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Mechanism of Lysophosphatidylcholine-Induced Lysosome Destabilization

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Abstract Lysosomal destabilization is critical for the organelle and living cells. Phospholipase A_2 (PLA_2) was shown to be able to destabilize lysosomes under some conditions. By what mechanism the enzyme affects lysosomal stability is not fully studied. In this study, we investigated the effects of lysophosphatidylcholine (lysoPC), a PLA_2-produced lipid metabolite, on lysosomal ion permeability, osmotic sensitivity and stability. By measuring lysosomal β-hexosaminidase free activity, membrane potential, proton leakage and their enzyme latency loss in hypotonic sucrose medium, we established that lysoPC could increase the lysosomal permeability to both potassium ions and protons and enhance lysosomal osmotic sensitivity. These changes in lysosomal membrane properties promoted entry of potassium ions into lysosomes via K^+/H^+ exchange. The resultant osmotic imbalance across the membranes led to losses of lysosomal integrity. The enhancement of lysosomal osmotic sensitivity caused the lysosomes to become more liable to destabilization in osmotic shock. These results suggest that lysoPC may play a key role in PLA_2-induced lysosomal destabilization.

Keywords Lysosome · Lysophosphatidylcholine · Ion permeability · Osmotic sensitivity

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Introduction

Loss of lysosomal integrity is critical for the organelle. The event can cause leakage of lysosomal protons, resulting in elevations of their internal pH and losses of their membrane potential. These changes may lead to lysosomal dysfunction. The most serious result of lysosomal destabilization is the leakage of their enzymes. Forty years ago, lysosomes were named a ''suicide bag'' of cells owing to the cytotoxic effects of their leaked hydrolases (De Duve and Wattiaux 1966). The destabilization of lysosomes can cause losses of lysosomal function and even cell death, including necrosis and apoptosis (Cirman et al. 2004; Erdal et al. 2005). In addition, the leaked lysosomal enzymes 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, Poole and Wildenthal 1980), poliovirus infection (Guskey, Smith and Wolff 1970), complement activation-produced lung injury (Hatherill et al. 1989), acute tissue injury (Fell and Dingle 1963), and so on. Since lysosomal destabilization can produce so many detrimental effects, a number of studies have focused on the mechanisms by which lysosomes are destabilized.

The lysosomal membrane is a barrier responsible for the maintenance of its integrity. Destruction of the membrane or change in the membrane structures may cause destabilization of the lysosomes. A line of evidence indicates that

phospholipase A_2 (PLA_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). A recent study showed that PLA_2 could also destabilize lysosomes in apoptosis (Zhao, Brunk and Eaton 2001). The leaked lysosomal enzyme cathepsins can cause apoptosis (Brunk et al. 1997). However, by what mechanism PLA_2 destabilizes lysosomes is not fully elucidated. Recently, we demonstrated that arachidonic acid, a product of PLA_2-catalyzed phospholipid hydrolysis, could destabilize lysosomes. Lysophosphatidylcholine (lysoPC), an amphipathic molecule, is another product of the PLA_2-hydrolyzed phospholipid. LysoPC is believed to be of biological importance. It may affect membrane order and membrane permeability. Whether lysoPC can destabilize lysosomes is not well studied. In this study, we established that lysoPC could increase the lysosomal membrane permeability to K^+ and H^+ and enhance lysosomal osmotic sensitivity. These changes in lysosomal membrane properties can destabilize the organelle. We provide new information for the mechanism by which PLA_2 destabilizes lysosomes.

Materials and Methods

Chemicals

Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), lysoPC, 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide, *p*-nitrophenol, oxonol VI and valinomycin were from Sigma (St. Louis, MO). Percoll was purchased from Amersham (Uppsala, Sweden). Other chemicals used were of analytical grade (Beijing Chemical Factory, Beijing, P. R. China).

Preparation of Lysosomes

Rat liver lysosomes were isolated by the Percoll gradient centrifugation methods of Jonas et al. (1983) with a minor modification to increase lysosome purity. Briefly, rat liver was homogenized in 0.25 M sucrose and centrifuged at $3{,}000 \times g$ 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, Hayashi and Natori 1984). Then, the supernatant was centrifuged at $20{,}000 \times g$ for 20 min. The pellet was resuspended in sucrose and mixed with Percoll (2:1, by vol) and centrifuged at $40{,}000 \times g$ for 90 min. The lower one-fourth volume of the gradient (lysosomal fraction) was pooled, mixed with 10 volumes of 0.25 M sucrose and centrifuged at $15{,}000 \times g$ for 15 min to remove Percoll. Purified lysosomes were resuspended in 0.25 M sucrose medium at 2.12 mg protein/ml for use. All performances were carried out at 4°C. Protein was determined according to Lowry et al. (1951).

