

Role of cathepsin A and cathepsin C in the regulation of glycosidase activity

Alina Minarowska¹, Łukasz Minarowski², Alicja Karwowska³,
Anna Justyna Milewska⁴, Marek Gacko⁵

¹Department of Human Anatomy, Medical University of Białystok, Poland

²Department of Lung Diseases and Tuberculosis, Medical University of Białystok, Poland

³Department of Epidemiology and Hygiene, Medical University of Białystok, Poland

⁴Department of Statistics and Medical Informatics, Medical University of Białystok, Poland

⁵Department of Vascular Surgery, Medical University of Białystok, Poland

Abstract: Increased tissue activity of cathepsin A and cathepsin C can be observed in many pathological conditions. It is associated with an enhanced degradation of glycosaminoglycans, proteoglycans, and glycoproteins, and results in their decreased tissue content. Cathepsin C releases the glycosidases from complexes formed with cathepsin A, and reinstates their activity. In this review a current state of knowledge is presented concerning the regulation of selected glycosidases activity by cathepsin A (EC 3.4.16.1) and C (EC 3.4.14.1). (*Folia Histochemica et Cytobiologica* 2012, Vol. 50, No. 1, 20–24)

Key words: cathepsin A, lysosomal carboxypeptidase A, cathepsin C, dipeptidyl peptidase I, glycosidase activity

More than sixty lysosomal hydrolases digest macromolecular compounds: proteins, polysaccharides, lipids, and nucleic acids at acidic pH. The lack or deficiency of a certain lysosomal enzyme resulting from genetic defect or inactivation can lead to the development of a storage disease [1]. A disease can be classified as a lysosomal storage disorder if it fulfils the following three criteria: 1) the lack or decreased activity of at least one lysosomal enzyme, 2) the stored substance is normally degraded in lysosomes, and 3) it is stored inside the lysosomes [2].

Cathepsin A (EC 3.4.16.1) prevents the processes involved in lysosomal storage. Cathepsin A forms complexes with glycosidases, protecting them in this way against proteolytic inactivation [3, 4]. Decreased lysosomal content of cathepsin A leads to the inactivation of several glycosidases and accumulation of glycosaminoglycans. Cathepsin C (EC 3.4.14.1) is also

involved in glycosaminoglycan metabolism. Cathepsin C releases the glycosidases from complexes formed with cathepsin A, and reinstates their activity [5].

Cathepsin A

Cathepsin A is multifunctional lysosomal protein that acts as a carboxypeptidase and forms complexes with glycosidases at pH between 4.5 and 5.5; it exhibits amidase and esterase activity at pH 7.0 [5, 6]. One molecule of cathepsin A is composed of 438 amino acid residues assembled into two subunits — cortical and apical one with molecular masses of 32 kDa (Ala1-Arg284) and 20 kDa (Met285-Tyr438), respectively (Figure 1). The subunits are held together with disulfide bonds C60-C361 and form a monomer of cathepsin A [7]. Its catalytic site is built of Ser150, Asp356, and His415 amino acid residues. Cathepsin A monomer has a molecular mass of 52 kDa and measures $60 \times 50 \times 70 \text{ \AA}$ [8].

Under acidic pH, 60–70% of cathepsin A exists as homodimers with 104 kDa molecular mass [6]. The remaining 30–40% is present in the form of a two-component, enzymatically active complex with beta-

Correspondence address: L. Minarowski,
Dept. of Lung Diseases and Tuberculosis,
Medical University of Białystok, Poland;
tel.: + 48 85 740 95 24, fax: + 48 85 732 41 49;
e-mail: lukasz.minarowski@gmail.com

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1      10      20      30      40      50
APDQDEIQRLPGLAKQPSFRQYSGYLKSSGSKHLHYWFVESQKDPENSPVVLWL

