Human cathepsin D

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Abstract: A literature survey was performed of human cathepsin D gene, cathepsin D biosynthesis, posttranslatory modifications, transport within the cell, substrate specificity and catalytic effect. Methods used to determine the activity and level of this proteinase as well as its role in the biochemistry and pathobiochemistry of cells, tissues and organs were considered.

Key words: Cathepsin D - Procathepsin D - Preprocathepsin D - Aspartic proteinase

The proteolytic activity in tissue extracts, reflected by acid pH, was described by Hedin in 1904 [1]. In 1928, the term cathepsin (from the Greek word meaning "to digest") was introduced by Wilstätter and Bamann [2] to define the enzyme showing this activity. In 1937, Anson [3] designed a method for cathepsin purification and used hemoglobin as a substrate to determine its activity. In 1952, lysosomal peptidases were described and called cathepsin A, B and C [4]. In 1955, De Duve [5] presented lysosomes as the site of cathepsin action. In Poland, cathepsin D and its hemoglobin substrate were studied by Czystohorski as early as in the 50s [6]. In 1960, Press et al. [7] used the term cathepsin D to differentiate it from other endopeptidases and exopeptidases.

At present, cathepsin D (EC 3.4.23.5) is defined as lysosomal aspartyl endopeptidase. It breaks down proteins into several polypeptide fragments that digest other lysosomal endopeptidases and exopeptidases.

Cathepsin D gene

The cathepsin D gene is located at the end of the short arm of chromosome 11, in the p15.5 region, close to the H-ras oncogene (Fig. 1). It consists of 9 exons and contains 11,106 base pairs. For a complete sequence of cathepsin D gene please refer to paper by Roedecker et al. [8]. Cathepsin D gene transcription leads to the formation of mRNA containing 1,988 bases. Its expression is regulated by steroid hormones, growth factors IGF-1, TNF-α and EGF and by retinoic acid [9-12]. Cathepsin D biosynthesis is inhibited by cycloheximide and colchicine [13-15].

Occurrence, isolation and purification

Cathepsin D can be found in nearly all cells, tissues and organs, but not in mature lysosome-free erythrocytes [16]. From tissue homogenate, cathepsin D is isolated and purified by means of ammonium sulphate saltation, organic solvent fractionation, molecular gel (sephadex) chromatography and ion-exchangeable chromatography. The terminal stages of purification involve affinity chromatography with application of pepstatin [17-20], synthetic inhibitors [21], antibodies, cathepsin D propeptide and hemoglobin [22-24], which are bound to a constant carrier. Differences in the pH values between procathepsin D and cathepsin D binding to pepstatinyl-agarose allowed isolation of these two forms of the enzyme [25]. Cathepsin D inhibitors can be isolated by means of cathepsin D bound to a constant carrier [22]. Cathepsin D is also obtained using genetic engineering techniques [10,26-28].

The method of cathepsin D isolation from the human liver [29-33], spleen [18], brain [37], uterus [34], placenta [35], gastric mucosa [36], thyroid [38] and leucocytes [39] has been described. The human liver cathepsin D preparation is produced by Calbiochem and Sigma.
Thanks to the disordered structure of the polypeptide chain (in approximately 70%) and the presence of four disulphate bridges and eight oligosaccharide chains, cathepsin D is well soluble and resistant to denaturing agents and proteolytic degradation [40-42].

