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ORIGINAL PAPER

Effects of arachidonic acid on the lysosomal ion permeability and osmotic stability

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Abstract In this study, we investigated the effects of arachidonic acid, a PLA_2-produced lipid metabolite, on the lysosomal permeability, osmotic sensitivity and stability. Through the measurements of lysosomal β-hexosaminidase free activity, membrane potential, intralysosomal pH, and lysosomal latency loss in hypotonic sucrose medium, we established that arachidonic acid could increase the lysosomal permeability to both potassium ions and protons, and enhance the lysosomal osmotic sensitivity. As a result, the fatty-acid-promoted entry of potassium ions into the lysosomes via K^+/H^+ exchange, which could produce osmotic imbalance across their membranes and osmotically destabilize the lysosomes. In addition, the enhancement of lysosomal osmotic sensitivity caused the lysosomes to become more liable to destabilization in osmotic shock. The results suggest that arachidonic acid may play a role in the lysosomal destabilization.

Keywords Lysosome · Arachidonic acid · Permeability · Potassium ion · Proton · Osmotic sensitivity

Abbreviations

CCCP	Carbonyl cyanide *m*-Chlorophenylhydrazone
FITC-dextran	Fluorescein isothiocyanate-dextran
AA	Arachidonic acid
PLA_2	Phospholipase A_2

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Introduction

Lysosomal destabilization is a crucial event not only for the organelle but also for living cells. It causes efflux of lysosomal protons, resulting in elevation of their internal pH and loss of their acidic hydrolase activities. In addition, leakage of lysosomal enzymes, especially cathepsins, can cause apoptosis and necrosis (Brunk *et al*, 1997). 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), acute tissue injury (Fell and Dingle, 1963) and so on. In the past years, effects of lysosomal destabilization on the organelle activities, cell viability and the pathogenesis of some diseases were extensively studied. However, mechanisms for lysosomal destabilization are not well understood. To elucidate this issue is important for the above studies.

As demonstrated previously, lysosomes are liable to destabilization in the cases of oxidation of their membrane lipids and proteins (Zhang and Yao, 1997), osmotic stresses (Yang *et al*, 2000), intralysosomal accumulation of some molecules such as glucagons (Deter and de Duve, 1967) and heavy metal ions (Abok *et al*, 1983). Recent evidence shows that lysosomes may disrupt in the initiation stage of necrosis and apoptosis, causing the cells to go to death (Brunk *et al*, 1997). Lysosomal membranes play a key role in the maintenance of lysosomal integrity.

Damage to the membranes or changes in the membrane structures may cause lysosomal destabilization. A line of evidence indicates that phospholipase A_2 may destabilize lysosomes in the presence of mercury and copper (Marchi *et al*, 2004). Importantly, PLA_2 can destabilize lysosomes and induce apoptosis by the leaked cathepsins (Zhao *et al*, 2001). By what mechanism PLA_2 destabilizes lysosomes is still unclear. Arachidonic acid is the product of PLA_2-catalyzed phospholipid hydrolysis. It is of interest to clarify whether this fatty acid can destabilize lysosomes and how it affects lysosomal membranes. The results of this study show that arachidonic acid can increase lysosomal membrane permeability to K^+ and H^+, and enhance lysosomal osmotic sensitivity. These membrane changes can destabilize the lysosomes

Materials and methods

Chemicals

4-Aminopyridine, arachidonic acid, CCCP, fluorescein isothiocyanate-dextran (FITC-Dextran, $M_r = 70,000$), 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide, quinine, tetraethylammonium, and valinomycin were from Sigma (St. Louis, MO). Percoll was purchased from Amersham (Uppsala, Sweden). Other chemicals used were of analytical grade from Beijing Chimical Factory.

