ANALELE UNIVERSITATII DIN ORADEA
FASCICULA: ECOTOXICOLOGIE, ZOOTEHNIE SI TEHNOLOGII DE INDUSTRIE ALIMENTARA, VOL. VII, ANUL 7, 2008

CATHEPSINS

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Abstract

The lysosome is a major site of mammalian protein degradation and is calculated to be capable of degradating as much protein as the digestive tract. Detailes characteristics are known for several of the enzymes involved in lysosomal proteolysis, although the enzymes are necessary tools for hydrolysis, they are only effective in the right environment.Proteins hydrolysis in the lysosome is performed by an array of endo – and exopeptidases working together to degrade the proteins to small peptides and amino acids. In 1941, Fruton and colleagues described four enzymes derived from crude tissue homogenates and named them cathepsins A, B, C, and D.

Key words: lysosome; protein degradation; cathepsins; proteolytic activity.

INTRODUCTION

Approximately 144 proteolytic enzymes of animal tissues have been classified. These can be divided into two main families: exopeptidases (proteinases), wich are restricted to terminal peptide linkages, and endopeptidases, wich are not restricted.

Cathepsins are a class of globular lysosomal proteases, most of which contain an active-site cysteine residue. This nomenclature initially described any group of intracellular peptide hydrolases, although several cathepsins also have extracellular roles. A main physiological role for cathepsins is general protein turnover in the lysosome. This role is exploited in the antigen presentation function of the immune system. Additionally, when cathepsins are directed to the outside of the cell, they can degrade proteins in the extracellular matrix.

Cathepsins A, B, C and D, represent the major types of proteases typically found in mammalian lysosomes. The collective name "cathepsin" was chosen to indicate that they were enzymes found primarily in the tissues rather than in extracellular fluids. Most of the cathepsins have subsequently been shown to function principally in the lysosome, with the possible exceptions of cathepsins G and E. The lysosomal proteases are all active at acidic pH. The aspartic proteases are represented by two enzymes, cathepsins D and E.

Cathepsins B and D are the best known lysosomal endopeptidases. The early discovery of these enzymes was possible because both enzymes are rahter stable (at least at pH 6 and below) and because there are no endogenous tightly binding inhibitors of these enzymes in mammalian

tissues. In some proteins there are regions of peptides that are resistant to some proteases and not others, suggesting that multiple endopeptidases offer advantages for efficient cleavage of the vast array of proteins that are degraded in lysosomes.

MATERIALS AND METHODS

Cathepsin H has been purified from tissues of several species and from tumor cells. Purified cathepsin H is stable in the pH range 5,0 – 6,5, but seems to be unstable in diluted tissue extracts after freezing. Cathepsin H is irreversibly inactivated above pH 7.0. With some tissues that can be obtained immediately after death, it is feasible to take advantage of a 50 – fold purification factor attainable by isolation of lysosomes. Otherwise, working with an extract of the whole tissue one encounters the difficulty that cathepsin H is partially complexed with endogenous inhibitors. Separation of cathepsin H from the enzyme – inhibitor complexes requires autolysis at acidic pH values followed by fractionation with ammonim suflate or acetone. The peptide bond specificity of the endopeptidase action of cathepsin H has not yet been determined. Cathepsin H was shown to cleave several proteins, but the endopeptidase activity of the enzyme is limited. Collagen, for instance, has not been degraded by cathepsin H.

Cathepsin B is a cysteine endopeptidase, reflecting the essential catalytic residue in the active site of the enzyme. It was originally isolated from liver lysosome and was later recognized as being comprised of two molecular components: B_1 and cathepsin B_2. Cathepsin B_1 is a thiol endopeptidase having maximum activity at pH 6.0 and is unstable above pH 7.0. In contrast, cathepsin B_2 hydrolyzes at pH 5.5 to 6.0, and shows amidase activity at pH 5.6.

Cathepsin L was purified from skeletal rabbit muscle, demonstrating that it was optimally active on myosin at pH 4.1. Taylor et al. identified cathepsin L in skeletal muscle by an immunohistochemical method. It degrades myosin heavy chain, actin, α – actinin, troponin T, and I. Cathepsin L has been estimated to have 10 times greater activity per protease molecule against myosin than cathepsin B. Yamashita and Konagaya reported that the enzyme most responsible for *post mortem* softening of salmon meat during spawning migration was cathepsin L. In continued research, they found about 80% of the autolyc activity at low pH from 3 to 5 was due to cathepsin L and the remaining was atributed to cathepsins D and E.