**Biosynthesis**

Cathepsin D is synthesized in the form of preprocathepsin [33,43-46] by the mRNA bound to the rough endoplasmic reticulum (RER). Translocation of preprocathepsin D to the cisterns of the endoplasmic reticulum (ER) is possible due to a signal sequence [47,48]. A signal recognition particle (SRP) binds to the sequence, at the same time blocking protein translation. Then, the SRP binds to its receptor, located in ER, which reactivates translation (Fig. 2). Binding of the SRP-receptor initiates the formation of hydrophobic protein channel in the ER membrane. Next, the SRP detaches from the complex and returns to the cytoplasm. Simultaneously, ribosomes bind to proteins of the ER membrane. The elongating polypeptide chain is introduced through the channel in the ER membrane to the cisterns due to a 20 amino acid sequence situated at the N-terminus. At the end of this channel, signal peptidase in ER cisterns cuts off the signal sequence, thus leading to the generation of procathepsin D that enters the ER cisterns. The signal sequence that has been detached undergoes degradation to amino acids. The amino acid sequence of procathepsin D determines its posttranslatory modifications: particle rugosity, formation of disulphate bridges, N-glycosylation and phosphorylation. About 5% of the polypeptide chain of procathepsin D has an alpha-helix conformation, 26% - beta conformation (rugose sheet), and the remaining part being a disordered structure. Disulphate bridges are formed between the cysteine residues Cys27-Cys96, Cys46-Cys53, Cys222-Cys226 and Cys265-Cys302, their location being determined by protein-disulphide isomerase (PDI). The N-glycosylation site is constituted by the Asn70 residue (heavy chain) and Asn199 residue (light chain).

**Glycosylation**

The oligoglycosylation chains are synthesized with the involvement of dolichol phosphate and then transferred onto asparagin residues [46,78], located on the triad sequences: Asn70-Gly71-Thr72 and Asn199-Val200-Phe201. The remaining 9/11 asparagin residues are not found on these sequences and are subject to glycolysation. Cathepsin D contains eight various mannose type oligosaccharide chains in the particle (Fig. 3). Five of them bind to the residue Asn-70 of the light chain and contain 3, 5, 6 or 7 mannose residues. All of them contain two GlcNAc, one has three GlcNAc residues. Three oligosaccharides bind to the residue Asn199 of the heavy chain and contain 5 mannose residues. One of them has three GlcNAc residues, one contains fucose and one has galactose. Each oligosaccharide chain contains two N-acetylgalactosamine molecules, whereas two of them have 3 molecules and from 3 to 7 mannose residues.

The phosphate acid residue is attached to the sixth carbon of mannose [81], which conditions binding to the mannose-6-phosphate receptor and translocation of cathepsin D to primary lysosomes. The GlcNAc residues found outside the oligosaccharide chain are not subject to phosphorylation and are not involved in the transport of cathepsin D.
Phosphorylation and binding to the receptor

The cis zone of the Golgi apparatus captures ER-derived transport vesicles that contain glycosylated procathepsin D, which is then transferred to its central and trans zones. In the trans zone, terminal mannose residues undergo phosphorylation to mannose-6-phosphate (M6P) [53,54]. By means of M6P residues, procathepsin D binds to mannose-6-phosphate receptors (M6PR) and in the form of vesicles is transported to primary lysosomes [55-60]. Primary lysosomes are formed by budding off from the membrane fragment of the smooth endoplasmic reticulum. The mannose-6-phosphate receptor (M6PR) is a heterogenic protein and occurs in two types: M6PR-46 and M6PR-300. The action of M6PR-46 (46 kDa) depends on the presence of bivalent cations, whereas that of M6PR-300 (300 kDa) is not bivalent cation-dependent. In the acid environment of primary lysosomes (pH=4.5-5.5), the procathepsin D M6P-M6PR complex undergoes dissociation. The receptor returns to the Golgi apparatus and transports M6P molecules of procathepsin D, whereas the receptor-free procathepsin D remains in lysosomes and undergoes dephosphorylation. The mannose-6-phosphate receptors occur in the trans-Golgi network and in primary lysosomes, but not in mature lysosomes. These differences help distinguish primary from mature lysosomes.