60      70      80      90      100
NGGPGCSSLDGLLTHEGPFVLPDGVLTLEYNPYSWNLIANVLYLESPAGVGFYSY

110     120     130     140     150     160
DDKFTATNDTEVAQSNFEALQDFRFLPEYKNNKFLTGESYAGIYIPTLAVLVMQD

170     180     190     200     210
PSMNLQGLAVGNGLSSYEQNDNSLVYFAYYHGLLGNRLWSSLQTHCCSQNKCNF

220     230     240     250     260     270
YDNKDLECVTNLQEVARIVGNSGLNIYNLYAPCAGGVPSHFRYEKDTVVVVQDLGN

280     290     300     310     320
IFTRLPLKR — MDPPCTNTTAASTYLNNPYVRKALNIPEQLPQWQMCNFLVNLQY

330     340     350     360     370     380
RRLYRSMNSQYLKLLSSQKYQILLYNGDVMACNFMGDEWFVDSLQKMEVQRR

390     400     410     420     430     438
PWLVKYGDSDGEQIAGFVKEFSHIAFLTIKGAGHMVPTDKPLAATTMTSRTLKQPY
    
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Figure 1. Amino acid sequence of cathepsin A: A1-R284 — 32 kDa subunit; M285-Y428 — 20kDa subunit; C — C disulfide bond binding the subunits; S150-D356-H415 — catalytic triad; N17, N291 glycosylated rests; Q76-Y84, V306-E401 — beta-galactosidase binding sequences [8]

Table 1. Amino acid composition of cathepsin A [44]

Amino acid*	Cathepsin A	Subunit	
		32 kDa	20 kDa
Ala (A)	23	14	9
Arg (R)	15	8	7
Asn (N)	33	22	11
Cys (C)	9	6	3
Phe (F)	20	14	6
Gln (Q)	26	15	11
Gly (G)	30	23	7
His (H)	8	6	2
Ile (I)	12	7	5
Asp (D)	22	15	7
Glu (E)	19	14	5
Leu (L)	51	35	16
Lys (K)	20	11	9
Met (M)	10	2	8
Pro (P)	25	16	9
Ser (S)	33	24	9
Thr (T)	21	11	10
Trp (W)	7	4	3
Tyr (Y)	27	18	9
Val (V)	27	19	8
Sum of amino acids	438	284	154

*Three-and one-letter code

-galactosidase [7]. Its beta-galactosidase-binding contact surface is formed of Gln76-Tyr84 and Val386-Glu391 sequences. Dimeric molecule of cathepsin A binds to the monomer of beta-galactosidase (64 kDa) forming a heterotrimer with 168 kDa molecular mass. Four molecules of the heterotrimer form macrocomplex with 680 kDa molecular mass [6]. At pH 7.5, this macrocomplex splits into 8 molecules of cathepsin A and 4 molecules of beta-galactosidase [7, 9] (Figure 2). Sodium dodecyl sulfate (SDS) dissociates this complex into monomers that are further split into sub-



Figure 2. Model of cathepsin A molecule monomer [adopted from 9]. • - • - • — amino acids of catalytic triad

Table 2. Glycosidases forming complexes with cathepsin A

Glycosidase	Molecular mass kDa	Substrate	Function	Literature citation
Beta-D galactosidase (β -Gal) EC 3.2.1.23	64.0	Galactosaminoglycans	Splits off galactose	[30]
Neuraminidase (Neu) EC 3.2.1.18	48.3	Sialoglycosaccharides (mucopolysaccharides)	Splits off neuraminic acid	[13]
6-N-galactosamine N-acetyl-6- -sulfate sulfatase (GALNS) EC 3.1.6.4	57.0	Glycosaminoglycans, keratan sulfate, chondroitin 6-sulfate	Splits off sulfate ion	[43]

