FOLIA HISTOCHEMICA
ET CYTOBIOLOGICA
Vol. 50, No. 1, 2012
pp. 20–24

REVIEW

VIA MEDICA

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

Alina Minarowska[1], Łukasz Minarowski[2], Alicja Karwowska[3], Anna Justyna Milewska[4], Marek Gacko[5]

[1]Department of Human Anatomy, Medical University of Bialystok, Poland
[2]Department of Lung Diseases and Tuberculosis, Medical University of Bialystok, Poland
[3]Department of Epidemiology and Hygiene, Medical University of Bialystok, Poland
[4]Department of Statistics and Medical Informatics, Medical University of Bialystok, Poland
[5]Department of Vascular Surgery, Medical University of Bialystok, 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, dipeptydyl 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].

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

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 × 50 × 70 Å [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-

```
1          10         20         30         40         50
APDQDEIQRLPGLAKQPSFRQYSGYLKSSGSKHLHYWFVESQKDPENSPVVLWL

      60         70         80         90        100
NGGPGCSSLDGLLTEHGPFLVQPDGVTLEYNPYSWNLIANVLYLESPAGVGFSYS

110        120        130        140        150        160
DDKFTATNDTEVAQSNFEALQDFFRLFPEYKNNKLFLTGESYAGIYIPTLAVLVMQD

      170        180        190        200        210
PSMNLQGLAVGNGLSSYEQNDNSL VYFAYYHGLLGNRLWSSLQTHCCSQNKCNF

220        230        240        250        260        270
YDNKDLECVTNLQEVARIVGNSGLNIYNLYAPCAGGVPSHFRYEKDTVVVQDLGN

      280        290        300        310        320
IFTRLPLKR — MDPPCTNTTAASTYLNNPYVRKALNIPEQLPQWQMCNFLVNLQY

330        340        350        360        370        380
RRLYRSMNSQYLKLLSSQKYQILLYNGDVDMACNFMGDEWFVDSLNQKMEVQRR

      390        400        410        420        430        438
PWLVKYGDSGEQIAGFVKEFSHIAFLTIKGAGHMVPTDKPLAATTMTSRTLNKQPY
```

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-

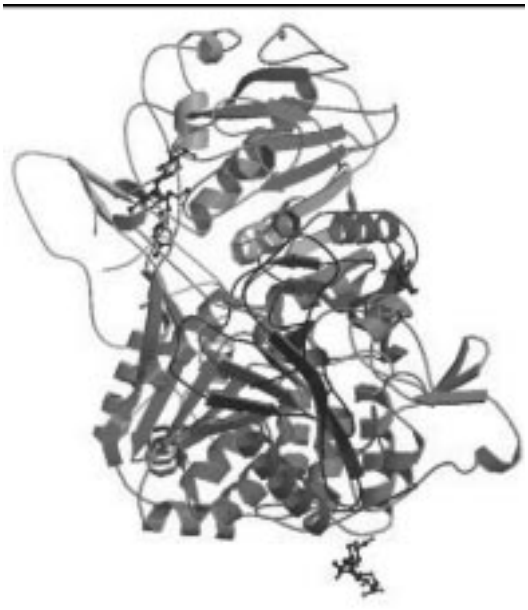

Figure 2. Model of cathepsin A molecule monomer [adopted from 9]. • – • – • — amino acids of catalytic triad

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 fractioned 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].

