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Modulation of the in vitro activity of lysosomal phospholipase A1 by membrane lipids

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Abstract

Lysosomal phospholipases play a critical role for degradation of cellular membranes after their lysosomal segregation. We investigated the regulation of lysosomal phospholipase A1 by cholesterol, phosphatidylethanolamine, and negatively-charged lipids in correlation with changes of biophysical properties of the membranes induced by these lipids.

Lysosomal phospholipase A1 activity was determined towards phosphatidylcholine included in liposomes of variable composition using a whole-soluble lysosomal fraction of rat liver as enzymatic source. Phospholipase A1 activity was then related to membrane fluidity, lipid phase organization and membrane potential as determined by fluorescence depolarization of DPH, ^{31}P NMR and capillary electrophoresis.

Phospholipase A1 activity was markedly enhanced when the amount of negatively-charged lipids included in the vesicles was increased from 10 to around 30% of total phospholipids and the intensity of this effect depended on the nature of the acidic lipids used (ganglioside GM1 < phosphatidylinositol ∼ phosphatidylserine ∼ phosphatidylglycerol ∼

Abbreviations: Chol, cholesterol; DPH, diphenylhexatriene; EDTA, ethylene-diamine-tetra-acetic acid; GM1, ganglioside GM1; LBPA, lysobisphosphatidic acid; LUV, large unilamellar vesicles; MLV, multilamellar vesicles; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PP, phosphatidylpropanol; PS, phosphatidylserine; SM, sphingomyelin; sPLA2, secreted phospholipase A2; SUV, small unilamellar vesicles

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phosphatidylpropanol < phosphatidic acid). For liposomes containing phosphatidylinositol, this increase of activity was not modified by the presence of phosphatidylethanolamine and enhanced by cholesterol only when the phosphatidylinositol content was lower than 18%.
Our results, therefore show that both the surface-negative charge and the nature of the acidic lipid included in bilayers modulate the activity of phospholipase A1 towards phosphatidylcholine, while the change in lipid hydration or in fluidity of membrane are less critical. These observations may have physiological implications with respect to the rate of degradation of cellular membranes after their lysosomal segregation.

Keywords: Lysosomal phospholipase A1; Cholesterol; Phosphatidylethanolamine; Negatively-charged lipid; Fluidity; Lamellar phase; Surface potential

Enzymes that hydrolyse lipids usually access their substrate from the membrane phase and must therefore undergo a process of interfacial activation in which the enzyme attaches to the membrane before acting on the substrate. This process, which has been extensively studied for phospholipase A2 (PLA2), critically depends on the physico-chemical nature as well as the organization and dynamics of the interface (Roberts, 1996; Berg et al., 2001; Berg and Jain, 2002). Kinetic and X-ray structural studies of sPLA2s have established that these enzymes contain a recognition site that allows their attachment to the interface, which is distinct from the catalytic site where the esterolysis of a phospholipid molecule occurs. As a consequence, the substrate specificity and the level of activity of sPLA2s is dictated by the type of membrane interface to which the enzyme preferentially binds (interfacial specificity) as well as by the type of phospholipid that is accommodated in the catalytic site (catalytic site specificity) (Singer et al., 2002; Tatulian, 2003).

In contrast to the large body of data available for sPLA2, little is known concerning intracellular phospholipases and especially the lysosomal phospholipase A1. This enzyme however plays a crucial role for the degradation of intracellular phospholipids (Mellors and Tappel, 1967; Stoffel and Greten, 1967; Stoffel and Trabert, 1969; Franson et al., 1971; Shinozaki and Waite, 1999). Impairment of its activity by polycationic antibiotics (Laurent et al., 1982; Montenez et al., 1999) or amphiphilic cationic drugs (Lullmann-Rauch, 1979; Kodavanti and Mehendale, 1990; Halliwell, 1997; Schneider et al., 1997; Reasor and Kacew, 2001) is considered to be responsible for the development of lysosomal phospholipidosis observed in cells and tissues upon exposure to those drugs.

The mode of access of the phospholipid molecule to the catalytic site of phospholipase A1 implies its removal from the bilayer, the physico-chemical structure of which is therefore susceptible to strongly affect the overall activity of the enzyme. For instance, the rate of hydrolysis of DL-α-dipalmitoylphosphatidylcholine and L-α-dimyristoylphosphatidylcholine incorporated into liposomes, by a soluble fraction of liver lysosomes is maximal near the transition temperature (Vandenbranden et al., 1985). We also know that the activity of lysosomal phospholipase A1 is reduced when the surface pressure exceeds 32 dynes/cm^2 (Robinson and Waite, 1983) and increases markedly when the amount of negatively-charged phospholipid present in the vesicles is raised from 10 to 30% of the total phospholipid content (Mingeot-Leclercq et al., 1988, 1990; Piret et al., 1992), which is within the range found in most natural membranes (Bode et al., 1976).

Unfortunately, no systematic data on the role played by the organization and dynamics of the interface (Zhou et al., 1997) on lysosomal phospholipase A1 activity, are currently available. Using models mimicking biological membranes, we investigated the effect of two major lipids present in these membranes (cholesterol and phosphatidylethanolamine) as well as the effect of an increase in the content of negatively-charged lipids (phospholipid versus glycosphingolipid) on the hydrolysis of phosphatidylcholine by phospholipase A1 present in lysosomal extracts. In parallel, we also examined whether the variations observed in terms of activity are related to potential modifications induced by these lipids on critical membrane properties such as fluidity, lipid organization and surface potential of the bilayer.