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Lysosomal destabilization via increased potassium ion permeability following photodamage

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Abstract

Isotonic K_2SO_4 solution protected lysosomes osmotically during a 20 min incubation, but lost its protective effect if the lysosomes were initially photooxidized after sensitization with Methylene blue. Increasing K_2SO_4 concentration promoted the latency loss of photodamaged lysosomes, but did not impair the integrity of unirradiated lysosomes. The results indicate that the photodamage enhanced lysosomal ionic permeability, with osmotic imbalance over the lysosomal membrane. Out of the decreased latency induced by the photodamage, 32% was prevented by the addition of 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid to the incubation solution, suggesting that electroneutral K^+/SO_4^{2-} co-uptake plays a role in the lysosomal destabilization. The photooxidation increased lysosomal H^+/K^+ exchange, which was confirmed by monitoring the H^+ leakage with the pH sensitive probe *p*-nitrophenol and examining the K^+ entry by membrane potential measurements. Addition of K_2SO_4 to a lysosomal suspension lowered the ΔpH of photodamaged lysosomes, presumably due to an increase in the exchange of internal H^+ for external K^+. Out of the photodamage-induced lysosomal latency loss, 50–60% was prevented by either lowering the external pH or preincubating the lysosomes with methylamine to elevate their internal pH. The results suggest that the photodamage-promoted K^+/H^+ exchange plays a major role in lysosomal osmotic destabilization.

Keywords: Lysosome; Latency loss; Proton; Exchange, K^+/H^+-; Photodamage; Methylene blue

1. Introduction

In the basic studies of photodynamic therapy (PDT) of cancers, identifying subcellular photosensitive targets and exploring photodamage mechanisms continues to be an area of active investigation [1–3]. A number of studies demonstrated that lysosomes are main photodamage site [4,5] and leakage of hydrolytic enzymes from photodestructed lysosomes may lead to cell death [6,7]. This is consistent with the 'suicide bag' concept of lysosomes [8]. In addition to lysosome-dependent photocytotoxicity, lysosomotropic photooxidation is recently suggested as a new approach for either delivering some drugs or reducing the pH of cytoplasm which may serve to augment the effects of a number of cancer treatments such as chemotherapy, hyperthermia and PDT [9]. Apparently, both lysosomal photocytotoxicity and ap-

Abbreviations: AO, Acridine orange; DIDS, 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid; $DiOC_6(3)$, 3,3′-dihexyloxacarbocyanine iodide; MB, Methylene blue; MES, 2-(*N*-morpholino)ethanesulfonic acid; UMBG,4-methylumbelliferyl-β-D-galactoside

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plications of lysosomotropic photooxidation are dependent on lysosomal destabilization. Lysosomes are photodamageable by either singlet oxygen or hydroxyl radicals. Although great efforts have been made to investigate photodamage-induced lysosomal disruption, exact mechanism of the lysis has not been completely elucidated.

In mammalian cells, K^+ and Na^+ are the major cytoplasmic cations. Since cytoplasmic [K^+] (140 mM) is generally more than 10-fold higher than [Na^+] (5–15 mM) [10], the danger of lysosomal osmotic disruption induced by abnormal entry and accumulation of K^+ in lysosomes has been emphasized for a long time [11–15]. In an earlier paper, we proved that lysosomal integrity can be photodestructed via the loss of osmotic protection against K^+ accumulation by proton pumping [16]. The limited lysosomal permeability toward K^+ is a barrier for the ion entry [17]. Any destructive factor against it may cause lysosomal osmotic lysis. Since membrane photomodification may increase ion permeation, it is likely that photodamage-induced lysosomal destabilization is caused by an osmotic imbalance via increased K^+ uptake. But previous studies neglected the effects of photodamage on lysosomal ionic permeability [4–7]. It is of interest to establish if such a mechanism for photodamaged lysosomal destabilization in K^+-containing media is really existed and what is the biophysical mechanism by which the inward K^+ permeation increases. The results of this paper indicate that MB mediated photodamage increases lysosomal permeability to both K^+ and H^+. The enhanced K^+ uptake, mainly through increased K^+/H^+ exchange, can cause lysosomal destabilization.