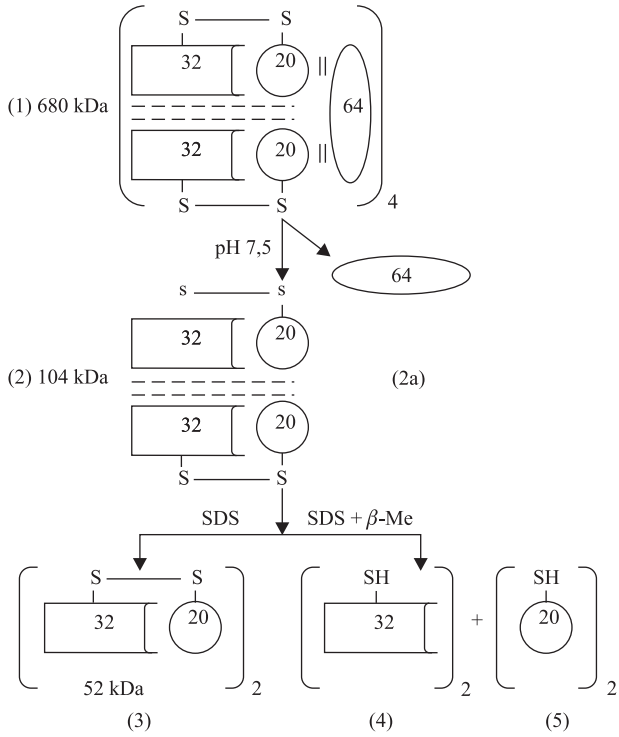

Figure 3. Macrocomplex of cathepsin A with beta-galactosidase [according to 41]. At pH 7.5 the complex of cathepsin A and beta-galactosidase (1) is dissociated into two cathepsin homodimers (2) and beta-galactosidase 2a); sodium dodecyl sulfate (SDS) dissociates homodimer of cathepsin A into two monomers (3), while beta-mercaptoethanol /ß-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

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, it hydrolyses dipeptide esters, amides, anilides, and beta-naphtylamides [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.

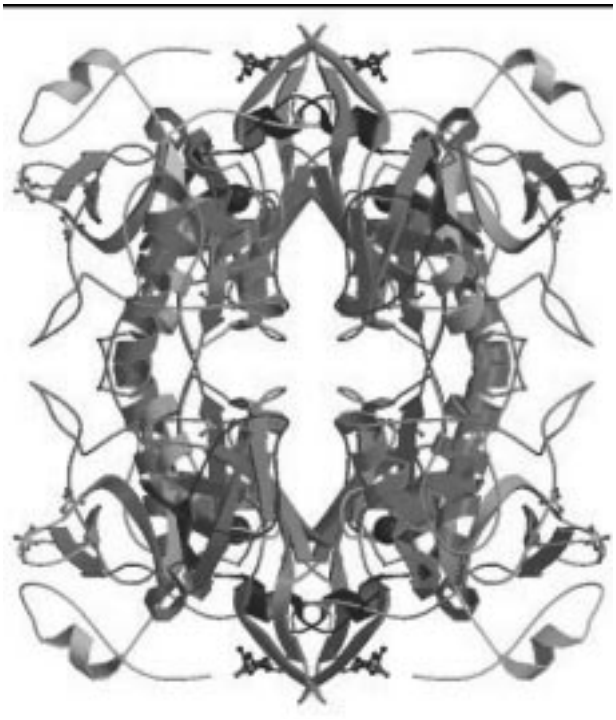

Figure 4. Model of cathepsin C molecule [adopted from 44]

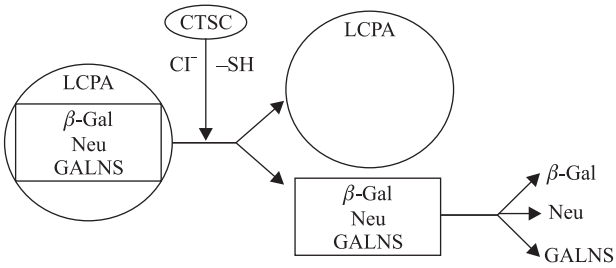

Figure 5. Proposed model of cathepsin C (CTSC) regulated dissociation of multienzyme complex: LCPA — cathepsin A; β-Gal — β-galactosidase; Neu — neuraminidase; GALNS — 6-N-galactosamine N-acetyl-6-sulfate sulfatase; Cl^- — chloride ions; –SH — sulfhydryl group

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].