Assay of Lysosomal Integrity

Lysosomal integrity was assessed by measurement of lysosomal enzyme latency, which refers to the percent of intact lysosomes as revealed by the inability of substrate to reach lysosomal enzymes until the organelles are deliberately ruptured (Greene and Schneider 1992). 4-Methylumbelliferyl *N*-acetyl-β-D-glucosaminide was used at 1 mM to measure lysosomal β-hexosaminidase activity (Bird, Forster and Lloyd 1987). The liberated 4-methylumbelliferone was determined by measuring its fluorescence (excitation 365 nm, emission 444 nm) on a Hitachi (Tokyo, Japan) F-4500 fluorescence spectrophotometer. Activities of the enzyme measured in the absence and presence of 0.36% Triton X-100 were designated the free activity and the total activity, respectively. Percentage free activity was calculated as (free activity/total activity) $\times$ 100. Lysosomal enzyme latency was defined as [1- (free activity/total activity)] $\times$ 100. Loss of lysosomal integrity was determined as increased percentage free activity or loss of lysosomal enzyme latency.

Assay of Lysosomal Permeability to K^+

The lysosomal permeability to K^+ can be assessed by the osmotic protection method, which is the most widely used method to determine whether a solute can enter 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 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 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 rate of entry of permeant ions. Lysosomes (7.5 µl) were incubated in 180 µl incubation medium (0.25 M sucrose or 0.125 M K_2SO_4, buffered at pH 7.0 with 10 mM 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid [HEPES]/KOH) in the presence or absence of lysoPC at 37°C for the indicated time. Then, a portion of this lysosomal suspension was used for the assay of

lysosomal integrity. Increases in the lysosomal permeability to K^+ were determined as increased loss of lysosomal enzyme latency.

Measurement of Lysosomal Membrane Potential

Lysosomal K^+ permeability can be assessed by measurement of membrane potential using oxonol VI as a probe (Zhong et al. 2000). The assay medium contained 0.25 M sucrose (pH adjusted to 6.7 with imidazole), oxonol VI (2 μM) and CCCP (2 μM). For measuring lysosomal membrane potential, 20 μl lysosomal sample was added to 1.8 ml assay medium at 88 μg protein/ml. The content of a blank cuvette were the same as that of a sample cuvette except for addition of lysosomal sample. Two hundred microliters of 0.75 M K_2SO_4 was added to the sample and blank cuvettes at the indicated times. Membrane potential was registered by the absorbance difference $\Delta A_{625-587}$ (Loh, Tam and Russell 1984). All measurements were performed at 25°C on a Hitachi 557 spectrophotometer.

Measurement of Lysosomal Proton Leakage

Lysosomal proton leakage can acidify the suspending medium. As described previously, the acidification of assay medium induced by lysosomal proton leakage can be measured by monitoring the absorbance decrease with pH-sensitive dye *p*-nitrophenol (Yao and Zhang 1997). Changes in absorbance are based on the property of the dye that the unprotonated *p*-nitrophenol molecules have a sufficiently larger extinction coefficient at 400 nm over that of protonated molecules (Zhang et al. 2000). The assay medium contained 0.25 M sucrose, 0.04 mM *p*-nitrophenol (Na salt) and 200 μl 0.75 M K_2SO_4. Absorbance (400 nm) of *p*-nitrophenol was measured immediately upon addition of lysosomal sample to the assay medium. All measurements were carried out at 25°C on a UNIC-2100 spectrophotometer (UNIC, Shangai, China).

Assay of Lysosomal Osmotic Sensitivity

Lysosomal osmotic sensitivity was assessed as described previously by examining the integrity after incubation in hypotonic sucrose medium (Wan et al. 2002). Lysosomes were treated with lysoPC at 37°C for 2 min. Then, the treated or control lysosomal samples were incubated in sucrose medium (concentration as indicated) at 37°C for the indicated time. A portion of this incubated lysosomal suspension was used for the assay of lysosomal integrity. Increases in free enzyme activity of the lysosomes incubated in hypotonic medium indicate increases in lysosomal osmotic sensitivity.

