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LYSOSOMAL HIGH MOLECULAR WEIGHT MULTIENZYME COMPLEX

HALINA OSTROWSKA*, KATARZYNA KRUKOWSKA, JOANNA KALINOWSKA, MIROSLAWA ORŁOWSKA and ILONA LENGIEWICZ

Department of Biology, Medical Academy of Białystok, Poland

Abstract: Three acidic glycosidases: β -galactosidase (β -GAL, EC 3.2.1.23), α -neuraminidase (NEUR, sialidase, EC 3.2.1.18), N-acetylaminogalacto-6-sulfate sulfatase (GALNS, EC 3.1.6.4) and serine carboxypeptidase cathepsin A (EC 3.4.16.1) form a functional high molecular weight complex in the lysosomes. The major constituent of this complex is cathepsin A, the so-called “lysosomal protective protein” (PPCA). By forming a multienzyme complex, it protects the glycosidases from rapid intralysosomal proteolysis, and it is also required for the intracellular sorting and proteolytic processing of their precursors. In man, a deficiency of cathepsin A leads to a combined deficiency of β -GAL and NEUR activities, called “galactosialidosis”. Multiple mutations identified in the cathepsin A gene are the molecular basis of this lysosomal storage disease. This review describes the structural organization of the lysosomal high molecular weight multienzyme complex and the importance of the protective protein/cathepsin A in physiology and pathology.

Key Words: Protective Protein/Cathepsin A, Acidic Glycosidases, Multienzyme Complex, Lysosome

INTRODUCTION

Lysosomes are the storage organelles for over 50 acidic hydrolases which can be the following groups of enzymes: proteases (cathepsins), glycohydrolases, phospholipases, phosphatases and sulfatases. They are responsible for the degradation of a wide spectrum of macromolecules within the hybrid organelles

* Corresponding author, E-mail: halost@amb.edu.pl

Abbreviations used: β -GAL - β -galactosidase; NEUR - neuraminidase; GALNS - N-acetylaminogalacto-6-sulfate sulfatase; PPCA - protective protein/cathepsin A; kDa - kilodalton; MPR - mannose-6-phosphate receptor; fMet - formylmethionyl; EBP - elastin binding protein.

produced by heterotypic fusion of the lysosomes with late endosomes, phagosomes and autophagosomes [1].

Many suggestions have been put forward that a strong tendency of some enzymes to aggregate in low pH range could be a storage mechanism for lysosomal hydrolases, and in addition could be important for the efficient stepwise catabolism of different macromolecules within the endolysosomal system [1]. The evidence for the existence of the functional lysosomal complex came from a number of biochemical and genetic studies of human autosomal recessive inherited lysosomal storage disease – galactosialidosis [reviewed in 2 and 3]. Independently, it was found that fibroblasts from galactosialidosis patients were deficient in β -GAL and NEUR activities and a 52-kDa lysosomal glycoprotein [4, 5]. This protein was named “protective protein” because of its ability to protect both glycosidases against intralysosomal proteolysis. Later studies demonstrated that the protective protein is a lysosomal serine carboxypeptidase [6] identical to cathepsin A/deamidase [7, 8], and further that the multiple mutations identified in the PPCA gene are the molecular basis of galactosialidosis [reviewed in 2 and 3]. Apart from lysosomal complex formation, the protective protein was shown to associate with NEUR and an alternatively spliced variant of β -GAL to form the cell surface receptor complex for elastin and laminin [9].

BIOSYNTHESIS OF PPCA AND COMPLEX ASSEMBLY

Lysosomal protective protein/cathepsin A (PPCA) is synthesized on membrane-bound polysomes as a 54-kDa precursor with a 28 amino acid N-terminal signal peptide [7, 10]. After the cleavage of the signal peptide, the precursor protein is folded and glycosylated at Asn117 and Asn305 in the endoplasmic reticulum lumen, which continues in the Golgi. Within the late Golgi compartment, the oligosaccharide chain at Asn117 is labeled by the addition of a manno-6-phosphate, and next the manno-6-phosphate receptors (MPRs) transport PPCA precursor to the endosomal/lysosomal compartment [10, 11]. In the lysosomes, the one-chain zymogen with a molecular weight of 54-kDa is processed to a mature form by the removal of a 2-kDa excision peptide [11]. The active 52-kDa PPCA monomer is composed of the 32-kDa and 20-kDa subunits linked together by disulfide bonds. The catalytic triad in the active site (¹⁵⁰Ser, ⁴²⁹His and ³⁷²Asp) is located in the 32-kDa subunit of cathepsin A.

At the early stage of biosynthesis, the protective protein precursor dimerizes and associates with NEUR and β -GAL precursors [10-12]. These events are essential for the correct intracellular routing of the precursors to the lysosomes, as well as for the proteolytic processing of 85-kDa β -GAL precursor to the mature 64-kDa enzyme, and for the activation of NEUR and stabilization of GALNS.