Proteolytic transformations

Preprocathepsin D is composed of 412 amino acid residues [63-68]. In its molecule, signal protease cleaves the peptide bond Ala20-Leu21 and 20-amino acid prepeptide is split off (Fig. 4), giving rise to procathepsin D, built up of 392-amino acid residues. Its autocatalytic activation takes place in primary lysosome.
some, in an acidic pH (Fig. 5). Intracellular cleavage of the Glu64-Gly65 bond and splitting-off of 44-amino acid propeptide occur (Fig. 6). An active mono-chain form of cathepsin D, composed of 348 amino acid residues, is generated (Fig. 7). The enumeration of amino acid residues of the mono-chain cathepsin D (from 1 to 348) has been used for tracing further transformations in the polypeptide chain. A non-defined endopeptidase cleaves the -Val6-Leu7- bond and the 6-amino acid fragment of the Ser-Ala-Ser-Ser-Ala-Val sequence is split off, giving rise to a molecule built up of 342 amino acid residues. The aminopeptidase cleaves the Leu7-Lys8- bond and a leucine residue is split off. A modified mono-chain cathepsin D molecule, composed of 341 amino acid residues, is split off the light chain N-terminus. Carboxypeptidase cleaves in turn the Arg347-Lys348 and then Ala346-Arg347 bonds and the residues Lys348 and Arg347 are split off the C-terminus of the light subunit. These protease inhibitors suppress cathepsin D maturation. The ultimate form of mature two-chain cathepsin D is composed of 337 amino acid residues. The heavy chain contains 196 and the light chain has 141 amino acid residues. These chains are linked by hydrophobic bonds (Fig. 8). The amino acid composition shows a substantial content of glycine and an approximately twofold level of dicarboxy amino acids and their amides as compared to alkaline amino acids (Table 1).

The proteolytic activation of procathepsin D via limited proteolysis is an irreversible process. An ultimate cathepsin D molecule has an approximate double symmetry. The proteolytic activity of the mono-chain and two-chain forms is the same (Fig. 9).

Proteolytic modifications of the molecule and diversity of oligosaccharide composition contribute to a marked molecular heterogeneity of cathepsin D [17,29,37,71,72].

**Substrate specificity**

Cathepsin D cleaves peptide bonds inside the polypeptide chain. These bonds are formed mainly by carboxyl groups of hydrophobic amino acids, especially aromatic amino acids [73,74].

Research studies performed with synthetic peptides (general formula Fig. 10) have provided information on the structure of the catalytic site of cathepsin D and the requirements each substrate should fulfill. The peptides contain different amino acid residues at positions X and Y. Only the peptides that have amino acids with ramified chains in the X-position are resistant to hydrolysis. Those containing at least five amino acid residues in a molecule are sensitive to cathepsin D effect. In the hexapeptide containing the sequence of Gly1-Phe2-Phe3-Tyr4-Thr5-Pro6-Lys7, cathepsin D cleaves two peptide bonds between Phe2-Phe3 and Phe3-Tyr4, whereas in the heptapeptide Phe1-Gly2-His3-Nph4-Phe5-Val6-Leu7-OMe the bond between Nph4-Phe5 is cleaved [76]. In micromolecular syn-
thetic substrates the number of peptide bonds cleaved by cathepsin D is considerably smaller even though they are formed by the same amino acid residues. The differences are due to additional sites in the enzyme molecule that bind macromolecular substrates [74,77].

In the oxidized insulin chain B, cathepsin D cleaves five peptide bonds, whereas all proteases of lysosomal extracts cleave as many as 27/29 peptide bonds in this polypeptide (Fig. 11). The peptide bonds cleaved by cathepsin D in some proteins have been presented in Table 2.

Mechanism

The catalytic site of cathepsin D is constituted by two asparagine acid residues - Asp33 and Asp231, located in the triad sequences of Asp33-Thr34-Gly35 and Asp31-Thr232-Gly233 [80]. In the acidic environment, the carboxyl group of Asp33 undergoes dissociation, whereas that of Asp231 does not. The carboxyl group of Asp33 activates the water molecule and allows proton release from this molecule. However, the protonated carboxyl group of Asp231 polarizes the carbonyl group of the peptide bond, facilitates formation of a tetraedric intermediate and allows cleavage of the bond. The reactions are performed by ionizing groups of cathepsin D and therefore their velocity is pH-dependent. Cathepsin D shows the highest activity in an acidic pH (pH=3.5-5.5).

