ELSEVIER

Biochimica et Biophysica Acta 1326 (1997) 75–82

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

The direct cause of photodamage-induced lysosomal destabilization

Guo-Jiang Zhang *, Junlan Yao

Department of Cellular Biophysics, Institute of Biophysics, Academia Sinica, 15 Datun Road, Chaoyang District, Beijing, 100101, People's Republic of China

Received 22 October 1996; revised 14 January 1997; accepted 15 January 1997

Abstract

Whether membrane lipid photoperoxidation is the immediate cause for lysosomal lysis is still unclear. In this study, we investigated the direct causal factor of photoinduced lysosomal destabilization in a K^+-containing solution. Methylene blue (MB)-mediated photodamage caused lysosomal membrane lipid peroxidation and loss of membrane fluidity. Compared with unirradiated lysosomes, the photodamaged lysosomes significantly lost enzyme latency in an isotonic K^+-containing solution during a 20-min period of incubation. It indicates an increase in lysosomal K^+ permeability. The inward K^+ permeation of photodamaged lysosomes was further proved by a K^+-induced elevation of internal membrane potential. In addition, the photodamaged lysosomes displayed an increased osmotic sensitivity, showing that MB-mediated photodamage promotes lysosomal osmotic fragility. Although these photoinduced alterations occurred, the lysosomes were relatively stable in an isotonic sucrose medium. In contrast, the organelle destabilized in a photodamage-dependent fashion in an isotonic K^+-containing solution. The results indicate that membrane lipid peroxidation does not definitely destabilize lysosomes. The direct cause for the lysosomal destabilization is photoinduced osmotic imbalance across its membrane via an increased K^+ uptake, while the increase in osmotic sensitivity favors the destabilization of photodamaged lysosomes.

Keywords: Lysosomal integrity; K^+ permeability; Osmotic sensitivity; Lipid peroxidation; Membrane fluidity; Photosensitization

1. Introduction

Lysosomes are acidic cellular organelles containing a variety of hydrolytic enzymes. Since leakage of the hydrolases may cause cell death [1], lysosomal integrity is of the utmost importance for cellular function and survival. In the basic studies of photodynamic therapy (PDT) of cancers, the photodestruction of lysosomes has been emphasized as a critical event for killing tumor cells [2–4]. A number of investigations demonstrated that lysosomes are main cellular photodamage site [5,6]. The photoinduced lysosomal disruption may cause some pathological disorders such as cell death and porphyria [7–9]. In addition to lysosomes-dependent photocytotoxicity, lysosomotropic photosensitization 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 [10]. Apparently, both lysosomal photocytotoxicity and applications of lysosomotropic photosensitization are dependent on lysosomal disruption. The important

Abbreviations: $DiOC_6(3)$, 3,3″-dihexyloxacarbocyanine iodide; DPH, 1,6-diphenyl-1,3,5-hexatriene; MB, methylene blue; MES, 2-(N-morpholino) ethanesulfonic acid; UMBG, 4-methylumbelliferyl-β-D-galactoside

* Corresponding author. Fax: +86 10 62027837.

PII S0005-2736(97)00009-6

role of lysosomes in PDT led to continuous investigations on photodamage-induced lysosomal lysis. In previous studies, we proposed that lysosomal integrity can be photodestructed via a loss of proton translocation [11] or through the increases in lysosomal permeability to both K^+ and H^+ [12].

Active oxygen, either singlet oxygen or hydroxyl radicals, can cause lysosomal membrane lipid peroxidation [13–17]. Some investigators proposed that membrane lipid peroxidation is the immediate cause for lysosomal destabilization [13,14], others questioned this point [18,19]. Whether membrane lipid photoperoxidation is the direct cause for lysosomal destabilization is still unclear. Under the conditions of this study, we demonstrated that membrane lipid peroxidation cannot immediately cause lysosomal destabilization in an isotonic sucrose medium, even though the lysosomes displayed an increased osmotic sensitivity. In contrast, the photodamaged lysosomes destabilized in an isotonic K^+-containing solution via an increased K^+ uptake.

2. Materials and methods

2.1. Chemicals

$DiOC_6(3)$, DPH, Hepes, MES, 4-methylumbelliferyl N-acetyl-β-D-glucosaminide and UMBG were from Sigma Chemicals (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. [20]. All procedures were carried out at 0–4°C. Lysosomes were resuspended in a 0.25 M sucrose medium at a final protein concentration of 25 mg/ml. Protein was determined according to Lowry et al. [21].

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. Lysosomes were photodamaged with light in the presence of MB. Incident light was from a REFLECTA slide reflector with a 670 nm filter. The light intensity at the sample position was 10 mW/cm^2. Samples were light-exposed on ice bath.