A large number of reports describe the supramolecular structure of lysosomal β -GAL-NEUR-GALNS-PPCA complex isolated from various mammalian cells and tissues [reviewed in 3 and 13]. For example, human placenta PPCA exists

within the lysosome in a 1270-kDa complex with β -GAL-NEUR-GALNS (about 1% of total PPCA), a 680-kDa complex with β -GAL (30 – 40% of total PPCA), and in a free 98-kDa dimer (60-70%) [3]. Unlike PPCA, the majority of β -GAL and all of the NEUR activity is associated with the protective protein in the multienzyme complex.

GALACTOSIALIDOSIS – A MOLECULAR DEFECT OF THE LYSOSOMAL PROTECTIVE PROTEIN

The association of lysosomal protective protein with acidic glycosidases is required for a stepwise glycolipid hydrolysis: NEUR catalyzes the catabolism of sialoglycoconjugates by releasing terminal sialic acid from their oligosaccharide side chains, β -GAL cleaves the terminal β -galactose residues from glycoconjugates, most notably GM1 ganglioside, and GALNS is involved in the first step of catabolism of keratan sulfate [reviewed in 3]. There are a number of reports indicating that a primary deficiency of PPCA results in a combined secondary deficiency of β -GAL and NEUR, called galactosialidosis [reviewed in 2 and 3]. This autosomal recessive lysosomal storage disease is characterized by the accumulation of various sialyloligosaccharides and gangliosides in patients' tissues and body fluids [reviewed in 2]. Patients with this disease show various clinical phenotypes as regards the age of onset and severity, a feature typical for many lysosomal disorders: there is an early infantile form, a late infantile form, and a juvenile-adult form. All these forms are characterized by a molecular defect of the lysosomal protective protein/cathepsin A [2, 14-16]. For example, the juvenile form of galactosialidosis is caused by the skipping of exon 7 in the cathepsin A gene termed SpDEX7, whereas mutations at the ⁴¹²Phe and ²²¹Tyr residues in the PPCA gene are typical in the late-infantile form of the disease [reviewed in 13]. Many of the missense mutations identified in mutant PPCAs alter the folding or the stability of the PPCA protein precursor [15, 16]. None of them was shown to occur in the active site or in the protein surface responsible for protective function [15]. These results indicate that the absence or inadequate processing of the protective protein/cathepsin A precursor lead to a partial or complete deficiency of PPCA activity in most galactosialidosis cells. The capacity of secreted PPCA to be taken up by galactosialidosis fibroblasts via the mannose-6-phosphate receptor [14], together with the fact that transplantation of cells overexpressing human PPCA into PPCA knockout mice corrected the deficient phenotype, may have a potential therapeutic significance for the treatment of galactosialidosis [17, 18].

OTHER POTENTIAL FUNCTIONS OF THE LYSOSOMAL PROTECTIVE PROTEIN/CATHEPSIN A

The structural function of the lysosomal protective protein/cathepsin A is distinct from its catalytic activity [7]. PPCA displays carboxypeptidase activity

at acidic pH and deamidase and esterase activities at neutral pH towards various synthetic and natural bioactive peptides [reviewed in 13 and 19]. *In vitro* studies have indicated that mammalian cathepsin A converts angiotensin I to angiotensin II, hydrolyzes endothelin-1, bradykinin and the chemotactic peptide fMet-Leu-Phe, and also deamidates substance P [8, 20]. PPCA, that is not involved in the formation of the complex can be released from activated cells such as blood platelets or lymphocytes [8, 21]. Therefore, it is suggested that it may act extracellularly in the local inactivation of small bioactive peptides. However, the significance of its function in the regulation of biologically active peptides *in vivo* as well as the clinical and pathological consequences associated with bioactive peptide dysfunction have not been investigated in cathepsin A knockout mice [17, 18].

Cathepsin A/deamidase is effectively inhibited by compounds that block the active site serine (diisopropylfluorophosphate or phenylmethylsulfonyl-fluoride as well as by non-specific SH-reactive compounds, such as p-chloromercuriphenylsulfonate or HgCl₂ [3,19]. Deamidase activity is inhibited by a potential antihypertensive agent ebalactone B [19], and the carboxypeptidase activity of cathepsin A is strongly inhibited by an effective anti-tumor and anti-inflammatory agent lactacystin/ β -lactone [22]. No highly-selective cell-permeable inhibitors of this enzyme are known as yet.

Apart from protective and enzymatic functions, PPCA has been shown to associate with NEUR and an alternatively spliced β -GAL (elastin binding protein, EBP) to form the cell-surface non-integrin laminin and elastin receptor complex expressed on fibroblasts, smooth muscle cells, chondroblasts, leukocytes and certain cancer cell types [9]. Little is known, however, about the nature and function of the PPCA in this complex.

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REFERENCES

1. Pillay, C.S., Elliott, E. and Dennison, C. Endolysosomal proteolysis and its regulation. **Biochem. J.** 363 (2002) 417-429.
2. d'Azzo, A., Andria, G., Strisciuglio, P. and Galjjaard, H. Galactosialidosis. In: **The Metabolic and Molecular Bases of Inherited Disease** vol. 2 (1995) 2835-2837.
3. Pshezhetsky, A.V. and Ashmarina, M. Lysosomal multienzyme complex: biochemistry, genetics and molecular pathophysiology. **Prog. Nucleic Acid Res. Mol. Biol.** 69 (2001) 81-114.
4. Hoogveen, A.T., Verheijen, F.W. and Galjaard, H. The relation between human lysosomal β -galactosidase and its protective protein. **J. Biol. Chem.** 258 (1983) 12143-12146.