Hydrolysis of the peptide bonds catalyzed by cathepsin D occurs in two stages and can be expressed as a general formula (Fig. 12). In the first stage, the

Fig. 7. Aminoacidic sequence of human, single-chain cathepsin D [46]; * - catalytic site - Asp33 and Asp231 residues; † - catalytic site Asn70 and Asn199 with added oligosaccharide chains.
enzyme (E) binds water and with a substrate (S) forms the enzyme-substrate complex (E-S), in which the components are bound by ionic and hydrogen bonds. Conformatory changes, intramolecular electron and proton regrouping within the peptide bond are observed, and a covalent intermediate compound (E-S') is formed. The intermediate compound is an aminoacyl enzyme. In the next stage, the intermediate compound undergoes breakdown, accompanied by the release of the first (R1) and the second (R2) reaction products.

The action of cathepsin D takes place not only with the involvement of asparagine acid residues but also with other amino acid residues, and it thus has a multifunctional and intracellular character. Multifunctional catalysis is based on simultaneous effects of nucleophilic and electrophilic groups of the enzyme catalytic site on the carbonyl group of the peptide bond. Attachment of the substrate is followed by two synchronized transfer reactions: in the first, the proton is transferred from a water molecule onto a carboxyl anion of Asp33, in the other, the proton derived from a carboxyl group of Asp231 is transferred onto the oxygen of the carbonyl group of the substrate, giving rise to a tetraedric intermediate [81-86].

Two protons are simultaneously transferred onto the peptide bond (Fig. 13a). The first originates from the water molecule activated by Asp33. The other is derived from the carboxyl group of Asp231 and is carried onto the oxygen atom of the carbonyl group of the peptide bond. These two transfers lead to the formation of an aminoacyl enzyme, being a tetraedric intermediate (Fig. 13b), sensitive to the action of the activated water molecule. The subsequent double proton transfer causes
breakdown of the tetraedric intermediate (Fig. 13c). The intra- and mono-molecular character ensures the maximum concentration of the catalytic groups, their optimal direction and spatial distribution. In consequence, these processes reduce the activation energy (Fig. 14). The peptide bond of the C-N substrate is broken down, which gives rise to the first product of reaction with a free carboxyl group (R1) and to an aminoenzyme. Then, the second product of reaction with a free amine group is released (R2). The catalytic site of cathepsin D changes into the anhydride form, which undergoes rapid hydrolysis and is thus reproduced as the primary structure of the catalytic site.

Pepstatin, a pentapeptide containing statin at position 4 (Sta4) was used to investigate the structure of the catalytic site. The Sta4 residue is a structural analog of

| Table 1. Amino acid composition of different molecular forms of cathepsin D. |
|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | Prepro | Prepeptide | Procathepsin | Prepeptide | One-chain cathepsin | Two-chain cathepsin |
|                  |        |           |              |            | Light chain | Light chain |
| Ala A             | 28     | 5         | 23           | 3          | 20           | 11           |
| Arg R             | 12     | 3         | 3            | 9          | 4            | 4            |
| Asn N             | 11     | 11        | 11           | 2          | 2            | 2            |
| Asp D             | 21     | 21        | 1            | 20         | 12           | 8            |
| Cys C             | 9      | 1         | 8            | 4          | 4            | 4            |
| Glu E             | 17     | 17        | 3            | 14         | 4            | 9            |
| Gln Q             | 17     | 16        | 1            | 15         | 10           | 5            |
| Gly G             | 39     | 39        | 3            | 36         | 19           | 16           |
| His H             | 6      | 6         | 1            | 5          | 4            | 1            |
| Ile I             | 23     | 23        | 3            | 20         | 11           | 8            |
| Leu L             | 41     | 6         | 35           | 3          | 32           | 15           |
| Lys K             | 23     | 23        | 3            | 20         | 11           | 8            |
| Met M             | 10     | 1         | 9            | 1          | 8            | 4            |
| Phe F             | 14     | 14        | 1            | 13         | 9            | 4            |
| Pro P             | 26     | 3         | 23           | 3          | 20           | 9            |
| Ser S             | 37     | 34        | 5            | 29         | 22           | 7            |
| Thr T             | 21     | 21        | 3            | 18         | 10           | 8            |
| Trp W             | 4      | 4         | 4            | 2          | 2            | 2            |
| Tyr Y             | 19     | 19        | 1            | 18         | 12           | 5            |
| Val V             | 34     | 34        | 6            | 28         | 14           | 13           |
| Sum               | 412    | 20        | 392          | 44         | 348          | 196          |
| Mr, kDa*          | 44550  | 1967      | 42601        | 47608      | 37850        | 21381        |
|                  |        | 15961     | 15181        |            |              |              |