2. Materials and methods

2.1. Chemicals

UMBG, 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide, DIDS, $DiOC_6(3)$, Hepes, MES, AO and valinomycin were from Sigma, (St. Louis, MO). The other chemicals used were of analytical grade from local sources. All aqueous solutions were prepared with deionized, glass-distilled water.

2.2. Preparation of lysosomes

Male Wistar rats starved for 24 h were killed by decapitation. Rat liver lysosomes were prepared by the method of Ohkuma et al. [18]. All procedures were carried out at 0–4°C. Lysosomes were resuspended in 0.25 M sucrose medium at a final protein concentration of 25 mg/ml. Protein was determined according to Lowry et al. [19].

2.3. Light exposure procedure

All photoreaction mixtures including control (unirradiated) samples consisted of MB (0.1 mM) and lysosomes (22.5 mg protein/ml) in 0.25 M sucrose. Incident light was from a REFLECTA slide reflector with 670 nm filter. The light intensity at the sample position was 10 mW/cm^2. Samples were light exposed on ice bath.

2.4. Solute permeability determination

Solute permeability of lysosomes can be assessed by the osmotic protection method [20,21]. The approach gives a semiquantitative measure of relative rates of entry of permeant solutes. The incubation medium contained 0.125 M K_2SO_4, buffered at pH 7.0 with 10 mM Hepes/KOH. A 30 μl photodamaged or control (sensitized with MB, not exposed to light) lysosomal sample was suspended in 1 ml incubation medium and incubated at 37°C for indicated time. After incubation, a 50 μl portion of this lysosomal suspension was used for the assay of lysosomal integrity. Difference in permeability was obtained by comparing the extent of lysosomal latency loss during the incubation period.

2.5. Assay of lysosomal integrity

Lysosomal integrity was assessed by measuring lysosomal enzyme latency [17,22,23]. The activity of lysosomal enzymes was measured fluorometrically by the method of Bird et al. [24]. β-Galactosidase was assayed using UMBG as substrate at a final concentration of 0.5 mM in the assay medium. β-hexosaminidase was assayed using 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide as substrate at a

final concentration of 2 mM in the assay medium. The liberated 4-methylumbelliferone was determined by measuring the fluorescence (excitation: 365 nm, emission: 444 nm) on a Hitachi 850 fluorescence spectrophotometer.

The activities of the enzyme measured in the absence and presence of Triton X-100 are designated the free activity and the total activity respectively [24]. 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 loss of lysosomal enzyme latency or increased percentage free activity.

2.6. Assay of lysosomal permeability to K^+

Lysosomal K^+ permeability was assessed by measuring the alteration of membrane potential upon addition of K_2SO_4 [15,22]. Membrane potential sensitive dye $DiOC_6(3)$ was used [25]. The assay medium contained 0.25 M sucrose, 3 μM $DiOC_6(3)$, buffered at pH 6.0 with 10 mM MES/Tris. Additions of lysosomal sample, K_2SO_4 and valinomycin to the assay medium are designated in the figure legend. The fluorescence measurements were conducted at 25°C with excitation and emission wavelength of 460 and 510 nm, respectively.

2.7. Miscellaneous assays

Lysosomal ΔpH was measured by following differential absorbance ($\Delta A_{492-540nm}$) of AO on a Hitachi 557 dual wavelength spectrophotometer [26]. Proton leakage induced acidification of lysosomal suspending medium was monitored by measuring the decrease in absorbance (400 nm) of *p*-nitrophenol [27]. Unless stated, all assays were carried out at 25°C. Calculation of medium osmolality was according to the handbook [28].