Preparation of lysosomes

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 $3000\times$ *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 mitochondrias (Yamada *et al*, 1984). Then, the supernatant was centrifuged for 20 min at $20,000\times$ *g*. The pellet was resuspended in sucrose and mixed with Percoll (2:1, v/v), and centrifuged at $40,000\times$ *g* for 90 min. The lower one fourth volume of the gradient (lysosomal fraction) was pooled and mixed with 10 volumes of 0.25 M sucrose, and centrifuged at $10,000\times$ *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 4°C. Protein was determined according to Lowry (Lowry *et al*, 1951).

Assay of lysosomal integrity

Lysosomal integrity was assessed by measuring the activity of lysosomal β-hexosaminidase using 1 mM 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide as substrate (Bird *et al*, 1987). The liberated 4-methylumbelliferone was determined by measuring its fluorescence (excitation 365 nm, emission 444 nm) on a Hitachi F-4500 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) $\times$ 100. Lysosomal enzyme latency can be 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. This approach gives a semiquantitative measure of relative rate of entry of permeant ions (Lloyd and Forster, 1986). A 8-μL lysosomes were incubated in 150 μl incubation medium (0.125 M K_2SO_4, buffered at pH 7.0 with 10 mM Hepes/KOH) in the presence or absence of 10 μM AA 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

The lysosomal K^+ permeability can be assessed by the 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. For measuring lysosomal membrane potential, 20-μL lysosomal sample and oxonol VI were added to 1.7 ml assay medium at 22 μg protein/ml and 2 μM, respectively. The contents of blank cuvette were same as that of sample cuvette except addition of lysosomal sample. Two hundred microliters of 0.75 M K_2SO_4 was added to the sample and blank cuvettes at indicated times. Membrane potential was registered by the absorbance difference $\Delta A_{625-587}$ (Loh *et al*, 1984). All measurements were performed at 25°C on a Hitachi 557 spectrophotometer.

Measurement of intralysosomal pH

The intralysosomal pH was measured by the method of Ohkuma (Ohkuma *et al*, 1982). Rat was injected intraperitoneally with FITC-Dextran (20 mg FITC-Dextran/150 g body weight) and starved for 16 h before decapitation. Lysosomes were prepared as described above. The measurement medium was composed of 0.25 M sucrose or 0.125 M K_2SO_4,

buffered at pH 7.0 with 20 mM Hepes/Tris. The fluorescence was measured at two excitation wavelengths (495 nm and 450 nm) with 510 nm as emission wavelength. Intralysosomal pH was determined from the fluorescence ratio (F_{495}/F_{450}) of the lysosomal sample relative to a standard curve generated as described by Ohkuma. All measurements were carried out at 37°C on a Hitachi F-4500 fluorescence spectrophotometer.

Assay of lysosomal osmotic sensitivity

Lysosomal osmotic sensitivity was assessed as described previously by examining their integrity after incubation in hypotonic sucrose medium (Wan *et al*, 2002). First, lysosomal samples (0.45 mg protein/ml) were treated with 15 μM arachidonic acid at 37°C for 10 min. Then, the lysosomal samples were incubated in sucrose medium (concentration as indicated) at 37°C for the indicated time. Then, a portion of this 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 the lysosomal osmotic sensitivity.

Results

Effects of arachidonic acid on the lysosomal K^+ permeability

Previous studies indicate that lysosomes are susceptible to the K^+ influx-induced osmotic stress (Ruth and Weglicki, 1982). Normal lysosomes show only a limited permeability toward K^+ (Harikumar and Reeves, 1983), which is favorable for the maintenance of lysosomal osmotic stability. Following experiments were carried out to assess the effects of AA on the lysosomal K^+ permeability. As shown in Fig. 1, free-enzyme activity of the AA-treated lysosomes is similar to that of control ones in sucrose incubation medium. It suggests that the AA treatment did not affect lysosomal integrity. In contrast to the enzyme latency loss in sucrose medium, the AA treatment increased the lysosomal free-enzyme activity from 22 to 54% in the K_2SO_4 medium. Based on the osmotic protection method used for the assay of lysosomal ion permeability (Lloyd and Forster, 1986), it indicates that AA increased the lysosomal K^+ permeability and destabilized the lysosomes in the K^+–containing medium. To examine whether K^+ entered the lysosomes through potassium channels, we used potassium channel blockers 4-Aminopyridine, Tetraethylammonium and Quinine in the above experiments. The results show that all of these blockers could not abrogate the effects of AA on the lysosomal K^+ permeability. By now, there is no evidence showing the existence of K^+ channels, carriers and K^+ pump on the lysosomal membranes. Our recent works are also failed to find the channels. It is likely that