Cathepsins D is an aspartic endopeptidase with two aspartic acid residues in its active site. It is related in catalytic mechanism to the gastric enzyme pepsin, and, like pepsin, it has an acidic pH optimum for hydrolysis of a range of proteins. Almost all the data on the activity of catepsin D indicate that it is only active below pH 6.0. The crystal structure of cathepsin D reveals a long substrate-binding cleft that is able to accommodate hydrophobic amino acids in synthetic substrates or inhibitors. Cathepsin D has no reportes exopeptidase activity, and its preference for hydrophobic amino acids might imply that this enzyme is primarily involved in the degradation of denatured proteins in the lysosome.

Cathepsin C, lysosomal dipeptidyl peptidase, is primarily considered to be an exopeptidase. Cathepsin C may have a important physiological role in removing dipeptide propeptides from the N – termini of a range of serinde endopeptidases of inflammatory cells.

RESULTS AND DISCUSSION

It is clear that lysosomes contain a wide range of proteases that are capable of degrading proteins down to smaller units. The ability of the enzymes to degradate any protein will depend upon the structure of the substrate. Substrate concentration in any individual lysosome is hard to determine partly due to heterogeneity of lysosomes and partly due to each protein having large numbers of peptide bonds that can potentially be cleaved by each of the enzymes. The concentraton of peptide bonds due just to enzymes within a lysosome is in excess of 1 M. Even though most of these bounds have presumably evolved to be resistant to proteolysis, it is possible to envisage that some amino acid side chains have affinity for the active sites of the enzymes, resulting in enzyme inhibition. Susbtrate proteins taken up into lysosomes must compete with these “inhibitory” interactions in order to be hydrolyzed.

In vitro experiments with millimolar concentrations of the lysosomal proteases have not been performed, owing to the difficulty in obtaining sufficient enzyme. However, mechanisms are available to dilute lysosomal enzyme concentrations in living cells. Sucrose is accumulated in lysosomes, and this causes the organelles to become enlarged, leading to dilution of lysosomal protein. Sucrose-induced lysosomal enlargement has been demonstrated to reduce pinocytosis by macrophages.

The influence of pH on degradation manifests like so: at very low pH (2-3), many proteins are completely denaturated; at the pH of the lysosome, estimated to be on the order of pH 5.0, only partial denaturation would be expected. Substrates that are delivered to lysosomes will typically go throught a pH gradient, with the pH droping gradually from 7.2 outside

the cells down to 6.5 to 6.0 in endosomes and then eventualy to 5.0 or less in the lysosome. For the lysosomal enzymes, evolution has favored stabilization of enzymes at low pH, such that these do not unflod at pH 5.0, and even though many of them are clipped by proteases, this does not seem to affect their overall stability. Human cathepsin L has a half-life of 80 sec at neutral pH. This slow inactivation of an enzyme that retains catalytic potential at neutral pH would not be an effective mechanism for the control of the activity of the enzyme *in vivo* should it escape from the lysosomal environment.

The pH optimal for the degradation of collagen fibres by cathepsins B and L are below 4.0, even though it is quite clear that these enzymes are catalytically competent at neutral pH. The low pH optimum is attributed to the denaturation of the collagen molecules at reduced pH. By contrast, the insoluble protein elastin can be degraded by cathepsin S at neutral pH. Cathepsin D has little activity above pH 6.0 and is optimally active against most proteins at pH 3-4.

The relative importance of the individual enzymes in degrading proteins down to amino acids or dipeptides will depend on rates of hydrolysis of the individual enzymes, as well as the concentration of the enzymes. Of the endopeptidases, cathepsins L and S seem to be the most catalytically efficient. Cathepsin D is very efficient at low pH, where as cathepsins B and H are much poorer endopeptidases.

So I have shown that the most important lysosomal proteins that have been isolated in skeletal muscle cells are cathepsin A, B_1, B_2 (lysosomal carboxypeptidase B), C, D, E, H and L.

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