3. Results

3.1. Photodamage increases lysosomal permeability to the ions of K_2SO_4

Lysosomal solute permeability is usually assessed by the osmotic protection method [20,21]. Parallel

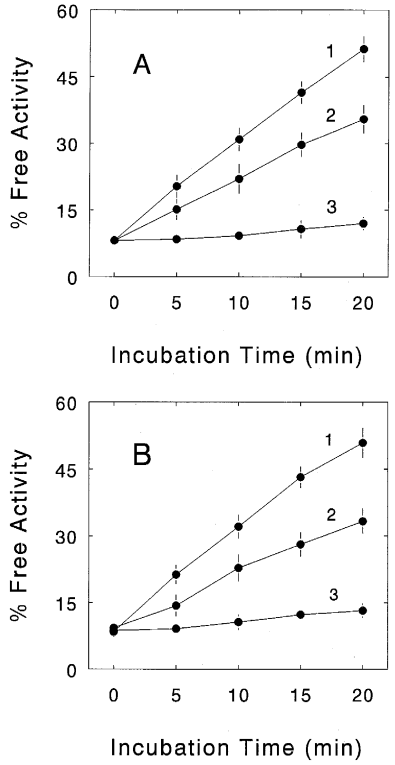

Fig. 1. Effects of MB mediated photooxidation on lysosomal ionic permeability in K_2SO_4 suspension. Lysosomes treated with MB were irradiated for indicated time before incubation: (1) 30 min, (2) 20 min, (3) 0 min (control). A 30 μl resulted sample (0.675 mg protein) was incubated in 1 ml medium at 37°C for indicated time. Incubation medium contained 0.125 M K_2SO_4, buffered at pH 7.0 with 10 mM Hepes/KOH. After incubation, 50 μl suspension was assayed for enzyme free and total activity by the method described in Section 2. (A) Percentage free activity of β-galactosidase. (B) Percentage free activity of β-hexosaminidase. Values are means $\pm$ S.D. of four measurements.

results from the assay of two lysosomal enzymes show that unirradiated lysosomes are relatively stable in isotonic K_2SO_4 suspension during a 20 min period of incubation (Fig. 1, curve 3), while photodamaged lysosomes increasingly lose latency with incubation. Longer periods of light-exposure cause more significant latency loss (Fig. 1, curves 1 and 2). It indicates that MB mediated photodamage increases lysosomal permeability toward either K^+ or SO_4^{2-} or both of them. Similar results were also obtained when the lysosomes were incubated in isotonic Na_2SO_4 medium for 20 min (data not shown).

Increasing K_2SO_4 concentration of the medium

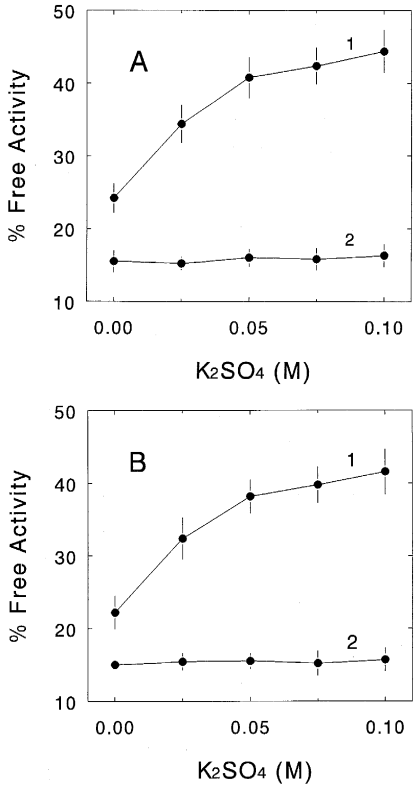

Fig. 2. Effects of medium K_2SO_4 concentration on the osmotic stability of MB photodamaged lysosomes. Incubation medium contained K_2SO_4 with indicated concentration and sucrose to give a total osmolality of 0.25 osM, buffered at pH 7.0 with 10 mM Hepes/KOH. 30 μl sample (0.675 mg protein, (1) photodamaged for 30 min; (2) control) were incubated in 1 ml medium at 37°C for 10 min. After incubation, 50 μl suspension was assayed for enzyme free and total activity as in Fig. 1. (A) Percentage free activity of β-galactosidase. (B) Percentage free activity of β-hexosaminidase. Values are means ± S.D. of four measurements.