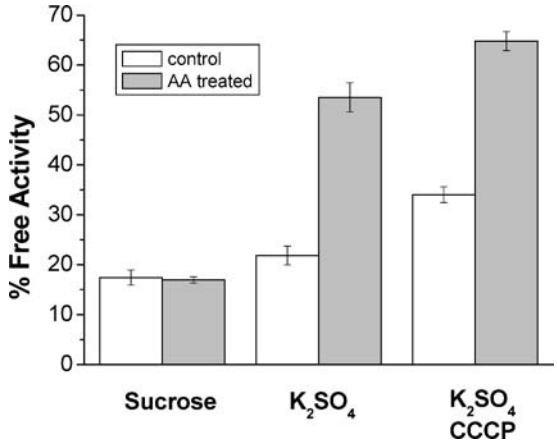

Fig. 1 Effects of arachidonic acid treatment on the lysosomal permeability to K^+ Lysosomal permeability to K^+ was assessed as described in Materials and Methods section. Lysosomes were incubated in 0.125 M K_2SO_4 medium or 0.25 M sucrose medium (buffered at pH 7.0 with 10 mM Hepes/KOH) at 37°C for 20 min. Lysosomal free-enzyme activity was measured immediately after the incubation. Open bar: lysosomes incubated in the absence of AA; Closed bar: lysosomes incubated in the presence of 10 μM AA. 1 μM CCCP was added to the K_2SO_4 medium as indicated. Values are mean ± SD of three measurements

K^+ entered the AA-treated lysosomes by a passive diffusion mechanism. The charges of K^+ entering lysosomes must be electroneutrally balanced by charge-compensating movements of another ion. To fully exhibit AA-induced increase in the lysosomal K^+ permeability, protonophore CCCP was added to the K_2SO_4 medium to promote K^+ entry via K^+/H^+ exchange. Thus, extent of the K^+ entry will depend solely on the K^+ permeability. The results show that free-enzyme activity of the AA-treated lysosomes increased more markedly in the presence of CCCP. It reinforces above conclusion and reflects the occurrence of K^+/H^+ exchange.

To clarify the response of lysosomes to the dose of arachidonic acid would help to establish physiological relevance of above observed effects. In the following experiments, the lysosomes were treated with AA at different concentrations under the conditions of Fig. 1. The results show that AA did not affect lysosomal integrity in the K^+-containing medium at concentrations less than 3 μM (Fig. 2). The lysosomes increasingly lost latency in the K_2SO_4 medium with increasing AA concentration above 3 μM. In contrast, the lysosomes did not lose latency in isotonic sucrose medium even though they were treated with 10 μM AA (as shown in Fig. 1). It indicates that K^+ may destabilize the AA-treated lysosomes and that more than 3 μM AA is required to increase the lysosomal K^+ permeability. Additional experiments were carried out to examine whether oleic acid also affects the lysosomal K^+ permeability and integrity. After treating the lysosomes with 50 and 100 μM oleic acid in 0.125 M K_2SO_4 medium (pH 7.0) at 37°C for 20 min, free-enzyme activity of the lysosomes was at 16 and 18% (similar to the value of normal lysosomes in the K_2SO_4 medium), respectively. Increasing

