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Effects of phospholipase A_2 on the lysosomal ion permeability and osmotic sensitivity

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Abstract

In this study, we investigated the mechanism of PLA_2-induced lysosomal destabilization. Through the measurements of lysosomal β-hexosaminidase free activity, their membrane potential, the intra-lysosomal pH and the lysosomal latency loss in hypotonic sucrose medium, we established that PLA_2 could increase the lysosomal membrane permeability to both potassium ions and protons. The enzyme could also enhance the organelle osmotic sensitivity. The increases in the lysosomal ion permeability promoted influx of potassium ions into the lysosomes via K^+/H^+ exchange. The resulted osmotic imbalance across the lysosomal membranes osmotically destabilized the lysosomes. In addition, the enhancement of the lysosomal osmotic sensitivity caused the lysosomes to become more liable to destabilization in the osmotic stress. The results explain how PLA_2 destabilized the lysosomes.
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Keywords: Lysosome; Phospholipase A_2; Permeability; Potassium ion; Proton; Osmotic sensitivity

1. Introduction

Lysosomes play important roles in the physiological turnover of cellular macromolecules such as proteins, lipids, nucleic acids and carbohydrates. The maintenance of lysosomal integrity is of the utmost importance for the organelle to carry out its functions. The destabilization of lysosomes influences their normal activities, may even lead to cell death. Forty years ago, lysosomes were named 'suicide bag' of cells, since the leaked lysosomal hydrolases had been shown to be cytotoxic (de Duve and Wattiaux, 1966). In recent years, interest in the lysosomal destabilization has heightened with the knowledge that leakage of lysosomal enzymes, especially cathepsins, can cause apoptosis or necrosis (Brunk et al., 1997; Erdal et al., 2005; Cirman et al., 2004). The leaked lysosomal enzymes can also bring about harmful effects in the pathogenesis of many diseases such as prion encephalopathies (Laszlo et al., 1992), Alzheimer's disease (Nixon et al., 1992), myocardial ischemia (Decker et al., 1980), poliovirus infection (Guskey et al., 1970), complement activation-produced lung injury (Hatherill et al., 1989), and acute tissue injury (Fell and Dingle, 1963). Since lysosomal integrity is critical for living cells, a great number of studies investigated the factors that are detrimental to lysosomal integrity. To elucidate

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Oxonol VI, bis(3-propyl-5-oxoisoxazol-4-yl)pentamethine-oxonol; FITC-Dextran, fluorescein isothiocyanate-dextran; PLA_2, phospholipase A_2

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the lysosomal destabilizing mechanisms is important for understanding apoptosis, necrosis and lysosomal pathophysiology (Ferri and Kroemer, 2001; Kroemer and Jaattela, 2005).

Lysosomal membrane is a barrier responsible for its integrity. Damages to lysosomal membranes or changes in the membrane structure may cause the organelle destabilization. A line of evidence indicates that phospholipase A_2 may destabilize lysosomes in the presence of mercury and copper (Marchi et al., 2004). The enzyme activated by the elevated cytosolic calcium concentration can destabilize lysosomes in leukocyte and mussel blood cell (Marone et al., 1983; Burlando et al., 2002). Recent studies showed that PLA_2 destabilized lysosomes under some apoptotic stimuli, the resulting leakage of lysosomal enzymes can induce apoptosis (Zhao et al., 2001; Brunk et al., 1997). However, it is still unclear how PLA_2 destablizes lysosomes. In this study, we established that PLA_2 increased the lysosomal membrane permeability to K^+ and H^+, and enhanced their osmotic sensitivity for the first time. These changes in the lysosomal membrane properties may account for the destabilization of the organelle.

2. Experimental procedures

2.1. Materials

Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), fluorescein isothiocyanate-dextran (FITC-Dextran, $M_r = 70{,}000$), 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide, phospholipase A_2 (from honey bee venom) and Valinomycin were from Sigma (St. Louis, MO). Bis(3-propyl-5-oxoisoxazol-4-yl)pentamethine-oxonol) (oxonol VI) was from Molecular Probes. Percoll was purchased from Amersham (Uppsala, Sweden). Other chemicals used were of analytical grade from Beijing Chemical Factory.

2.2. Preparation of lysosomes

Male Wistar rats were starved for 24 h and killed by decapitation. Rat liver lysosomes were isolated by the Percoll gradient centrifugation methods of Jonas with a minor modification to increase lysosomal purity (Jonas et al., 1983). Briefly, rat liver was homogenized in 0.25 M sucrose and centrifuged at 3000 × *g* for 8 min. The supernatant was incubated at 37 °C for 5 min in the presence of 1 mM $CaCl_2$ to promote separation of lysosomes from mitochondria (Yamada et al., 1984). Then, the supernatant was centrifuged for 20 min at 20,000 × *g*. The pellet was resuspended in sucrose and mixed with Percoll (2:1, by vol.), and centrifuged at 40,000 × *g* for 90 min. The lower 1/4 volume of the gradient (lysosomal fraction) was pooled and mixed with 10 volumes of 0.25 M sucrose, and centrifuged at 10,000 × *g* for 13 min to remove Percoll. The purified lysosomes were resuspended in 0.25 M sucrose medium at 2.12 mg protein/ml for use. All performances were carried out at 0–4 °C. Protein was determined according to (Lowry et al., 1951).

2.3. Assay of lysosomal integrity

Lysosomal integrity was assessed by the measurement of lysosomal enzyme latency. The latency of a lysosomal enzyme refers to the percent of intact lysosomes as revealed by the inability of substrate to reach the lysosomal enzyme until the organelles are deliberately ruptured (Greene and Schneider, 1992). 4-Methylumbelliferyl *N*-acetyl-β-D-glucosaminide, the substrate of lysosomal β-hexosaminidase, was used at 1 mM to measure the enzyme activity (Bird et al., 1987). The liberated 4-methylumbelliferone was determined by measuring its fluorescence (excitation 365 nm, emission 444 nm) on a Hitachi F-4010 fluorescence spectrophotometer. Activities of the enzyme measured in the absence and presence of 0.36% Triton X-100 was designated the free activity and the total activity, respectively. Percentage free activity was calculated as (free activity/total activity) × 100. Lysosomal enzyme latency can be defined as [1 − (free activity/total activity)] × 100. Loss of lysosomal integrity was determined as increased percentage free activity or loss of lysosomal enzyme latency.

2.4. Assay of lysosomal permeability to K^+

The lysosomal permeability to K^+ was assessed by the osmotic protection method. It is the most widely used method to determine whether a solute can enter the lysosomes (Lloyd and Forster, 1986; Forster and Lloyd, 1988; Reign and Tager, 1977). According to the principle of this method, an impermeable solute can provide perfect osmotic protection to the lysosomes suspended in the isotonic solution. However, the solute that penetrates into the lysosomes can break the initial osmotic balance across the lysosomal membranes. A progressive osmotic imbalance develops with increasing the solute concentration inside the lysosomes. As a result, the lysosomes swell and burst. Thus the rupture of the lysosomal membrane induced by swelling, and hence the permeability to a solute including ions, can be monitored by measuring changes in the latency of a lysosomal enzyme after incubating the lysosomes in a solution of that solute. This approach gives a semiquantitative measure of relative

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