units as a result of the addition of the reducing compound. After reducing pH to 4.5, macrocomplex with 680 kDa molecular mass is formed again. The formation of this complex protects beta-galactosidase against degradation and proteolytic inactivation [10, 11]. Complex of cathepsin A with beta-galactosidase is isolated by means of affinity chromatography on *p*-aminophenyl-beta-D-thiogalactopyranoside-agarose [14]. Obtained complex of cathepsin A and beta-galactosidase is dissociated at pH 7.5 and fractionated into its components by means of gel chromatography technique with Shim-pack Dial-3000 column. About 1% of cathepsin A molecules is present as a polyenzymatic macrocomplex with beta-galactosidase, N-acetyl-alpha-neuraminidase, and N-acetylgalactosamine-6-sulfate sulfatase [12, 13]. This macrocomplex has a molecular mass of approximately 1280 kDa [7, 14, 15]. Table 2 summarizes the characteristics of glycosidases that are bound by cathepsin A.

Inherited deficiency or point mutations in the amino acid sequence of cathepsin A (Q21R, S23Y, W37R, S61L, V104M, L208P, Y221N, Y351C, M365T, G389S, F398V) inhibit the formation of dimeric forms and complexes with glycosidases [16–18]. Degradation and inactivation of beta-galactosidase and neuraminidase are reflected by a secondary deficiency of those enzymes, leading to the accumulation of galactosaminoglycans and sialoglycosaccharides, and as a consequence to the storage disease – mucopolysaccharidosis IV B and galactosialidosis [19, 20]. Degradation of beta-galactosidase is catalyzed by cysteine cathepsins [21]. Leupeptin, an inhibitor of cathepsins, halts this process. Decreased activity of cathepsin A can be observed in the course of muscular dystrophy [22] and in multiple sclerosis [23].

Cathepsin C

Cathepsin C also participates in the regulation of glycosidase activity. It is a lysosomal cysteinyl peptidase — an enzyme that cleaves off dipeptides from the N-terminus of peptides and proteins [24, 25]. Moreover,

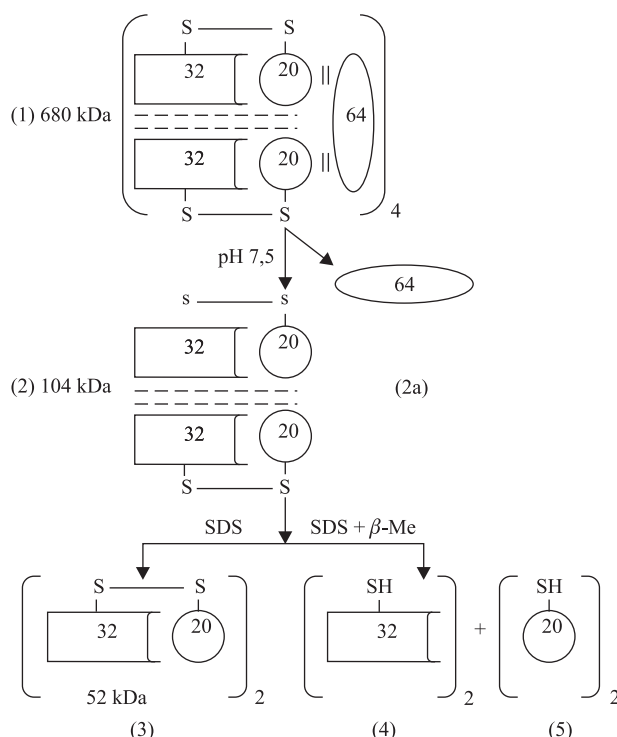


Figure 3. Macrocomplex of cathepsin A with beta-galactosidase [according to 41]. At pH 7.5 the complex of cathepsin A and beta-galactosidase (1) is dissociated into two cathepsin homodimers (2) and beta-galactosidase (2a); sodium dodecyl sulfate (SDS) dissociates homodimer of cathepsin A into two monomers (3), while beta-mercaptoethanol / β -Me/ splits them further into 32 kDa (4) and 20 kDa subunits (5). S-S — disulfide bonds binding subunits of the monomer; — — hydrophobic bonds that bind the monomers

it hydrolyses dipeptide esters, amides, anilides, and beta-naphthylamides [26]. Additionally, cathepsin C shows the activity of transpeptidase [27]. It catalyzes hydrolysis at pH 5.0–6.0 and transpeptidation at pH 6.8–7.0 [4]. Cathepsin C is activated by chloride anions and sulfhydryl compounds [28].