*calculated from amino acid composition, without sugar moieties.

Cathepsin D


Fig. 11. Cleavage of B-chain of insulin by cathepsin D and lysosomal extracts [78,79].
Table 2. Preferential cleavage sites in various protein substrates found upon mild cleavage by cathepsin D [75]. Homologous amino acid residues at the various positions are enclosed by boxes.

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Fig. 12. Simplified diagram presenting hydrolysis of peptide bond.

Fig. 13. Cathepsins D mechanism of action: (a) transfer of two protons, (b) formation of tetraedric intermediate, (c) breakdown of the tetraedric intermediate.
the substrate and of the transitory state, and exerts an inhibitory effect due to structural similarity to the tetraedric intermediate, formed during cleavage of peptide bond by asparagin acid residues of the catalytic site (Fig. 15). The interaction of cathepsin D with pepstatin is illustrated by a spatial model (Fig. 16).

Activators and inhibitors
The activity of cathepsin D is increased by glycine ethyl ester [90], phospholipids [91], polyphosphates [92] and probably by a protein activator (20000 Da) isolated from the spleen [93]. The role of activating compounds in studies on cathepsin D is slight.

No typical cell cathepsin D inhibitors have been revealed. The inhibitory effect is demonstrated by a propeptide split off during procathepsin D autoactivation [73,94,95] and by respective fragments of its structure [96]. Alpha2-macroglobulin is an endogenous plasma inhibitor of this protease [97,98]. The activity is also suppressed by antibodies [99,100], some glycosaminoglycans [101] and fragments of DNA structure [102]. 1,2-epoksy3-(p-nitrofenyloksy)propan reacting with Asp33 [103] and methyl ester of diacetylo-DL-norleucine reacting with Asp231 are the synthetic inhibitors of cathepsin D [104]. Cathepsin D inhibitors, called pepstatins, are synthesized by bacteria of the genus Streptomyces [105-107]. Polypeptide inhibitors of this proteinase occur in spare organs of many plants [108] and in tissues of some lower ani-
Cathepsin D inhibitors have played a substantial role in the studies concerning the structure and mechanism of action of this protease and its tissue location [111-115].

The effects of cathepsin D also depend on the structure of substrate protein. Denatured or heme-free hemoglobin shows increased susceptibility to the action of this proteinase as compared to native hemoglobin [18]. However, hemoglobin complexification with haptoglobin reduces its susceptibility to cathepsin D [116].

**Role in protein degradation**

Cathepsin D takes part in digestion of exhausted and denatured cell proteins or proteins showing abnormal structure and those which entered the cell via endocytosis [14,25,26,96]. It initiates proteolytic degradation of proteins, cleaving it into large fragments. This causes increased accessibility of the subsequent peptide bonds and thus they are further digest-

![Fig. 17. Site of enzymes action on proteoglycans [121]. □□□ protein core; □□□ polysaccharide chain; C - cathepsin D; H - hialuronidase; X - β-xilosidase.](image)

![Fig. 18. Activation of proenzymes and inactivation of proteinase inhibitors by cathepsin D [123-128].](image)

<table>
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<th>Protein</th>
<th>Cathepsin D activity (%)</th>
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ed by other lysosomal proteins to dipeptides and amino acids. Cleavage of even a single peptide bond in a protein molecule may cause its structural denaturation and functional inactivation. The state of the protein molecule (native, denatured) defines accessibility of the protease peptide bonds. In proteoglycans, cathepsin D cleaves peptide bonds located in the central part of the polypeptide chains (Fig. 17), which results in the formation of large peptide glycan fragments. Collagen is also sensitive to the action of cathepsin D [122].