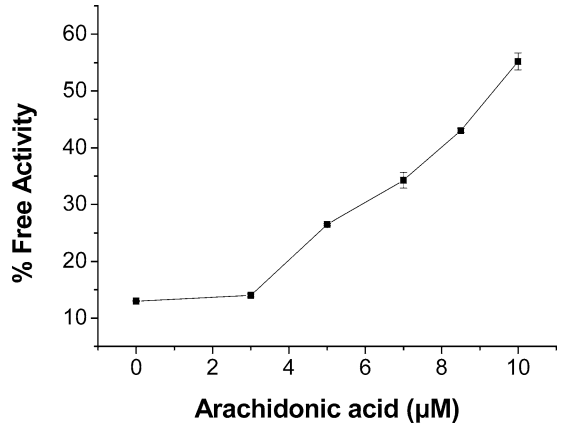

Fig. 2 Effects of arachidonic acid concentration on the lysosomal latency loss Lysosomes were treated with AA at indicated concentration in 0.125 M K_2SO_4 medium (buffered at pH 7.0 with 10 mM Hepes/KOH) at 37°C for 20 min. Then, the lysosomal free-enzyme activity was measured immediately as described in Materials and Methods section. Values are mean ± SD of three measurements

oleic acid concentration to 150 μM raised the value only to 22%. The results indicate that oleic acid did not markedly affect the lysosomal K^+ permeability and integrity. In contrast, only 10 μM AA increased the free-enzyme activity to 55%.

The effects of AA on the lysosomal K^+ permeability was re-examined by measuring their membrane potential with oxonol VI. According to the property of this probe, increases in the differential absorbance ($A_{625-587}$) of the dye, indicating a more positive interior potential of the membrane, will be observed when K^+ are allowed to enter (Zhong *et al*, 2000). As shown in Fig. 3, differential absorbance of the AA-treated lysosomal sample (line 2) increased more markedly than that of control lysosomes (line 3) upon addition of K^+ to the medium. The result indicates that the lysosomal permeability to K^+ increased by the AA treatment. It is supported by additional evidence that the dye absorbance of the AA-treated lysosomal sample approached the absorbance of valinomycin-treated lysosomes (line 1) more closely than the control lysosomes. It indicates that the potassium ion permeability of the former is greater than that of the latter. As shown in Fig. 1, 10 μM AA caused marked lysosomal latency loss during a 20-min treatment, which reflected an increase in the lysosomal K^+ permeability. However, 10 μM AA could not produce marked change in the lysosomal membrane potential until 20 μM AA was used in the above measurement. It may be attributed to two reasons. First, measurement of membrane potential is possible less sensitive than the assay of lysosomal latency loss to the changes in the lysosomal K^+ permeability. In other words, the former method is possible not as efficient as the latter for estimating lysosomal K^+ permeability. Therefore, higher concentration of AA was needed to produce a larger increase in the lysosomal K^+ permeability, which could produce marked changes in the lysosomal membrane potential. Second, the lysosomes were treated with AA for only 3 min in the membrane potential measurement, while AA treated lysosomes for 20 min in the assay of lysosomal latency loss. Therefore, higher concentration of AA is required to produce increases in the lysosomal K^+ permeability in a 3-min membrane potential measurement.

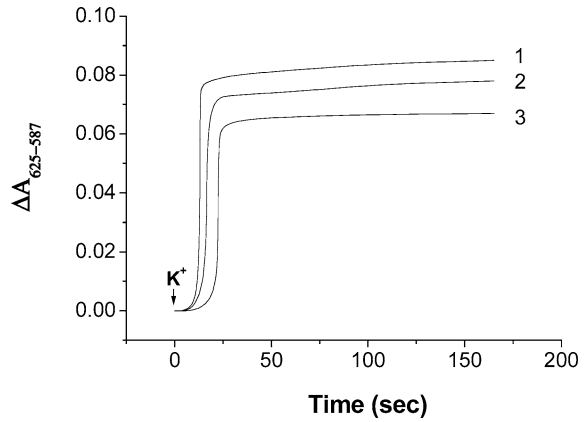

Fig. 3 Effects of arachidonic acid treatment on the lysosomal membrane potential Lysosomal membrane potential was measured as described in Materials and Methods section. Assay medium contained 0.25 M sucrose (pH adjusted to 6.7 with imidazole) and 2 μM oxonol VI. Lysosomal sample was added to the assay medium at 22 μg protein/ml. 200 μl 0.75 M K_2SO_4 was added to the assay medium at indicated times. Line 1: assay medium contained 6 μM valinomycin; line 2: assay medium contained 20 μM AA; line 3: control sample (no AA and valinomycin). A typical result out of three experiments is shown