Results

Effects of LysoPC on Lysosomal K^+ Permeability

Normal lysosomes show only a limited permeability toward K^+ (Harikumar and Reeves 1983), which is favorable for the maintenance of lysosomal osmotic stability. Whether lysoPC affects lysosomal K^+ permeability was examined. As shown in Figure 1, control lysosomes maintained their enzyme latency in both the sucrose medium and the K_2SO_4 medium during 20 min incubation. Similarly, lysoPC-treated lysosomes also maintained their integrity in sucrose medium. The results indicate that lysosomes were stable in the media and that lysoPC itself did not destabilize the lysosomes. However, lysoPC-treated lysosomes lost their enzyme latency markedly in K_2SO_4 medium (free activity increased from 7% at 0 min to 67% at 20 min). According to the osmotic protection method used for the assay of lysosomal ion permeability (Lloyd and Forster 1986), the results indicate that lysoPC treatment increased lysosomal K^+ permeability and destabilized the lysosomes in K^+–containing medium.

The effect of lysoPC on lysosomal K^+ permeability was further examined by measuring membrane potential with oxonol VI. According to the property of the probe, increases in differential absorbance ($\Delta A_{625-587}$) of the dye, indicating a more positive interior potential of the membrane, will be observed when K^+ is allowed to enter (Zhong et al. 2000). As shown in Figure 2, absorbance of the dye increased greatly when valinomycin was used to permeablize the lysosomes to K^+ (line 1). The differential

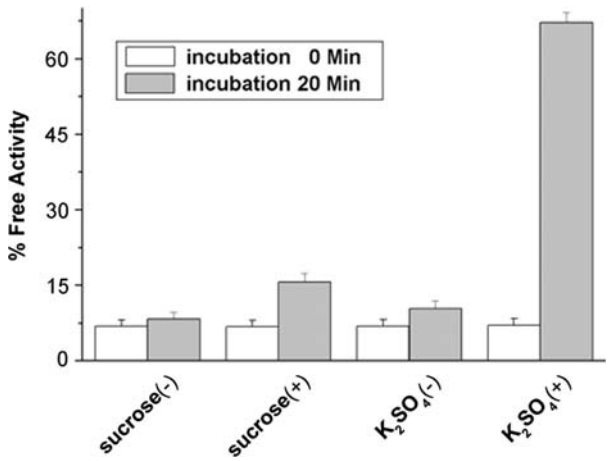

Fig. 1 Effects of lysoPC treatment on lysosomal permeability to K^+. Lysosomes were incubated in 0.25 M sucrose medium or 0.125 M K_2SO_4 medium (both buffered at pH 7.0 with 10 m M HEPES/KOH) at 37°C for 0 or 20 min. Lysosomal free enzyme activity was measured immediately after incubation. -, lysosomes incubated in the absence of lysoPC; +, lysosomes incubated in the presence of 20 μ M lysoPC. Values are means ± standard deviation of three measurements

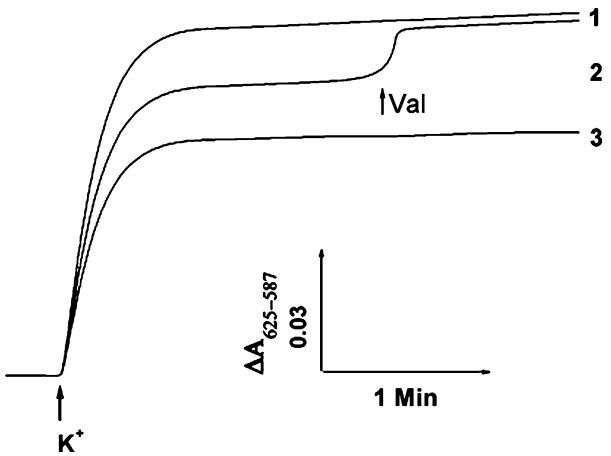

Fig. 2 Effects of lysoPC treatment on lysosomal membrane potential. Lysosomal sample was added to 1.8 ml assay medium at 88 μg protein/ml. We added 200 μl 0.75 M K_2SO_4 to the medium at indicated times. *Line 1*, assay medium contained 5 μM valinomycin; *line 2*, assay medium contained 20 μM lysoPC and 5 μM valinomycin added at the indicated time; *line 3*, control sample (no lysoPC and valinomycin added). A typical result out of three experiments is shown