enhanced the latency loss of lysosomes photodamaged for 30 min. As shown in Fig. 2, when K_2SO_4 concentration increases but the total osmolality of the medium is maintained with sucrose, percentage free activity of two enzymes remains constant for unirradiated lysosomes (curve 2), yet significantly increases for light exposed lysosomes (curve 1). The photodamaged lysosomes lose latency in a K_2SO_4 concentration-dependent manner. Based on the results in Figs. 1 and 2, it suggests that the latency loss of photodamaged lysosomes is due to an increased ion permeability.

3.2. Photodamage-induced increase in lysosomal K^+ permeability

Lysosomal membrane potential can be measured using membrane potential sensitive dye. In the assay using this method, an increase of relative fluorescence of cyanine dye, indicating a less negative interior, will be observed when K^+ are allowed to enter the lysosomes [15,22]. As shown in Fig. 3, the fluorescence increase of photodamaged lysosomal sample upon addition of K_2SO_4 (Fig. 3B) is larger than that of control sample (Fig. 3A). It indicates that the larger magnitude of elevated internal potential of photodamaged lysosomes is due to an increased permeability to K^+. Addition of K^+ ionophore valinomycin causes additional fluorescence increase, indicating a further enhanced K^+ permeability.

3.3. K^+/SO_4^{2-} co-uptake of photodamaged lysosomes

Lysosomal membrane shows a very limited permeability toward sulfate [21]. Sulfate uptake by rat liver

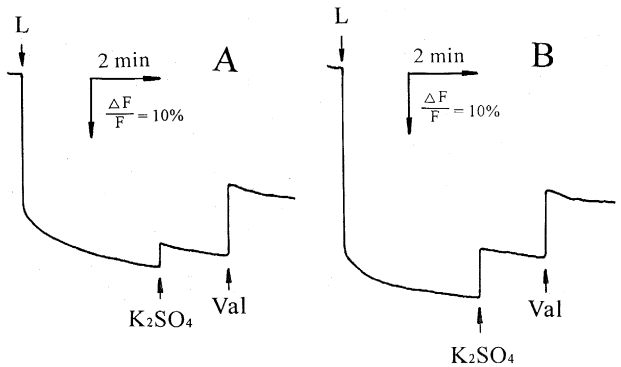

Fig. 3. Effects of K_2SO_4 on the membrane potential of MB photodamaged lysosomes. The medium(2 ml) contained 0.25 M sucrose and 3 μM $DiOC_6(3)$, buffered at pH 6.0 with 10 mM MES/Tris. As indicated, 4 μl lysosomes (0.09 mg protein), 50 μl 0.75 M K_2SO_4 and 10 μl 1 mM valinomycin were added to the cuvette. Lysosomes were designated as 'L'. Fluorescence of $DiOC_6(3)$ was measured at 25°C with excitation and emission wavelength of 460 and 510 nm, respectively. The fluorescence intensity is expressed as a percentage of its intensity just before addition of the lysosomes ($\Delta F/F$). (A) Control. (B) Lysosomes photodamaged for 30 min. A typical result out of three experiments is shown.