5. Verheijen, F.W., Palmeri S., Hoogeveen, A.T. and Galjaard, H. Human placenta neuraminidase: activation, stabilization and association with β -galactosidase and its protective protein. **Eur. J. Biochem.** 149 (1985) 315-321.
6. Tranchemontagne, J., Michaud, L. and Potier, M. Deficient lysosomal carboxypeptidase activity in galactosialidosis. **Biochem. Biophys. Res. Commun.** 168 (1990) 22-29.
7. Galjart, N.J., Morreau, H., Willemsen, R., Gillemans, N., Bonten, E.J. and D'Azzo, A. Human lysosomal protective protein has cathepsin A-like activity distinct from its protective function. **J. Biol. Chem.** 266 (1991) 14754-14762.
8. Jackman, H.J., Tan, F., Tamei, H., Beurling-Harbury C., Li X.-Y., Skidgel, R.A. and Erdos, E.G. A deamidase in human platelets that deamidates tachykinins: probable identity with the lysosomal 'protective protein'. **J. Biol. Chem.** 265 (1990) 11265-11272.
9. Hinek, A. Biological roles of the non-integrin elastin/laminin receptor. **Biol. Chem.** 377 (1996) 471-480.
10. Morreau, H., Galjart, N.J., Willemsen, R., Gillemans, N., Zhou, X.Y. and D'Azzo, A. Human lysosomal protective protein glycosylation, intracellular transport and association with β -galactosidase in the endoplasmic reticulum. **J. Biol. Chem.** 267 (1992) 17949-17956.
11. Rudenko, G., Bonten, E., d'Azzo, A. and Hol, W.G.J. Three-dimensional structure of the human protective protein: structure of the precursor form suggests a complex activation mechanism. **Current Biol.** 3 (1995) 1249-1259.
12. Bonten, E. J. and d'Azzo, A. Lysosomal neuraminidase catalytic activation in insect cells is controlled by the protective protein/cathepsin A. **J. Biol. Chem.** 275 (2000) 37657-37663.
13. Hiraiwa, M. Cathepsin A/protective protein: an unusual lysosomal multifunctional protein. **Cell. Mol. Life Sci.** 56 (1999) 894-907.
14. d'Azzo, A., Hoogeveen, A.T., Reuser, J.J., Robinson, H. and Galjaard, H. Molecular defect in combined β -galactosidase and neuraminidase deficiency in man. **Proc. Natl. Acad. Sci. USA** 79 (1982) 4535-4539.
15. Rudenko, G., Bonten, E., Hol, W.G.J., and D'Azzo, A. The atomic model of the human protective protein/cathepsin A. **Proc. Natl. Acad. Sci. USA.** 95 (1998) 621-625.
16. Zhou, X.Y., Galjart, N.J., Willemsen, R., Gillemans, N., Galjaard, H. and d'Azzo, A. A mutation in a mild form of galactosialidosis impairs dimerization of protective protein and renders it unstable. **EMBO J.** 10 (1998) 4041-4048.
17. Zhou, X.H., Morreau, H., Rottier R., Davis, D., Bonten, E., Gillemans, N., Wenger, D., Grosveld, F.G., Doherty, P., Suzuki, K. et.al. Mouse model for the lysosomal disorder galactosialidosis and correction of the phenotype

- with overexpressing erythroid precursor cells. **Genes Dev.** 9 (1995) 2623-2634.
18. Hahn, C.N., Del Pilar, M., Zhou, X.Y., Mann, L.W. and d'Azzo, A. Correction of murine galactosialidosis by bone marrow-derived macrophages overexpressing human protective protein/cathepsin A under control of the colony-stimulating factor-1 receptor promoter. **Proc. Natl. Acad. Sci. USA** 95 (1998) 14880-14885.
 19. Skidel, R.A. and Erdős, E.G. Cellular carboxypeptidases. **Immunol. Rev.** 161 (1998) 129-141.
 20. Itoh, K., Kase, R., Shimmoto, M., Satake, A., Sakuraba, H. and Suzuki, Y. Protective protein as an endogenous endothelin degradation enzyme in human tissues. **J. Biol. Chem** 270 (1995) 515-518.
 21. Hanna, W.L., Turbov, J.M., Jackman, H.L., and Tan, H. Dominant chymotrypsin-like esterase activity in human lymphocyte granules is mediated by the serine carboxypeptidase called cathepsin A-like protective protein. **J. Immunol.** 153 (1994) 4663-4672.
 22. Ostrowska, H., Wójcik, C., Omura, S. and Worowski, K. Lactacystin, a specific inhibitor of the proteasome, inhibits human platelet lysosomal cathepsin A-like enzyme. **Biochem. Biophys. Res. Commun.** 234 (1997) 729-732.