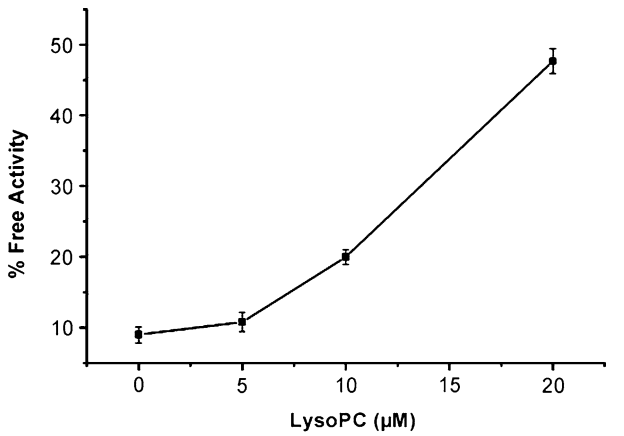

Fig. 3 Effects of lysoPC concentration on lysosomal latency loss. Lysosomes were treated with lysoPC at the indicated concentration in 0.125 M K_2SO_4 medium (buffered at pH 7.0 with 10 mM HEPES/KOH) at 37°C for 15 min. Lysosomal free enzyme activity was measured immediately after incubation. Values are means ± standard deviation of three measurements

absorbance of the lysoPC-treated lysosomal sample (line 2) increased more markedly than that of the control lysosomes (line 3) upon addition of K^+ to the medium. This indicates that the lysosomal permeability to K^+ increased by lysoPC treatment. The dye absorbance of the lysoPC-treated lysosomal sample (part of line 2 before addition of valinomycin) approached that of the valinomycin-treated lysosomes (line 1) more closely than the control lysosomes (line 3). This result indicates that the potassium ion permeability of the former is greater than that of the latter. Addition of valinomycin to the medium of the lysoPC-treated lysosomal sample (line 2) caused the absorbance to approach that of the valinomycin-treated lysosomes (line 1). This further established that lysoPC increased lysosomal K^+ permeability. We should explain why the protonophore CCCP was used in the above measurements. The pH gradient of lysosomes produces an internal negative membrane potential. An increase in the lysosomal H^+ permeability causes the internal membrane potential to become more negative (Harikumar and Reeves 1983). As shown in the following experiments, lysoPC could increase the lysosomal H^+ permeability. The prerequisite for the assessment of lysosomal K^+ permeability by the measurement of membrane potential is to maintain the proton permeability of both control lysosomes and the lysoPC-treated ones at the same level; thus, changes in membrane potential will depend solely on their own K^+ permeability. For this reason, CCCP was used to permeabilize all of the measured lysosomes to protons.

The response of lysosomal K^+ permeability to the dose of lysoPC was examined. The results show that treatment of the lysosomes with 5 μM lysoPC did not affect the lysosomal integrity in K_2SO_4 medium. Lysosomes increasingly lost their enzyme latency in the medium with increasing lysoPC concentration above 10 μM (Fig. 3). It would help to establish the physiological relevance of the above observed effects. To examine whether K^+ enters the lysosomes through potassium channels, we used 4-aminopyridine, tetraethylammonium and quinine, all blockers of the potassium channel, in the above experiments. The results show that all of these blockers could not prevent entry of K^+ into the lysoPC-treated lysosomes (*data not shown*). To date, no evidence is available concerning the existence of K^+ channel, carrier and K^+ pump on the lysosomal membranes. It is likely that K^+ entered the lysoPC-treated lysosomes by a passive diffusion mechanism.

Effects of LysoPC on Lysosomal H^+ Permeability

Normally, lysosomes exhibit a limited permeability toward H^+, which is favorable for the maintenance of intralysosomal acidic pH (Harikumar and Reeves 1983). Increases in lysosomal H^+ permeability can cause leakage of their protons. A previous study demonstrated that lysosomal proton leakage could be assessed by measurement of the suspending medium acidification with the pH-sensitive dye *p*-nitrophenol (Yao and Zhang 1997). Using this method, we examined the effect of lysoPC on lysosomal proton permeability. As shown in Figure 4, absorbance of the lysoPC-treated lysosomal sample decreased more markedly (line 3) than that of the control ones (line 2). Since unprotonated *p*-nitrophenol molecules have a sufficiently larger extinction coefficient at 400 nM over that of protonated molecules (Lozier et al. 1976), the marked