Effects of arachidonic acid on the lysosomal H^+ permeability

The intralysosomal acidity is maintained by their H^+-ATPase-mediated proton translocation and the limited membranes permeability toward H^+ (Harikumar and Reeves, 1983). Increases in the lysosomal H^+ permeability can cause leakages of their protons. Experiments were carried out to examine the effects of AA on the lysosomal H^+ permeability. As shown in Fig. 4, treatment of the lysosomes with 4 μM AA increased their internal pH from 5.71 (line 1, not treated with AA) to 5.79 (line 2) in the sucrose medium and from 6.01 (line 3, not treated with AA) to 6.41 (line 4) in the K_2SO_4 medium within 3 minutes. It indicates that the AA treatment increased lysosomal H^+ permeability. 4 μM AA could induce marked elevations of intralysosomal pH but only a slight increase in the lysosomal free enzyme activity (Fig. 2). It suggests that lysosomal H^+ permeability might be more sensitive to the perturbation of AA than the response of their K^+ permeability. Compared with the samples measured in the sucrose medium, the larger pH increase of the lysosomes measured in K_2SO_4 medium is due to a K^+/H^+ exchange. The effect of external K^+ on the proton efflux is further established by the evidence that the lysosomal

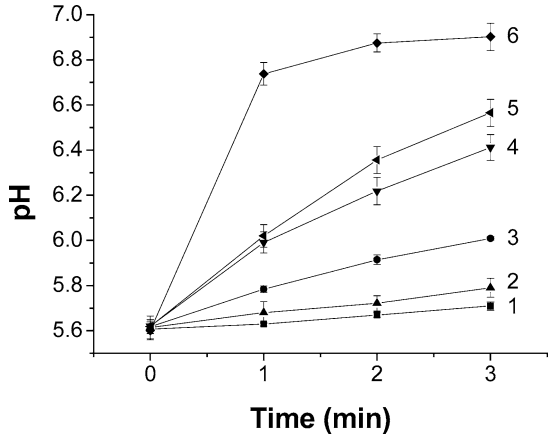

Fig. 4 Effects of arachidonic acid treatment on the intralysosomal pH intralysosomal pH was measured as described in Materials and Methods section. Lysosomal sample (0.45 mg protein/ml) was added to 1 ml following medium: Line 1: 0.25 M sucrose medium; line 2: 0.25 M sucrose medium containing 4 μM AA; line 3: 0.125 M K_2SO_4 medium; line 4: 0.125 M K_2SO_4 medium containing 4 μM AA; line 5: 0.125 M K_2SO_4 medium containing 4 μM valinomycin; line 6: 0.125 M K_2SO_4 medium containing 4 μM AA and 4 μM valinomycin. Intralysosomal pH was measured at indicated time after addition of lysosomes to the measuring medium. Values are means of three measurements

pH increased more markedly upon addition of valinomycin (ionophore of K^+) to the medium (compare line 6 with line 5). K^+/H^+ exchange is a major pathway for the entry of K^+ into lysosomes (Casey *et al*, 1978). To what extent K^+ enters lysosomes correlates not only to their K^+ permeability but also to the membrane permeability to H^+. Above results suggest that AA-induced increase in the lysosomal H^+ permeability may promote entry of K^+ into the lysosomes and therefore destabilize the organelle.