References

1. Neutfeld ET. Lysosomal storage disease. *Ann Rev Biochem.* 1991;60:257–280.
2. Holtzman E. *Lysosoms*. Plenum Press, New York, London 1989.
3. D'Azzo A, Hoogeveen AT, Reuser JJ, Robinson H, Galjaard H. Molecular defect in combined β-galactosidase and neuraminidase deficiency in man. *Proc Natl Acad Sci USA*. 1982;79:4535–4539.
4. D'Agrosa RM, Callahan JW. In vitro activation of neuraminidase in the β-galactosidase-neuraminidase-protective protein complex by cathepsin C. *Biochem Biophys Res Commun*. 1988;157:770–775.
5. Siergiejuk M, Gacko M, Worowska A. Cathepsin A activity of a parietal thrombus of an abdominal aortic aneurysm. *Folia Histochem Cytobiol.* 2011;49:10–12.
6. Pshezhetsky AV. Lysosomal carboxypeptiodase A. In: *Handbook of Proteolytic Enzymes.* Acad Press, San Diego, USA, Barrett AJ, Rawling ND, Woessner JF eds. 1998, vol. II:1923––1929.
7. Pshezhetsky AV, Potier M. Direct affinity purification and supramolecular organization of human lysosomal cathepsin A. *Arch Biochem Biophys.* 1994;313:64–70.
8. Rudenko G, Bonten E, d'Azzo A, Hol WGJ. The atomic model of the human protective protein/cathepsin A. *Proc Natl Acad Sci USA.* 1998;621–625.
9. Rudenko G, Bonten E, d'Azzo A, Hol WGJ. Three-dimensional structure of the human human "protective protein": structure of the precursor form suggest a complex activation mechanism. *Current Biol.* 1995;3:1249–1259.
10. Pshezhetsky AV, Elsliger MA, Vinogradova MV, Potier M. Human β-galactosidase-cathepsin A complex: definition of the β-galactosidase binding interface on cathepsin A. *Biochemistry* 1995;34:2431–2440.
11. Galjart NJ, Morrean H, Willemsen R, Gillemans N, Bonten EJ, d'Azzo A. Human lysosomal protective has cathepsin A-like activity distinct from its protective function. *J Biol Chem*. 1991;226:14754–14762.
12. Hoogeveen AT, Verheijen FW, Galjaard H. The relation between human lysosomal β-galactosidase and its protective protein. *J Biol Chem.* 1983;258;12143–12146.
13. Bonten EJ, d'Azzo A. Lysosomal neuraminidase catalytic activation in insect cells is controlled by the protective protein/cathepsin A. *J Biol Chem.* 2000;275:37657–37663.
14. Pshezhetsky AV, Potier M. Stechiometry of the human lysosomal carboxypeptidase- β-galactosidase complex. *Biochem Biophys Res Commun*.1993;195:354–362.
15. Pshezhetsky AV, Potier M. Association of N-acetylo-galactosamine-6-sulfate sulphatase with the multienzyme lysosomal complex of β-galactosidase, cathepsin A and neuraminidase: possible implication for intralysosomal catabolism of keratin sulphate. *J Biol Chem.* 1996;271:28359–28365.
16. Shimoyo M. Fukuhara Y, Otoh K, Oshima A, Sakuraba H, Suzuki Y. Protective protein gene mutations in galactosialidosis. *J Clin Invest*. 1993;91:2393–2398.
17. Zhou XY, Galjart NJ, Willemsen R, Gillemans N, Galjard H. A mutation in a mild form of galactosialidosis impairs dimerization of the protective protein and renders it unstable. *EMBO J.* 1991;10:4041–4048.