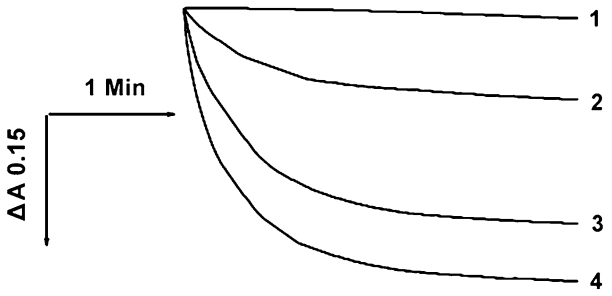

Fig. 4 Effect of lysoPC treatment on lysosomal proton leakage. All assay media contained 0.25 M sucrose, 75 mM K_2SO_4 and 0.04 mM *p*-nitrophenol. Additional components are added to individual media as follows: assay medium of *lines 1* and *3* contained 20 μM lysoPC; assay medium of *line 1* was buffered at pH 7.0 with 20 mM HEPES/KOH; assay medium of *line 4* contained 2 μM CCCP and 5 μM valinomycin. Lysosomal sample was added to 2 ml assay medium at 88 μg protein/ml. Then, absorbance (400 nm) was measured immediately at 25°C. *Line 1*, lysoPC-treated lysosomes; *line 2*, lysosomes were not treated with lysoPC; *line 3*, lysoPC-treated lysosomes; *line 4*, lysosomes were not treated with lysoPC. A typical result out of three measurements is shown

absorbance decrease of the lysoPC-treated lysosomes reflected greater proton leakage. The effect of proton leakage on dye absorbance was confirmed by the observation that the absorbance decrease of the lysoPC-treated lysosomes could be abolished in the buffered assay medium (compare line 1 with line 3). An additional experiment showed that the absorbance decreased more markedly upon addition of CCCP and valinomycin to the medium (line 4). This result is consistent with previous results showing that the ionphores could promote efflux of lysosomal protons through K^+/H^+ exchange (Casey, Hollemans and Tager 1978). It further verified that lysosomal proton leakage caused the absorbance decrease. These results indicate that lysoPC increased lysosomal H^+ permeability.

K^+/H^+ Exchange Mechanism of LysoPC-Treated Lysosomes

Passive diffusion of K^+ into lysosomes should be accompanied by the charge-compensating ions to maintain the electroneutrality of the ion movement. Since K^+/H^+ exchange is a major pathway for the entry of K^+ into lysosomes (Casey et al. 1978), the influx of K^+ into lysosomes correlates with their membrane permeability to both K^+ and H^+. The above results indicate that lysoPC increased the lysosomal permeability to K^+ and H^+. To determine whether potassium ions enter lysoPC-treated lysosomes through K^+/H^+ exchange, we suspended treated lysosomes in K^+-containing media that were buffered at different pH to provide different pH gradients across the membranes. Because different ΔpH across the lysosomal membrane could generate different driving forces for the exchange of lysosomal protons with external K^+, lysoPC-treated lysosomes should exhibit different latency losses after incubation in K^+-containing medium. As shown in Figure 5, free enzyme activity of lysoPC-treated lysosomes increased markedly from 35% to 68% when the medium pH was elevated from 6.6 to 7.4. In contrast, control lysosomes maintained their free enzyme activity at each medium pH. This indicates that potassium ions entered lysoPC-treated lysosomes via K^+/H^+ exchange and, therefore, destabilized the organelle.

Effects of LysoPC on Lysosomal Osmotic Sensitivity

Lysosomes were named intracellular ''osmometers'' owing to their relatively higher sensitivity to osmotic shock (Lloyd and Forster 1986). The osmotic sensitivity of lysosomes reflects their ability to resist hypotonic pressures. Increases in lysosomal osmotic sensitivity can cause the organelle to become more susceptible to osmotic stress. Lysosomes are more liable to osmotic swell and even lysis in osmotic shock because more water may enter the lysosomes. To assess the effects of lysoPC on lysosomal osmotic sensitivity, we examined lysosomal integrity after incubating lysosomes in hypotonic sucrose medium for a period of time. As shown in Figure 6, lysoPC-treated lysosomes lost their enzyme latency more markedly than control lysosomes in hypotonic sucrose medium. This indicates that lysoPC-treated lysosomes lost their normal ability to resist osmotic stress; i.e., their osmotic sensitivity increased. This conclusion is supported by the results