In addition to the lysosomal permeability toward K^+ and H^+, the oppositely directed transmembrane concentration gradients of H^+ and K^+ are required to drive exchanges of lysosomal H^+ for the external K^+. In order to further establish the occurrence of K^+/H^+ exchange, we suspended lysosomes in K^+-containing mediums that were buffered at different pH to provide different pH gradient across their membranes. Thus, the lysosomes should exhibit different latency loss in these mediums. As shown in Fig. 5, treatment of lysosomes with AA increased their free-enzyme activity in K_2SO_4 medium. Extent of the lysosomal latency loss increased with elevating the medium pH from 6.0 to 7.4. Increased pH gradient across the membranes promoted entry of K^+ into the lysosomes, suggesting that K^+ entered the AA-treated lysosomes via K^+/H^+ exchange.

Effects of arachidonic acid on the lysosomal osmotic sensitivity

Lysosomes are sensitive to osmotic stresses (Lloyd and Forster, 1986). An increase in the lysosomal osmotic sensitivity can cause the organelle to become more susceptible to osmotic shock, *i.e.* the lysosomes are prone to osmotic swell and lysis. Above results showed that the AA treatment enhanced the lysosomal permeability to K^+ and H^+. Increased uptake of K^+ can cause osmotic imbalance across the lysosomal membranes. Whether the lysosomes can resist such osmotic stresses correlates to their osmotic sensitivity. Lysosomes were incubated in hypotonic sucrose medium to examine their osmotic sensitivity. The results show that the lysosomes treated with 15 μM AA lost their enzyme latency markedly in the hypotonic sucrose medium (Fig. 6, line

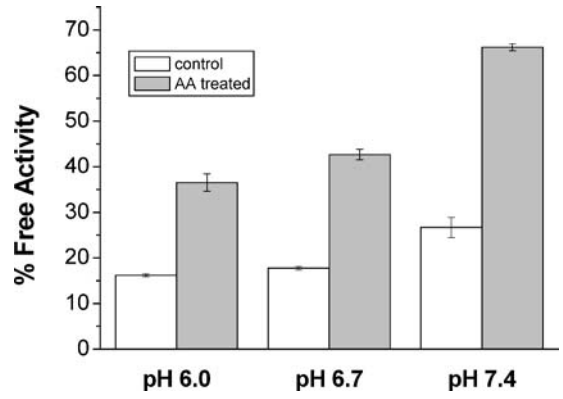

Fig. 5 Effects of K_2SO_4 medium pH on the integrity of arachidonic acid-treated lysosomes A 8.0-μl lysosomes sample was incubated in 120 μl 0.125 M K_2SO_4 medium in the presence or absence of 20 μM AA at 37°C for 30 min. The K_2SO_4 medium was buffered at indicated pH with 10 mM Citric acid/Hepes or KOH/Hepes. Lysosomal free-enzyme activity was measured immediately after the incubation. Open bar: lysosomes incubated in the absence of AA; closed bar: lysosomes incubated in the presence of AA. Values are mean ± SD of three measurements

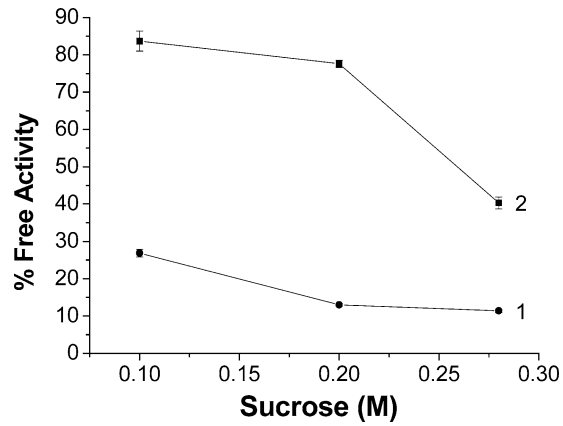

Fig. 6 Effects of arachidonic acid treatment on the lysosomal osmotic sensitivity Lysosomal sample (0.45 mg protein/mL) was treated with 15 μM AA at 37°C for 10 min. Then, the sample was incubated in sucrose medium (concentration as indicated) at 37°C for 20 min. Lysosomal free-enzyme activity was measured immediately after the incubation. Line 1: lysosomes were not treated with AA; line 2: lysosomes were treated with 15 μM AA. Values are mean ± SD of three measurements