Table 1
Effects of DIDS on the osmotic stability of MB photodamaged lysosomes

DIDS addition	Light exposure time (min)	% Free β-galactosidase activity
None	0	12.3 ± 1.3
None	30	52.4 ± 2.6
Addition before incubation	30	39.3 ± 2.2
Addition after incubation	30	51.5 ± 2.4

Incubation medium contained 0.125 M K_2SO_4, buffered at pH 7.0 with 10 mM Hepes/KOH. 30 μl MB sensitized lysosomal samples (0.675 mg protein) irradiated for indicated times were incubated in 1 ml medium at 37°C for 20 min. DIDS was added to the medium at a final concentration of 0.1 mM before or after incubation as indicated. After incubation, 50 μl suspension was assayed for β-galactosidase free and total activity. Values are means $\pm$ S.D. of four measurements.

lysosomal membrane vesicles has been proved to be strongly inhibited by DIDS [25]. To investigate the photodamage effect on lysosomal permeability to sulfate, we used DIDS in the osmotic protection experiments (Table 1). Lysosomes were incubated in isotonic K_2SO_4 medium for 20 min, then the activity of β-galactosidase was measured. As shown in Table 1, percentage free activity of unirradiated and photodamaged lysosomes is at 12% and 52%, respectively. It is consistent with the results in Fig. 1 (right end of curves 1 and 3), showing a photodamage-induced increase in lysosomal permeability to the ions. When photodamaged lysosomes were incubated in the presence of DIDS, the enzyme free activity decreased to 39%, suggesting that either the lysosomes were osmotically protected or the enzyme was inhibited by DIDS. To examine the effect of DIDS on the enzyme activity, the enzyme sample (30 μl lysosomal sample treated by 0.2% Triton X-100) was incubated under the same conditions for 20 min with or without 0.1mM DIDS, then the enzyme activity was assessed using the same method. The results show that the enzyme was not inhibited whether DIDS was added to the medium before or after incubation (data not shown). Therefore, the decrease in enzyme free activity from 52% to 39% is caused by a promoted osmotic protection to the lysosomes, presumably due to a reduction in sulfate uptake. As shown in Table 1, out of photodamage-induced lysosomal latency loss (enzyme free activity increases from 12% to 52%), 32% is protected by DIDS (free activity decreases to 39%). It suggests that the photodamage increases lysosomal permeability to sulfate and the photodamage-induced co-uptake of K^+/SO_4^{2-} plays a role in the lysosomal destabilization.

3.4. Photodamage-induced increase in lysosomal H^+ permeability

A pH difference exists across lysosomal membrane (ΔpH, acid inside). This is chiefly resulted from the active transport of a membrane bound proton pump [29]. The negatively charged molecules in the lysosomes reach Donnan equilibrium with the protons and maintain a stable acidic pH [30]. Limited lysosomal permeability to H^+ favors maintenance of the ΔpH. Photodamage-induced alteration in the permeability to H^+ was investigated using a pH sensitive dye. The unprotonated *p*-nitrophenol molecules have a sufficiently larger extinction coefficient at 400 nm over that of protonated molecules [27]. Lysosomal H^+ leakage results in acidification of suspending medium which can be semiquantitatively examined by measuring absorbance decrease of the dye. As shown in Fig. 4, the absorbance of the dye decreases slightly for unirradiated lysosomes (line 2), while pronouncedly for photodamaged lysosomes (line 3 and 4). The reduction in absorbance is not observed when the medium is buffered at the same pH (line 1), indicating that the decrease in absorbance of *p*-

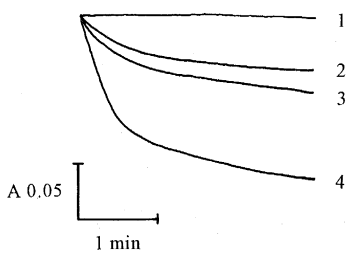

Fig. 4. Effects of MB photodamaged lysosomes on *p*-nitrophenol absorbance. Assay medium contained 0.25 M sucrose and 0.1 mM *p*-nitrophenol. Absorbance (400 nm) was measured at 25°C immediately after adding 50 μl lysosomes (1.125 mg protein) to 2 ml medium. (1) Medium was buffered at pH 6.5 with 10 mM MES/Tris, lysosomes were photodamaged for 30 min; (2, 3, 4) Medium pH at 6.5, without buffer, lysosomes treated with MB were light-exposed for different time before the assay: (2) 0 min, (3) 15 min, (4) 30 min. A typical result out of three experiments is shown.