One molecule of human cathepsin C is built of 206 amino acid residues, arranged in four polypeptide chains with a total molecular mass of approximately 200 kDa [29]. Its spatial model is presented in Figure 4.

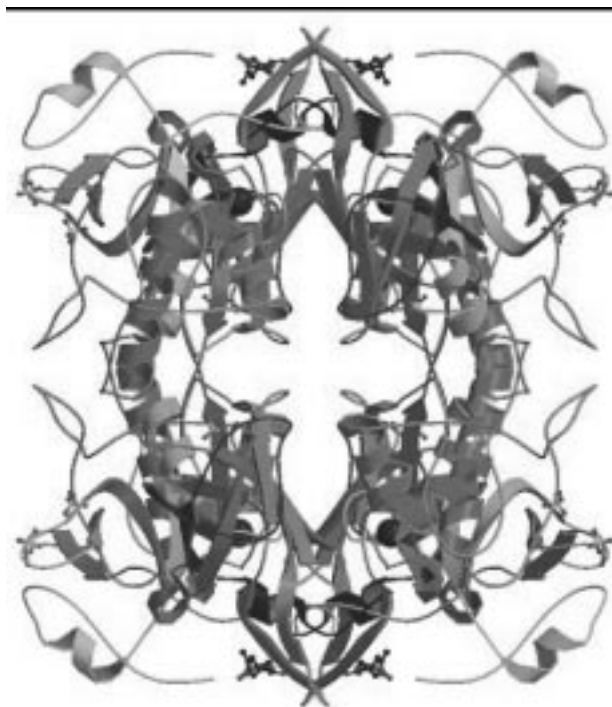


Figure 4. Model of cathepsin C molecule [adopted from 44]

The role of cathepsin C in the regulation of lysosomal enzymatic activity involves the release of beta-galactosidase and neuraminidase from complex with cathepsin A (Figure 5). This process requires the presence of chloride anions (Cl^-) and sulfhydryl compounds [4, 30]. Released beta-galactosidase, neuraminidase, and cathepsin A exhibit normal activity. Genetic mutation and reduced activity of cathepsin C cause Papillon-Lefevre syndrome characterized by palmoplantar keratoderma, periodontitis, and muscular dystrophy [3, 42].

Increased tissue activity of cathepsin A and cathepsin C can be observed in many pathological conditions [24, 26, 31–34]. It is associated with an enhanced degradation of glycosaminoglycans, proteoglycans, and glycoproteins, and results in their decreased tissue content [35–40].

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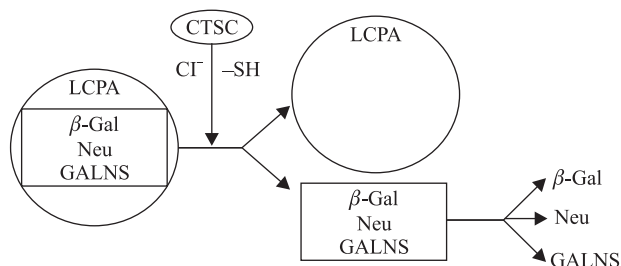


Figure 5. Proposed model of cathepsin C (CTSC) regulated dissociation of multi-enzyme complex: LCPC — cathepsin A; β -Gal — β -galactosidase; Neu — neuraminidase; GALNS — 6-N-galactosamine N-acetyl-6-sulfate sulfatase; Cl^- — chloride ions; $-\text{SH}$ — sulfhydryl group

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