2). In contrast, control lysosomes could relatively resist the hypotonic pressures (line 1). In other words, the AA-treated lysosomes lost their normal ability to resist osmotic stress. It suggests that the AA treatment increased lysosomal osmotic sensitivity. In the above measurements, 10 μM AA could not produce marked increase in the lysosomal osmotic sensitivity (data not shown). It is likely that the osmotic sensitivity property is less sensitive to AA than the response of permeability to K^+ and H^+.

Discussion

Lysosomal permeability to K^+ and H^+, and lysosomal osmotic sensitivity are important properties of the organelle. Changes in these properties may cause loss of their functions and even destabilization of the lysosomes. The lysosomes in vivo are surrounded by a high concentration of cytoplasmic K^+ (140 mM). To what extent potassium ions enter lysosomes correlates to their permeability to K^+. Normal lysosomes show only a limited permeability toward K^+ (Harikumar and Reeves, 1983). However, some factors or events such as low temperature (Reijngoud and Tager, 1977) and oxidation to lysosomal membranes (Zhang and Yao, 1997) can increase lysosomal ion permeability. As demonstrated previously, increases in the lysosomal K^+ permeability caused increases in the lysosomal uptake of K^+, which may produce osmotic stresses to their membranes and eventually disintegrate the organelle (Ruth and Weglicki, 1982). Although lysosomal K^+ permeability is an important property of the organelle, not much is known about the factors that affect the ion permeability. The evidence presented in this study may have some significance for the studies of lysosomal pathophysiology.

Lysosomal H^+ permeability directly affects intralysosomal acidic pH and indirectly affects lysosomal integrity. Previous studies indicated that K^+/H^+ exchange is a major pathway for the lysosomal uptake of K^+ (Casey *et al*, 1978). Increases in lysosomal H^+ permeability can promote influx of K^+ into the lysosomes via K^+/H^+ exchange. Lysosomal membranes normally exhibit a limited permeability to H^+ (Harikumar and Reeves, 1983). It has been established that the physical state of lysosomal membranes (Zhang *et al*, 2000) and oxidation of the thiol groups of their membrane proteins (Wan *et al*, 2001) can affect the membrane permeability to H^+. Other factors affecting the ion permeability are not well studied. Phospholipase A_2 may destabilize lysosomes under some conditions (Marchi *et al*, 2004; Zhao *et al*, 2001), but mechanisms for the events are unclear. As established in this study, arachidonic acid can increase lysosomal permeability to H^+ and K^+. K^+ entered the lysosomes via K^+/H^+ exchange and destabilized the lysosomes. It should be clarified whether arachidonic acid plays a role in the PLA_2-induced lysosomal destabilization.

Lysosomes are named intracellular "osmometer" owing to their susceptibility to osmotic stress (Lloyd and Forster, 1986). As shown earlier, arachidonic acid promoted entry of K^+ into the lysosomes, causing osmotic shock to their membranes. Moreover, AA enhanced lysosomal osmotic sensitivity. Thus, the lysosomes are more liable to disintegrate in the K^+-induced osmotic stress. Our previous studies show that changes in the membrane physical state and photooxidation of lysosomal membrane thiol groups can increase lysosomal 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 the increases in their permeability to water and the enhancement of membrane osmotic fragility. Exact mechanism remains for further study.