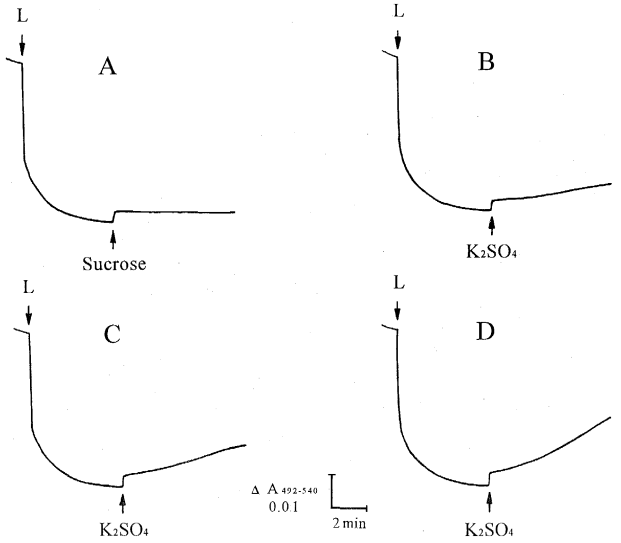

Fig. 5. Effects of K_2SO_4 on the proton gradient of MB photodamaged lysosomes. Assay medium contained 0.25 M sucrose and 5 μM AO, buffered at pH 7.0 with 10 mM Hepes/Tris. The ΔpH was monitored at 25°C by measuring AO differential absorbance ($\Delta A_{492-540nm}$). 100 μl lysosomes (2.25 mg protein) were added to 2 ml medium at indicated time. Uptake of the dye was allowed to reach steady state, then 100 μl 0.75 M K_2SO_4 or 100 μl 0.25 M sucrose was added. Lysosomes treated with MB were light-irradiated for different time before the assay: (A, D) 30 min, (B) 0 min, (C) 15 min. A typical result out of three experiments is shown.

nitrophenol was due to H^+ leakage induced acidification of lysosomal suspension. It suggests that the photodamage to lysosomes increases their permeability to H^+.

3.5. K^+/H^+ exchange on photodamaged lysosomes

Lysosomal H^+ efflux in exchange for external K^+ can be examined by monitoring the decrease in lysosomal ΔpH [31]. Acridine orange, a lysosomal ΔpH sensitive dye, was used in the measurements. As shown in Fig. 5, differential absorbance of AO decreases after adding lysosomes, demonstrating the existence of membrane ΔpH (acid inside). Compared with control sample (Fig. 5B), addition of K_2SO_4 causes apparent absorbance increase for photodamaged lysosomes (Fig. 5C,D), indicating that the alkalization inside photodamaged lysosomes is caused via K^+/H^+ exchange. Since no absorbance increase was observed upon addition of sucrose with the same volume (Fig. 5A), the dilution effect can be ruled out. The absorbance increase induced by adding K_2SO_4 depends on the exposure time, suggesting that the photodamage to lysosomes causes an increased K^+/H^+ exchange.