Cathepsin D also performs a limited proteolysis, referring to single peptide bonds, thus leading to the activation of proenzymes of certain proteases and inactivation of their inhibitors (Fig. 18). It is also involved in the conversion of prohormones and precursor forms of biologically active peptides to active forms [132] and contributes to their inactivation. (Table 3). From hemoglobin, cathepsin D releases biologically active hemorphins [131,132]. Thus, it plays a regulatory role.

The optimum pH for the action of cathepsin D bound to the lysosomal membranes is higher as compared to the free enzyme [133]. Also cathepsin D bound in vitro by a constant carrier shows higher pH optimum [134]. A small amount of cathepsin D can be found on cell surface, due to the fusion of lysosomes with the plasma membrane, it also enters the intercellular environment and passes to the blood [135].

**Determination of cathepsin D activity and concentration**

Cathepsin D does not need activators in order to act and has no endogenous inhibitors. Procathepsin D present in a sample undergoes rapid autoactivation in test conditions (pH=3.5-4.0) and therefore total activity of cathepsin D can be determined in the material examined. Bovine globin [136,137] and bovine hemoglobin [138] are most sensitive to cathepsin D action. The enzyme activity is reflected by the quantity of TCA-soluble tyrosine released from these substrates. Cathepsin D shows a considerably lower affinity as compared to other proteins (Table 3). The activity of this protease is also determined using micromolecular synthetic substrates [140-143].

The content of active cathepsin D in tissue homogenates in blood plasma and body fluids is determined by means of a graph showing the relationship between the activity and the level of highly-purified cathepsin D preparation [144] (Fig. 19). Another method employed to assess cathepsin D content is titration of the catalytic site using pepstatin and its derivatives [107,145] (Fig. 20). Pepstatin binds to cathepsin D in the mole ratio of 1:1. The total content of cathepsin D, inactive and active in tissues and body fluids, is determined by means of specific antibodies [18,146].

Cell and tissue localization of cathepsin D is performed using the immunohistoenzymatic methods, with specific antibodies [147-150] or labeled pepstatin [151-153].

Differences in substrate specificity, pH optima, sensitivity to activators and inhibitors, and in antigen properties allow identification of cathepsin D and determination of its activity and concentration in tissue homogenates, blood plasma and body fluids [154-157].

**Final comments**

Cathepsin D plays an essential role in metabolism as an enzyme degrading the exhausted cell proteins and restoring their amino acids to syntheses, as well as a modulator of proteolysis, activating precursor forms of many proteases and inactivating their inhibitors. The role of cathepsin D in biomedical sciences extends its involvement in many pathological processes such as...
apoptosis, inflammatory states, rheumatic diseases, neoplasms, muscle dystrophy, Alzheimer disease, as well as in their diagnostics [158-168]. The use of inhibitors of cathepsin D biosynthesis, activation and its active form allows regulation of cathepsin D activation.

Human proprocathepsin, procathepsin and cathepsin D differ from their equivalents isolated from the mammalian tissue in a number of particulars concerning the amino acid sequence, posttranslatory modifications and conformational structure [169]. Due to the differences, human anti-cathepsin D antibodies do not react with cathepsin D of other species [170].

References:


Kawrowska A, Chlabicz M. Bovine globin as substrate of cathepsin D. In preparation.


Ravdin PM, Tandon ASK, Allred DC. Cathepsin D by western blotting and immunohistochemistry: failure to con-


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