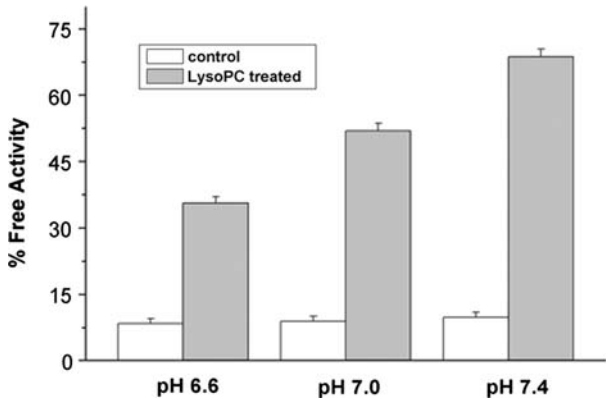

Fig. 5 Effects of K_2SO_4 medium pH on the integrity of lysosomes. Lysosomes were treated with 20 μM lysoPC in sucrose at pH 7.0 for 10 min, then washed two times to remove lysoPC. We incubated 7.5 μl of the treated lysosomal sample in 180 μl 0.125 M K_2SO_4 medium (at 88 μg protein/ml) at 37°C for 15 min. The K_2SO_4 medium pH was adjusted to the indicated value with 10 mM HEPES/Tris. Lysosomal free enzyme activity was measured after incubation. *Open bar*, control lysosomes, not treated with lysoPC; *closed bar*, lysosomes treated with 20 μM lysoPC. Values are means ± standard deviation of three measurements

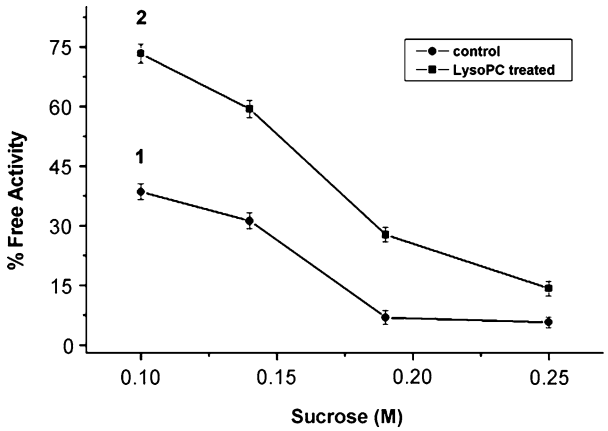

Fig. 6 Effects of lysoPC treatment on lysosomal osmotic sensitivity. Lysosomes (0.21 mg protein/ml) were treated with 80 μM lysoPC at 37°C for 2 min. Then, 80 μl of the treated sample was incubated in 400 μl sucrose medium (concentration as indicated) at 37°C for 5 min. LysoPC was diluted to 16 μM in sucrose medium. Lysosomal free enzyme activity was measured after incubation. *Line 1*, lysosomes were not treated with lysoPC; *line 2*, lysosomes were treated with 80 μM lysoPC. Values are means ± standard deviation of three measurements

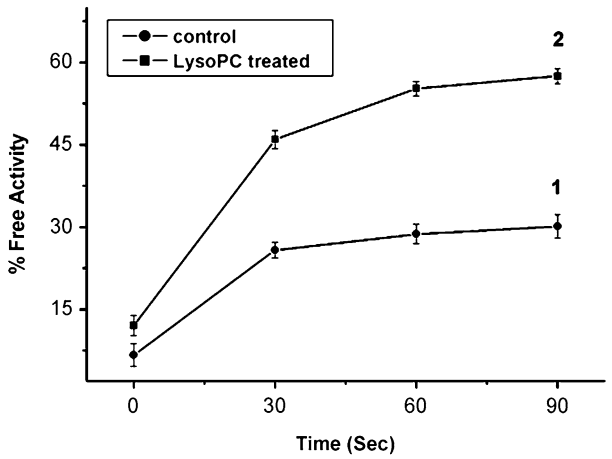

Fig. 7 Effects of incubation duration on osmotic stability of lysosomes. Lysosomes (0.21 mg protein/ml) were treated with 80 μM lysoPC at 37°C for 2 min. Then, 80 μl of the treated sample was incubated in 400 μl 0.14 M sucrose medium at 37°C for the indicated time. LysoPC was diluted to 16 μM in sucrose medium. Lysosomal free enzyme activity was measured immediately after incubation. *Line 1*, lysosomes were not treated with lysoPC; *line 2*, lysosomes were treated with 80 μM lysoPC. Values are means ± standard deviation of three measurements

shown in Figure 7. LysoPC-treated lysosomes lost enzyme latency to a greater extent and more rapidly than control lysosomes in hypotonic sucrose medium. The above results indicate that lysoPC could increase lysosomal osmotic sensitivity.

