Biochimica et Biophysica Acta 1793 (2009) 674–683

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Principles of lysosomal membrane degradation
Cellular topology and biochemistry of lysosomal lipid degradation

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ARTICLE INFO

Article history:
Received 4 July 2008
Received in revised form 24 September 2008
Accepted 30 September 2008
Available online 1 November 2008

Keywords:
Lysosome
Glycosphingolipid
Sphingolipid activator protein

ABSTRACT

Cellular membranes enter the lysosomal compartment by endocytosis, phagocytosis, or autophagy. Within the lysosomal compartment, membrane components of complex structure are degraded into their building blocks. These are able to leave the lysosome and can then be utilized for the resynthesis of complex molecules or can be further degraded. Constitutive degradation of membranes occurs on the surface of intra-endosomal and intra-lysosomal membrane structures. Many integral membrane proteins are sorted to the inner membranes of endosomes and lysosome after ubiquitinylation. In the lysosome, proteins are degraded by proteolytic enzymes, the cathepsins. Phospholipids originating from lipoproteins or cellular membranes are degraded by phospholipases. Water-soluble glycosidases sequentially cleave off the terminal carbohydrate residues of glycoproteins, glycosaminoglycans, and glycosphingolipids. For glycosphingolipids with short oligosaccharide chains, the additional presence of membrane-active lysosomal lipid-binding proteins is required. The presence of lipid-binding proteins overcomes the phase problem of water soluble enzymes and lipid substrates by transferring the substrate to the degrading enzyme or by solubilizing the internal membranes. The lipid composition of intra-lysosomal vesicles differs from that of the plasma membrane. To allow at least glycosphingolipid degradation by hydrolases and activator proteins, the cholesterol content of these intraorganellar membranes decreases during endocytosis and the concentration of bis(monoacylglycero)phosphate, a stimulator of sphingolipid degradation, increases. A considerable part of our current knowledge about mechanism and biochemistry of lysosomal lipid degradation is derived from a class of human diseases, the sphingolipidoses, which are caused by inherited defects within sphingolipid and glycosphingolipid catabolism.

1. Lysosomal membrane digestion

Lysosomes are major degradative compartments of eukaryotic cells. In contrast to the proteasome, lysosomes degrade a wide variety of structurally diverse substances, such as proteins, glycosaminoglycans, nucleic acids, oligosaccharides, and complex lipids, into their building blocks [1]. These can leave the lysosomes either via diffusion, or with the aid of specialized transporters [2]. In the cytosol, the building blocks can be further degraded to fuel energy metabolism or can re-enter biosynthetic pathways. To provide building blocks of complex macromolecules for salvage and recycling pathways seems to be an important function of lysosomes. It has been shown, that in not very rapidly dividing cells, glycosphingolipids (GSL) are synthesized predominantly from sphingoid bases, carbohydrates and sialic acids released by lysosomes. In human foreskin fibroblasts for example, 90% of the glucosylceramide derives from recycling of sphingoid base, only 10% is synthesized *de novo* [3]. Under this aspect, the concept of lysosomes as waste dumps within cells would be a misleading association and should be replaced by the idea of lysosomes as stomachs of the cell, that provide macromolecule constituents and ensure lipid homeostasis.

1.1. Endocytosis and autophagy

Eukaryotic cells maintain highly regulated transport systems that convey cargo into the cell or exchange membranes and cargo between cellular organelles. Cellular and foreign cargo, but also membranes can reach the endosomal–lysosomal system via endocytosis, phagocytosis, autophagy, or direct transport. The various cellular functions associated with this process require degradation steps within the lysosomes, where proteins, complex cargo constituents, or complex membrane lipids have to be cleaved. During endocytosis, cargo enters the cell via clathrin-dependent or -independent mechanisms in a

Abbreviations: BMP, bis(monoacylglycero)phosphate; CADs, cationic amphiphilic drugs; GlcCer, glucosylceramide; GM3, NeuAcα2,3Galβ1,4Glcβ1ceramide; GM2, GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glcβ1ceramide; GM2-AP, GM2 activator protein; GSL, glycosphingolipids; LLBP, lysosomal lipid binding proteins; MVBs, multivesicular bodies; NPC, Niemann–Pick disease Type C; SAPs, sphingolipid activator proteins (Sap A–D and GM2-AP); Sap, saposin A–D

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doi:10.1016/j.bbamcr.2008.09.020

constitutive or ligand-induced manner [4]. Parts of the plasma membrane with and without receptor proteins are internalized, traffic through the endosomal compartment, and undergo different steps of sorting, before they are either recycled to the plasma membrane, or delivered to the lysosome for degradation. They reach the lysosome either as intra-lysosomal membrane structures or as part of the perimeter membrane [5,6]. During endosomal maturation, the luminal pH value decreases from values of about 7.2 to below 5 [7].

The endosomal membrane consists of different domain arrangements, in which Rab proteins are localized in morphologically distinct domains, like in a mosaic. Endosomes comprised of different domain arrangements display biochemical and possibly functional diversity [8].

Cellular macromolecules can be degraded by different pathways in eukaryotic cells. Ubiquitinylated proteins are degraded by the proteasomal system in the cytosol, bulk cytoplasma and organelles are delivered to the lysosome by (macro)autophagy [9] and cellular membranes are degraded in the lysosome after endocytosis. Autophagy requires a membrane degradation step, before cargo can be degraded by the lysosomal degradation system. Autophagy represents a unique form of membrane trafficking, in which membrane compartments (autophagosomes) engulf organelles or cytosolic cargo and deliver them to the lysosome for degradation [10]. Under normal growth conditions, autophagy occurs at a basal level. Starvation dramatically induces autophagy to maintain a pool of basic nutrients. Autophagy is evolutionary conserved in eukaryotes. Insights into the molecular pathways of autophagy were mainly gained by genetic approaches in yeast mutants defective in autophagy. Degradation of autophagic bodies occurs in yeast in the vacuole.

