by a novel pathway is a major determinant of cathepsin L levels in human macrophages. J. clin. Invest. *86* (1990) 176–183.
62 Rivett, A. J., Intracellular Protein Breakdown. Essays Biochem. *25* (1990) 39–81.
63 Rozhin, J., Wade, R. L., Honn, K. V., and Sloane, B. F., Membrane-associated cathepsin L: a role in metastasis of melanomas Bioch. biophys. Res. Commun. *164* (1989) 556–561.
64 Salminen, A., and Gottesman, M. M., Inhibitor studies indicate that active cathepsin L is probably essential to its own processing in cultured fibroblasts. Biochem. J. *272* (1990) 39–44.
65 Schiessler, H., Schleuning, W. D., and Fritz, H., Cleavage specificity of boar acrosin on polypeptide substrates, ribonuclease and insulin B-chain. Hoppe-Seylers Z. Physiol. Chem. *356* (1975) 1931–1936.
66 Schmidt, M. T., and Kirschke, H., Degradation of proteoglycans by cathepsin L and H, in: Proteinases and Their Inhibitors: Regulation of Cellular Metabolism, pp. 117–126. Ed. A. Dubin. Nakl Univ. Jagiellonskiego, Warszawa–Krakow 1990.
67 Schwertner, R., Untersuchungen zum Disulfid- und Sulfhydrylgehalt in Lebern von Wistarratten. Thesis Martin-Luther-Universität, Halle–Wittenberg 1980.
68 Seglen, P. O., Regulation of autophagic protein degradation in isolated liver cells, in: Lysosomes: Their Role in Protein Breakdown; pp. 371–414. Academic Press, London 1987.
69 Sloane, B. F., Rozhin, J., Moin, K., Buck, M. R., Day, N. A., and Helmer, K. M., Cathepsin B and endogenous cysteine proteinase inhibitors in metastatic tumors, in: Intracellular Proteolysis Mechanisms and Regulations, pp. 527–533. Japan Scientific Societies Press, Tokyo 1989.
70 Stambaugh, R., and Buckley, J., Identification and subcellular location of the enzymes effecting penetration of the zona pellucida by rabbit spermatozoa. J. Reprod. Fert. *19* (1969) 423–432.
71 Turnsek, T., Kregar, I., and Lebez, D., Acid sulphhydryl protease from calf lymph nodes. Bioch. biophys. Acta *403* (1975) 514–520.
72 Vensel, W. H., Komender, J., and Barnard, E. A., Non-pancreatic proteases of the chymotrypsin family II. Two proteases from a mouse mast cell tumor. Bioch. biophys. Acta *250* (1971) 395–407.
73 Wiederanders, B., and Kirschke, H., The processing of lysosomal proteinases, in: Proteinases and Their Inhibitors: Regulation of Cellular Metabolism; pp. 77–85. Ed. A. Dubin. Nakl Univ. Jagiellonskiego, Warszawa–Krakow 1990.
74 Willstätter, R., and Bamann, E., Über die Proteasen der Magenschleimhaut. Erste Abhandlung über die Enzyme der Leukozyten. Hoppe-Seylers Z. physiol. Chemie *180* (1929) 127–143.
75 Yamaguchi, N., Chung, S.-M., Shiroeda, O., Koyama, K., and Imanashi, J., Characterization of a cathepsin L-like enzyme secreted from human pancreatic cancer cell line HPC-YP. Cancer Res. *50* (1990) 658–663.
76 Yamamoto, K., Kamata, O., Katsuda, N., and Kato, K., Immunochemical difference between cathepsin D and cathepsin E-like enzyme from rat spleen. J. Biochem. *87* (1980) 511–516.
77 Yonezawa, S., Takahashi, T., Wang, X.-j., Wong, R. N. S., Hartsuck, J. A., and Tang, J., Structures at the proteolytic processing region of cathepsin D. J. biol. Chem. *263* (1988) 16504–16511.

0014-4754/92/020151-07$1.50 + 0.20/0