Discussion

The maintenance of lysosomal integrity is of the utmost importance for organelle activities. Lysosomal destabilization can cause losses of membrane ΔpH and membrane potential, leading to dysfunction of lysosomes. Normally, lysosomes show a limited permeability toward various ions. Peptides and uncharged carbohydrates with molecular weight above 220 Da have difficulty permeating lysosomal membranes. A remarkable feature of destabilized lysosomes is the enhancement of their membrane permeability. This increases the influx of external ions and molecules into the lysosomes, producing osmotic stress and destabilizing the lysosomes. The most serious change in destabilized lysosomes is the loss of their enzyme latency. In recent years, a number of studies have demonstrated that cathepsins, a family of lysosomal proteases, could modify Bid (a member of the Bcl-2 family) and directly activate caspase-3 after leaking into the cytoplasm (Cirman et al. 2004). These events can induce apoptosis (Erdal et al. 2005). Extensive leakage of lysosomal hydrolases can also cause necrosis (Brunk et al. 1997). In addition, leaked lysosomal enzymes play important roles in the pathogenesis of many diseases (Laszlo et al. 1992; Nixon et al. 1992; Decker et al. 1980). These data heightened interest in the study of lysosomal destabilization. An important question is how lysosomes are destabilized under various conditions.

A recent study showed that PLA_2 could destabilize lysosomes and induce apoptosis by the leaked enzymes (Zhao et al. 2001). However, by what mechanism PLA_2 destabilizes lysosomes is not fully understood. In this study, we demonstrated that lysoPC could destabilize lysosomes. Obviously, lysosomal destabilization is an abnormal event in living cells. It means that the cytosolic lysoPC should not affect lysosmal integrity under normal conditions. A great number of studies have reported that PLA_2 was activated under various pathological conditions. In these cases, the enzyme produces more lysoPC, which may destabilize the lysosomes. It has been reported that physiological concentrations of lysoPC are in the range of 5–180 μM in different tissues and body fluids (Croset et al. 2000; Xu 2002). In pathophysiological situations, such as ischemia, lysoPC may reach concentrations of up to 200 μM (Akita et al. 1986; Sedlis, Sequeira and Altszuler 1990). Previous studies in cardiac muscle have found that lysoPC concentrations of 10–75 μM are sufficient to produce electrophysiological derangements in multicellular (Pogwizd et al. 1986) and isolated myocyte preparations (Kiyosue, Aomine and Arita 1984). However, little information is available concerning the physiological concentration of cytosolic lysoPC. In this study, we demonstrated

that lysosomes lost latency after treatment with more than 10 μM lysoPC. This suggests that lysosomal integrity is not affected by cytosolic lysoPC at a concentration less than 10 μM. These results will help the study of how PLA_2 destabilizes lysosomes under various conditions.

Lysosomal ion permeability and osmotic sensitivity are important properties for organelle stability. Changes in these properties may destabilize lysosomes. Lysosomes *in vivo* are surrounded by a high concentration of cytoplasmic K^+ (140 mM). Previous studies showed that excessive entry of K^+ into lysosomes could produce osmotic stresses to their membranes and eventually disintegrate the organelle (Ruth and Weglicki 1982). Whether cytoplasmic K^+ can enter the lysosomes and affect their stability correlates with the lysosomal permeability to K^+. Normal lysosomes show only a limited permeability toward K^+ (Harikumar and Reeves 1983), which prevents abnormal entry of K^+ into the lysosomes and osmotically protects the organelle. However, some factors, such as low temperature (Reign and Tager 1977) and oxidation to lysosomal membranes (Zhang and Yao 1997), can increase lysosomal K^+ permeability. Although lysosomal K^+ permeability is an important property of the organelle, not much is known about the factors that affect the ion permeability. The results of this study provide new information in this respect and explain why lysoPC can destabilize lysosomes.

Passive diffusion of K^+ into lysosomes must be accompanied by charge-compensating ions to maintain the electroneutrality of ion movements. As demonstrated previously, K^+/H^+ exchange is a major pathway for lysosomal uptake of K^+ (Casey et al. 1978). Therefore, the extent of K^+ entry into lysosomes correlates with lysosomal H^+ permeability. Lysosomal membranes normally exhibit a limited permeability to H^+ (Harikumar and Reeves 1983). An increase in lysosomal H^+ permeability can promote influx of K^+ into the organelle through K^+/H^+ exchange. It has been established that the physical state of lysosomal membranes (Zhang et al. 2000) and oxidation of the thiol groups of the lysosomal membrane proteins (Wan, Wang and Zhang 2001) can affect the membrane permeability to H^+. By now, not much information is available concerning the factors that affect lysosomal H^+ permeability. Recently, we found that arachidonic acid could increase lysosomal H^+ permeability. The results of this study show that lysoPC produces a similar effect on lysosomes. The lysoPC-produced K^+/H^+ exchange explains how K^+ entered the lysosomes.