Autophagy starts with the formation of autophagosomes, double-membrane-layered vesicles, which enclose cytosol or organelles [11]. In yeast, after fusion with the vacuole, the autophagosome is released into the lumen as a single-membrane vesicle and termed autophagic body. The breakdown of this subvacuolar vesicle depends on the acidic pH of the vacuole [12], and on vacuolar proteinase A and proteinase B (Prb1) [13]. However, the function of Prb1 might be to activate vacuolar zymogens that play a direct role in the breakdown process [14]. Two other proteins have also been implicated in membrane degradation, the putative lipases Aut5 [11] and Aut4 [15].

Another role of autophagy in membrane degradation is that it is a source of bis(monoacylglycero)phosphate (BMP, erroneously also called lysobisphosphatidic acid, Fig. 1). This negatively charged lipid is highly enriched in the internal membranes of the lysosome and required for degradation of small GSL [16]. Biosynthetically, BMP is formed during the degradation of phosphatidylglycerol and cardiolipin, presumably on the surface of intra-lysosomal vesicles [17,18]. Cardiolipin in turn, reaches the lysosome as a component of mitochondria by macroautophagy. Its degradation leads to formation of BMP on internal membranes.

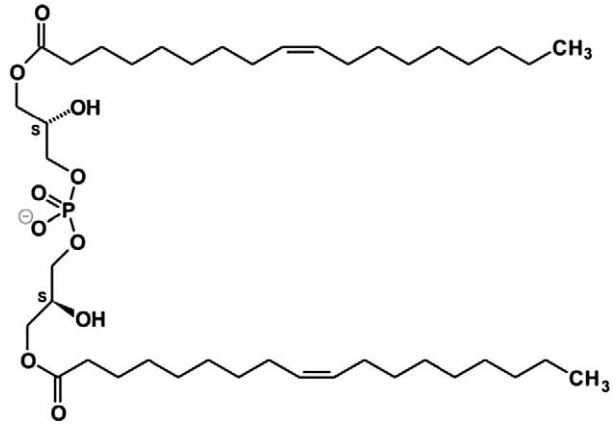

Fig. 1. Structure of bis(monoacylglycero)phosphate (BMP).

In eukaryotes, transmembrane proteins destined for lysosomal degradation are in eukaryotes often monoubiquitinylated and sorted in endosomal multivesicular bodies (MVBs) [19]. MVB formation requires the sequential action of three endosomal sorting complexes needed for transport (ESCRT-I,-II,-III) [20]. MVBs follow the pathway from early to late endosomes, and are eventually delivered to lysosomes, where they are degraded together with their protein cargo [21].

1.2. Topology of degradation

In the endosomal–lysosomal system, a variety of hydrolytic enzymes with acidic pH-optima cleave macromolecules such as proteins, polysaccharides, nucleic acids, glycoconjugates, and phospholipids. To protect the interior of the cell from these degradative enzymes, the integrity of the limiting membrane has to be preserved during the process of lysosomal degradation. This is achieved by a thick glycocalyx [22] composed of the carbohydrate part of lysosomal integral membrane proteins (LIMPS) and lysosomal associated membrane proteins (LAMPS) [23]. The enzymes required for lipid degradation cannot be expected to reach their substrates through this glycocalyx, which is composed of glycoproteins highly *N*-glycosylated with polylactosamine units. Since the perimeter membrane is protected from degradation, a second distinct pool of membranes has to be present in the endosomal/lysosomal compartment. This has been proposed in 1992 [5] and has been confirmed by independent groups. According to our current view, former parts of the plasma membrane destined for degradation reach the lysosome as part of small intra-lysosomal vesicles, in which the former extracytoplasmic membrane leaflet faces the lumen of the lysosome (Fig. 2). These intra-lysosomal vesicles provide the platform of membrane degradation. As indicated by studies with human patients with defects in GSL degradation, the surface of the inner lysosomal membranes represents the main site of membrane degradation in eukaryotic cells. Intra-lysosomal membranes have been initially observed, when different membrane degradation steps are defective. This was the case in cells from patients with sphingolipid storage diseases such as GM1 gangliosidosis [24] or combined sphingolipid activator protein (Sap) deficiency [25], where they accumulate as multivesicular storage bodies. Later on, they have been identified as MVBs [21,26]. Complementing the media of Sap-precursor-deficient fibroblasts with nanomolar concentrations of purified Sap-precursor reversed the aberrant accumulation of multivesicular structures, and restored the cells ability to degrade glycosphingolipids [27].

1.3. Lipid composition of lysosomal membranes

The lipid composition of limiting membrane and the intra-lysosomal membrane structures differs considerably from each other and from that of plasma membrane or the limiting membranes of other cellular organelles. In addition, the luminal pH value steadily decreases to achieve optimal conditions for the action of lysosomal enzymes. Besides the lipid composition, also the protein composition of the internal membranes is adjusted during endosomal sorting [28]. The intra-lysosomal membranes are formed by a lipid-sorting process along the endocytic pathway, during which its cholesterol content decreases and that of the negatively charged lipid BMP increases [16,29]. Due to its unusual sn1,sn1′-configuration, BMP has a sufficiently long lifetime in spite of the presence of the lysosomal phospholipases [30]. While intra-lysosomal membranes are enriched in BMP, this lipid is almost absent in the perimeter membrane, distinguishing the two membrane pools [16,29]. Other anionic lipids like phosphatidylinositol and dolichol phosphate, albeit in smaller