Arachidonic acid plays important roles in various cellular activities. It acts as a precursor in the synthesis of prostaglandin and leukotrienes. AA and its metabolites are also associated with cell growth, regeneration, and development (Gentili *et al*, 2004). The level of AA in living cells can markedly increase under some conditions such as aging (Gentili *et al*, 2004), transient ischemia (Rao *et al*, 1999), apoptosis (Yoshihiro, 2004) and so on. As reported previously, increased arachidonic acid can produce cytotoxic effects. It causes damage to mitochondria with initiation of a membrane permeability transition and a decline in membrane potential, followed by release of cytochrome c, caspase 3 activation, and apoptosis (Yoshihiro, 2004; Wu and Cederbaum, 2002). As demonstrated previously, PLA_2 may destabilize lysosomes in leukocyte and mussel blood cell (Marone *et al*, 1983; Bruno *et al*, 2002). Recent study shows that phospholipase A_2 may destabilize lysosomes in apoptosis (Zhao *et al*, 2001). The resultant leakage of lysosomal enzymes can induce apoptosis (Brunk *et al*, 1997). Whether arachidonic acid plays a role in this event is unclear. The results of this study suggest that AA possibly participates in the PLA_2 -mediated initiation of apoptosis. Further studies are required to verify this possibility.

As shown in this study, AA can increase the permeability of lysosomes to K^+ and H^+. Previous study demonstrated that AA can affect the permeability of squid giant axon to Na^+ but has little effect on K^+ permeation (Takenaka *et al*, 1988). By now, how fatty acids affect membrane permeability is not well elucidated. Two mechanisms have been proposed to account for solute permeation of membranes. To cross membranes by solubility-diffusion mechanism, the permeating particle must partition into the hydrophobic region, diffuse across, and leave by re-dissolving into the second aqueous phase.

For this mechanism, 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 high dielectric constant (∼80) than in a typical bilayer with low dielectric constant (∼2) (Orme *et al*, 1988). Membrane-transient defects are the second pathway for solute permeation. Thermal fluctuation of membrane lipids and incorporation of external perturbants can produce transient defects in the 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 membrane exclusively by the solubility-diffusion mechanism due to their high solubility in a hydrocarbon phase (Paula *et al*, 1996). As proposed previously, the amounts of defect in membranes and consequently the permeability increase with the increasing degree of unsaturation of membrane lipids. It has been proved that fatty acids can readily partition into membranes (Pjura *et al*, 1984). Arachidonic acid has four double bonds. The incorporation of AA into membrane can increase the degree of lipid unsaturation. It might be a reason for the AA-induced increase in the lysosomal permeability to K^+ and H^+. Compared with arachidonic acid, oleic acid almost did not affect the lysosomal K^+ permeability and integrity. Oleic acid has only one double bond. Whether the inefficiency of oleic acid on the lysosomes correlates to its low degree of unsaturation remains for further study.

In living cells, normal unbound fatty acid concentrations are $<10^{-7}$ M in the cytosol and $<10^{-6}$ M in the plasma (John, 1988). It was reported that cytosolic concentration of unbound fatty acid in the range of 10^{-7}–10^{-6} M are potentially toxic to mitochondria (Piper *et al*, 1983). In this study, we established that arachidonic acid (above 3–4 μM) could perturb some lysosomal membrane properties and eventually destabilize the organelle. The results provide new information for the pathological roles of arachidonic acid.

Lysosomes can be destabilized by the damages and alternations in their membranes due to increased membrane permeability and membrane fragility. In this respect, effects of membrane lipid peroxidation (Zhang and Yao, 1997) and heavy metal ions (Abok *et al*, 1983) on the lysosomal stability were studied. The occurrence of osmotic stress can also destabilize lysosomes even their membranes are at normal state. Certainly, the lysosomes suffered from some damages or membrane structure alternations are more liable to disrupt in osmotic shock. The destabilizing effect of AA on the lysosomes may correlate to these two mechanisms. AA increased lysosomal ion permeability, producing osmotic stress to their membranes. The increase in their osmotic sensitivity caused the lysosomes to become more sensitive to the osmotic shock. The incorporation of AA into lysosomal membranes can perturb the membrane lipid order, which may cause changes in their membrane properties and losses of the membrane barrier function.

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