Lysosomes are called intracellular ''osmometers'' owing to their susceptibility to osmotic stress (Lloyd and Forster 1986). A line of evidence showed that lysosomal osmotic sensitivity is an important property for organelle stability (Yang et al. 2000; Wan et al. 2002). The ability of lysosomes to resist osmotic stresses correlates with their osmotic sensitivity. The lysosomes are more liable to disrupt in an osmotic stress when their osmotic sensitivity increases. As shown above, lysoPC could increase lysosomal uptake of K and therefore caused osmotic shock to the membranes. Meanwhile, lysoPC enhanced lysosomal osmotic sensitivity. Thus, lysosomes are more liable to disintegrate in K^+-induced osmotic stress. As shown in our previous studies, changes in the physical state of lysosomal membranes and photooxidation of lysosomal membrane thiol groups can increase their osmotic sensitivity (Yang et al. 2000; Wan et al. 2002). At present, by what mechanism lysosomal osmotic sensitivity increases is unclear. It might be caused by increases in the permeability to water and an enhancement of membrane osmotic fragility. How lysoPC enhances lysosomal osmotic sensitivity remains for further study.

LysoPC can induce a wide range of important cellular effects, such as enhancement of vascular smooth muscle cell proliferation, attracting monocytes, inhibiting endothelium-dependent relaxation and retarding endothelial cell migration. It can also alter the expression of multiple genes, including increasing nitric oxide synthase in endothelial cells, upregulating growth factors such as heparin binding epidermal growth factor and platelet-derived growth factor in various cells, suppressing endothelin 1 in endothelial cells and enhancing the expression of adhesion molecules in endothelial cells (Colles and Chisolm 2000). In addition to these physiological effects, lysoPC has been implicated in the pathogenesis of diseases such as atherosclerosis, ischemia and certain inflammations (Wilson-Ashworth et al. 2004). LysoPC can also promote or prevent the occurrence of apoptosis under different conditions (Kogure et al. 2003; Yen et al. 2001). Importantly, lysoPC produces various effects on membranes. It affects membrane order and undulation forces (Golzio, Teissie and Rols 2000). As reported previously, nuclear membranes (Leno and Munshi 1994) and fibroblast membranes (Colles and Chisolm 2000) can be permeabilized with lysoPC. LysoPC can increase the ion permeability of membranes. It promotes a transient calcium influx in lymphoma cells (Wilson-Ashworth et al. 2004). A recent study demonstrated that lysoPC activates store-operated calcium channels, causing capacitative calcium influx (Smani et al. 2004). Other evidence shows that lysoPC can alter gramicidin channel function by altering the membrane deformation energy (Lundbaek and Andersen 1994) and activate non-selective cation channels that are permeable for monovalent and divalent cations (Schilling et al. 2004). The above studies show the various effects of lysoPC on the membranes and cells, but the mechanisms through which it evokes such diverse cellular responses are not clearly defined. These remain for further study to clarify.

In this study, we established that lysoPC increased the lysosomal membrane permeability to K^+ and H^+ and enhanced lysosomal osmotic sensitivity. We provide new information concerning the effects of lysoPC on membranes. At present, we are not able to explain how lysoPC produced such effects on lysosomes. It may correlate with the mechanisms proposed previously. To cross membranes by a solubility-diffusion mechanism, the permeating solute must partition into the hydrophobic region of the membrane, diffuse across and leave by redissolving into the aqueous phase outside the other side of the membrane. In this case, Born energy is required to transfer water or other charged particle from the high dielectric aqueous phase to the low dielectric membrane interior because the electrostatic energy of the ion is much lower in a water medium with a high dielectric constant (~80) than in a typical bilayer with a low dielectric constant (~2) (Orme, Moronne and Macey 1988). The second pathway for solute permeation is through the transient defects of membranes. The incorporation of external perturbants and thermal fluctuation of membrane lipids can produce transient defects in membranes. The resultant defects act as mobile free volumes that can carry small molecules and ions across the membrane (Trauble 1971). Most ions and polar molecules are difficult to dissolve in the hydrophobic region of membrane owing to the Born energy barrier, but they can readily enter the water-filled defects on the membrane surface. Therefore, transient defects seem to be the dominant permeation mechanism for ions, while neutral molecules cross the membrane exclusively by the solubility-diffusion mechanism due to their high solubility in a hydrocarbon phase (Paula et al. 1996). Whether lysoPC can produce transient defects in lysosomal membranes or increase the dielectric constant of lysosomal membranes is unknown. This possibly correlates with the effects of lysoPC on lysosomes.

As introduced above, PLA_2 could destabilize lysosomes under various conditions, but mechanisms for the events are not fully elucidated. The results of this study indicate that lysoPC may play a role in PLA_2-induced lysosomal destabilization.

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