The level of lysosomal K^+/H^+ exchange is dependent not only on lysosomal permeability to K^+ and H^+ but also on their electrochemical gradient across lysosomal membrane. Therefore, the level of K^+/H^+ exchange can be diminished by lowering lysosomal ΔpH. Through comparison of latency loss between lysosomes with different ΔpH, the effect of K^+/H^+ exchange on photodamage-induced lysosomal destabilization was investigated. Lysosomal ΔpH can be lowered by either reducing external pH or elevating intralysosomal pH by using of the lysosomotropic weak amine base methylamine [32]. As

Table 2
Effects of methylamine and lowering medium pH on the osmotic stability of MB photodamaged lysosomes

Conditions	Light exposure time (min)	% Free β-galactosidase activity
Methylamine addition		
None	0	13.4 ± 0.9
None	30	54.4 ± 2.3
Addition in preincubation	30	28.8 ± 1.5
Addition after incubation	30	53.3 ± 2.5
Medium pH		
7.0	0	11.2 ± 1.3
7.0	30	51.8 ± 2.1
6.0	30	30.6 ± 1.7

For methylamine effect assay, the medium of preincubation contained 0.25 M sucrose, buffered at pH 7.0 with 10 mM Hepes/KOH. 60 μl MB sensitized lysosomal samples (1.35 mg protein) irradiated for indicated times were preincubated in 0.3 ml medium for 5 min at 25°C in the presence or absence of 0.1 mM methylamine, then mixed with 1.7 ml medium containing 0.147 M K_2SO_4, 10 mM Hepes/KOH, pH 7.0 (final concentration of K_2SO_4 at 0.125 M) and incubated at 37°C for 20 min. After incubation, 50 μl suspension was assayed for β-galactosidase free and total activity. For medium pH effect assay, incubation medium contained 0.125 M K_2SO_4, buffered at pH 7.0 with 10 mM Hepes/KOH or buffered at pH 6.0 with 10 mM MES/KOH. 30 μl MB sensitized lysosomal samples (0.675 mg protein) irradiated for indicated times were incubated in 1 ml medium at 37°C for 20 min. After incubation, 50 μl suspension was assayed for β-galactosidase free and total activity. Values are means ± S.D. of four measurements.

previously measured, the interior pH of lysosomes isolated by this method is about 5 [18]. As shown in Table 2, when photodamaged lysosomes are suspended in isotonic K_2SO_4 medium, the latency loss in the medium of pH 6 is less than that in the medium of pH 7. Out of photodamage-induced latency loss (enzyme free activity increases from 11% to 52%), about 54% is protected by lowering external pH (free activity decreases from 52% to 30%). Furthermore, less latency loss was detected if photodamaged lysosomes were preincubated with methylamine. Since 0.1 mM methylamine does not inhibit β-galactosidase under the same conditions (data not shown), the reduction in enzyme free activity is presumably due to a promoted osmotic protection to the lysosomes. As shown in Table 2, 30 min photodamage causes percentage free activity of β-galactosidase to increase from 13% (control) to 54%, while preincubation with methylamine reduces the enzyme free activity of photodamaged lysosomes to 29%, indicating that 60% of the increased latency loss is protected. Since lysosomal ΔpH has been suggested to be an electrochemical driving force for K^+/H^+ exchange [21] and the latency loss of photodamaged lysosomes can be mostly prevented by lowering the ΔpH, it suggests that photodamage-induced lysosomal destabilization is caused mainly through a K^+/H^+ exchange pathway under the conditions of this study.

4. Discussion

K^+ is the most abundant cation in cytoplasm [10]. The osmotic effects of K^+ on lysosomes has been an interesting topic of study [11–15]. As shown in this paper, photodamage-induced lysosomal latency loss is closely related to increased influx of K^+. A large inwardly directed K^+ gradient across lysosomal membrane and increased K^+ permeability are favorable for K^+ entry. It accounts for the effect of increasing K_2SO_4 concentration on photodamage-induced lysosomal destabilization (Fig. 2). It has been pointed out that ion flux across lysosomal membrane must be accompanied by charge-compensating movements of another ion in order to maintain electroneutrality [33]. Lowering lysosomal ΔpH mostly prevents photodamaged lysosomes from K^+ entry induced osmotic destabilization (Table 2). It indicates that the entry of K^+ is mainly through an electroneutral K^+/H^+ exchange pathway. In addition, photodamaged lysosomes are partly protected against osmotic destabilization via an inhibition of sulfate uptake (Table 1). It suggests that the electroneutral co-uptake of K^+ and SO_4^{2-} also plays a role in the lysosomal osmotic destabilization. As indicated in Section 3, about 60% and 32% of the increased latency loss is protected by the treatment of methylamine and DIDS, respectively. Thus, K^+ uptake by other mechanism appears unlikely under the conditions of this study.