18. Zhou XY, Van der Spoel A, Rottier R, Hale G, Willemsen R, Berry GT. Molecular and biochemical-analysis of protective protein cathepsin A mutations: correlation with clinical severity in galactosialidosis. *Hum Mol Genet.* 1996;5:1977––1987.
19. Tranchemontagne J, Michaud L, Potier M. Deficient lysosomal carboxypeptidase activity in galactosialidosis. *Biochem Biophys Res Commun*. 1990;168:22–29.
20. Verhijem F, Palmeri S, Hoogeveen AT, Galjard H. Human placental neuraminidase: activation, stabilization and association with β-galactosidase and its "protective" protein. *J Biochem*. 1985;149:315–321.
21. Hiraiwa M. Cathepsin A/protective protein: an unusual lysosomal multifunctional protein. *Cell Mol Life Sci*. 1999;56:894–907.
22. Hinek A, Phazhetsky AV, von Itzstein M, Starcker B. Lysosomal sialidase (neuraminisae-1) is target to the cell surface in a multiprotein complex that facilitates elastic fiber assembly. *J Biol Chem.* 2006;281:3598–3710.
23. Feng C, Li J, Zuo Z. Expression of the transcription factor regulatory factor X1 in the mouse brain. *Folia Histochem Cytobiol.* 2011;49:344–351.
24. Kase R, Itoh K, Takiyama N, Oshima A, Sakuraba H, Suzuki Y. Galactosialidosis: simultaneous deficiency of esterase, carboxy-terminal deamidase and acid carboxypeptidase activities. *Biochem Biophys Res Commun.* 1990;172: 1175–1179.
25. Hiruma H, Kawakami T. Characteristics of weak base-induced vacuoles formed around individual acidic organelles. *Folia Histochem Cytobiol.* 2011;49:272–279.
26. Kotani M, Yamada H, Sakuraba H. Cytochemical and biochemical detection of intracellularly accumulated sialyl glycoconjugates in sialidosis and galactosialidosis fibroblasts with *Macckia amurensis*. *Clin Chim Acta*. 2004;344:131––135.
27. Pagano MB, Bartoli MA, Ennis TL, Mao D, Simmonds PM, Thompson RW, Han CT. Critical role of dipeptidyl peptidase I in neutrophil recruitment during the development of experimental aortic aneurysms. *Proc Natl Acad Sci USA.* 2007;104:2855–2860.
28. D'Azzo A, Andria G, Strisciuglio I, Galjaijaard H. Galactosalidosis. In: Scriver CR, Beaduet AL, Sly WS eds, *The metabolic and molecular bases of inherited disease*, Valle DMc Graw-Hill, New York. 1995, vol. 2:2835–2837.
29. Pshezhetsky AV, Ashmarina M. Lysosomal multienzyme complex: biochemistry, genetics and molecular pathophysiology. *Prog Nucl Acid Res Mol Biol*. 2001;69:81–114.
30. D'Agrosa R, Hubbes M, Zhang S, Shankaran R, Callahan JW. Characteristics of the b-galactosidase-carboxypeptidase complex in GMJ-gangliosidosis and b-galactosialidosis fibroblast. *Biochem J*. 1992;285:833–838.
31. Bierc M, Minarowski L, Woźniak L, Chojnowska S, Knas M, Szajda S, Zwierz K. The activity of selected glycosidases in salivary gland tumors. *Folia Histochem Cytobiol.* 2010;48:471–474.
32. Dariusz Szajda S, Waszkiewicz N, Stypułkowska A, Dadan J, Zwierz K. Lysosomal exoglycosidases in serum and urine of patients with pancreatic adenocarcinoma. *Folia Histochem Cytobiol.* 2010;48:351–357.
33. Dice JT. *Lysosomal pathways of protein degradation.* Landes Biosciences, Austin, TX, 2000.
34. Morreau H, Galjart NJ, Willemsen R, Gillemans N, Zhou XY, d'Azzo A. Human lysosomal protective protein glycosylation, intracellular transport and association with b-galactosidase in the endoplasmic reticulum. *J Biol Chem.* 1992;67:17949–17956.
35. Ostrowska H, Krikowska K, Kalinowska J, Orłowska M, Lengiewicz I. Lysosomal high molecular Wright multienzyme complex. *Cell Molec Biol Lett*. 2003;8:19–24.
36. Galjard NJ, Gillemans N, Mejer D, a'Azzo A. Mouse" protective protein" cDNA cloning sequense comparison and expression. *J Biol Chem*. 1990;265:4678–4684.
37. Ostrowska H, Wójcik C, Worowski K. Cathepsin A and chymotrypsin-like activity of the proteasome in human blood platelets. *Folia Histochem Cytobiol.* 1999;37:153–154.
38. Minarowska A, Minarowski L, Karwowska A, Gacko M. Regulatory role of cathepsin D in apoptosis. *Folia Histochem Cytobiol.* 2007;45:159–163.
39. Hiraiwa M, Saitoh M, Arai N, Shiraishi T, Odani S, Uda Y. Protective protein in the bovine lysosomal b-galactosidase complex. *Biochim Biophys Acta*. 1997;1341:189–199.
40. Jackman HJ, Tan F, Tamei H, Beureling-Harbury C, Li XY, Skidet RA, Erdos EG. A deamidase in human platelets that deamidates tachykinins: probable identity with the lysosomal "protective protein". *J Biol Chem.* 1990;265:11265–11272.
41. Gacko M, Worowska A, Woźniak A, Jedynak M, Panek B, Lapiński R. Lysosomal carboxypeptidase A]. Postepy Biochem. 2005;51:162–70.
42. Lehman A, Mattman A, Sin D, Pare P, Zong Z, d'Azzo A, Campos Y, Sirrs S, Hinek A. Emphysema in an adult with galactosialidosis linked to a defect in primary elastic fiber assembly. *Mol Genet Metab.* 2012; epub ahead of print doi: 10.1016/j.ymgme.2012.02.004.
43. Bielicki J, Hopwood JJ. Human liver N-acetylgalactosamine 6-sulphatase. Purification and characterization. *Biochem J.* 1991;279(Pt 2):515–520.
44. Turk D, Janjic V, Stern I, Podobnik M, Lamba D, Dahl SW, Lauritzen C, Pedersen J, Turk V, Turk B. Structure of human dipeptidyl peptidase I (cathepsin C): exclusion domain added to an endopeptidase framework creates the machine for activation of granular serine proteases. *EMBO J.* 2001;20:6570–6582.

Submitted: 6 August, 2011
Accepted after reviews: 20 December, 2011