It has long been suggested that incubating lysosomes in K^+-containing medium can elevate intralysosomal pH via K^+/H^+ exchange [18,34,35]. Abnormal uptake of K^+ by lysosomes, induced by either low temperature [36,37] or ionophore treatment [21], is generally believed to be through K^+/H^+ exchange pathway or via K^+/anion co-uptake. Preincubation of lysosomes with methylamine decreases photodamage-induced latency loss (Table 2). The promotion of osmotic protection to lysosomes is presumably due to the decrease in K^+ uptake via depressing K^+/H^+ exchange. The effect of K^+/H^+ exchange on the lysosomal osmotic destabilization is estimated from the extent of decreased latency loss induced by the amine treatment. Two possible factors may cause underestimation of the exchange level. First, lysosomal ΔpH is probably not completely abolished by the amine treatment, since a considerable buffering capacity was found inside lysosomes [38]. A slight K^+/H^+ exchange may still exist after the amine treatment. Second, uptake of methylamine to some extent results in lysosomal swelling via an osmotic action [32]. Since methylamine-induced osmotic protection is probably more or less counteracted by its osmotic action, it is likely that the level of K^+/H^+ exchange is underestimated from the extent of decreased latency loss induced by the amine treatment. Therefore, the role of K^+/H^+ exchange in the lysosomal destabilization is probably more important than that estimated from Table 2.

Lysosomal membrane is relatively impermeable to inorganic sulfate [21,25,39,40]. The inside negative lysosomal membrane potential is apparently unfavorable for the uptake of negatively charged sulfate. As shown in the results, photodamage to lysosomes in-

duces an increased ion permeability. Both efflux of H^+ and uptake of sulfate may cause a more negative internal potential, while influx of K^+ makes lysosomal interior more positive. The total effect of the ion movements was shown to elevate the internal potential of photodamaged lysosomes when K_2SO_4 was added to the suspension (Fig. 3). Depolarization of membrane potential to the direction of inside positive is electrically favorable for the uptake of negatively charged sulfate. Furthermore, increased influx of K^+ may promote the entry of sulfate via electroneutral co-uptake. These photodamage-induced electrochemical alterations on lysosomes may be responsible for the slightly increased sulfate uptake.

In previous studies, we have shown that impairment of active proton transport is a possible mechanism for photodamage-induced lysosomal destabilization [16]. The destructive effect is solely due to failure of the proton pump to protect the lysosomes osmotically by lowering their K^+ content. This occurred in an early stage of photodamage when only alteration in H^+-ATPase was observed. In this paper, the assay was carried out in a relatively severe photodamage condition which caused increase in membrane ionic permeability and lysosomal destabilization. It suggests that different mechanism exists at different stage of lysosomal photodamage.

Photodynamic lipid peroxidation has been suggested to be detrimental to membrane structure and function [41]. It may be linked to effects such as increased ion permeability and inactivation of membrane proteins. Under the conditions of this study, formation of lipid peroxides on lysosomal membrane is also observed after MB mediated photodamage (data not shown). It remains to be determined whether the increased permeability is induced directly by lipid peroxidation and how they each contribute to the lysosomal destabilization.

Photodamage-induced lysosomal disintegration is a crucial event in photocytotoxicity, but mechanism of the lysis has not been completely elucidated. In the present study, we propose a new mechanism for this event. Main points of this investigation are focused on the biophysical properties of photodamage-induced K^+ permeation. Biochemical structure analysis for elucidating increased ion permeability remains for further study.

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