2.4. Lipid peroxidation measurements

Photogenerated lipid hydroperoxides (LOOHs), including those derived from cholesterol, were determined by iodometric assay [22,23]. At various intervals during irradiation, 0.3 ml control or photodamaged lysosomal samples (6.75 mg protein) were mixed with 0.2 ml 0.1 mM EDTA solution and extracted with 0.8 ml chloroform/methanol (2:1, V/V). Aliquots of 0.3 ml from the organic phase were evaporated under nitrogen, and the recovered LOOHs were analyzed iodometrically. A molar extinction of $2.5 \times 10^4\ M^{-1}\ cm^{-1}$ was used for quantitation. The formation of TBARS during irradiation was determined as described previously [24]. A 50-μl control or photodamaged lysosomal sample (1.125 mg protein) was used for the measurement. Absorbance reading at 532 nm were converted to TBARS values (nmol/mg protein), using an extinction coefficient of $1.57 \times 10^5\ M^{-1}\ cm^{-1}$.

2.5. Membrane fluidity measurement

We used the hydrophobic probe DPH to assess lysosomal membrane fluidity by monitoring the anisotropy of its fluorescence [25]. A solution of 2 mM DPH in tetrahydrofuran was diluted 500 fold by injection into vigorously stirred PBS buffer (0.1 M, pH 7.4), resulting in a clear medium. For labeling, 2 ml of this medium was incubated with a 50-μl lysosomal sample at 37°C for 30 min. Fluorescence was measured immediately on a Hitachi 850 fluorescence spectrophotometer with excitation and emission at 350 and 452 nm, respectively. The emission intensity was detected through an analyzer oriented parallel ($I_{\parallel}$) or perpendicular ($I_{\perp}$) to the direction of polarization of the excitation light. The degree of fluorescence polarization are calculated by the equation: $P = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$.

2.6. Solute permeability determination

Solute permeability of lysosomes can be assessed by the osmotic protection method [26,27]. The approach gave a semiquantitative measure of relative rates of entry of permeant solutes. The incubation medium contained 0.125 M K_2SO_4 or 0.25 M sucrose, buffered at pH 7.0 with 10 mM Hepes/KOH. A 30-μl photodamaged or control lysosomal sample (0.675 mg protein) 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 period of incubation.

2.7. Assay of lysosomal osmotic sensitivity

According to the method of Neely et al. [28], the osmotic sensitivity of lysosomes was evaluated from the increase in free enzyme activity occurring between 0.25 and 0.10 M sucrose. A 30-μl photodamaged or control lysosomal sample was suspended in 1 ml sucrose medium and incubated at 37°C for 10 min. After incubation, a 50-μl portion of this lysosomal suspension was used for the assay of lysosomal integrity.

2.8. Assay of lysosomal integrity

Lysosomal integrity was assessed by measuring lysosomal enzyme latency [29]. The activity of lysosomal enzymes was measured fluorometrically by the method of Bird et al. [30]. 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 [30]. 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.

2.9. Assay of lysosomal permeability to K^+

Lysosomal K^+ permeability was assessed by measuring the alteration of membrane potential upon addition of K_2SO_4 [31,32]. Membrane potential sensitive dye $DiOC_6(3)$ was used [33]. 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 legends. The fluorescence measurements were conducted at 25°C with excitation and emission wavelength of 460 and 510 nm, respectively.

3. Results

3.1. Photodamage causes lysosomal membrane lipid peroxidation

MB-mediated photodamage caused lysosomal membrane lipid peroxidation. As shown in Fig. 1, the productions of lipid peroxides TBARS and LOOHs follow a similar exposure time course and both ex-

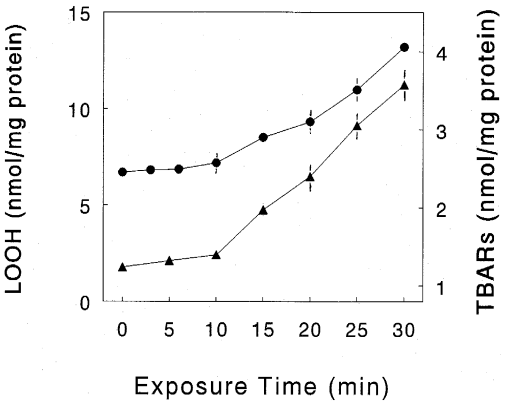

Fig. 1. Lipid peroxidation of lysosomes following MB-mediated photodamage. MB-sensitized lysosomes were irradiated for indicated time. Formations of LOOHs (▲, left-hand scale) and TBARS (●, right-hand scale) were determined as described in Section 2. Values are means ± S.D. of 4 measurements.

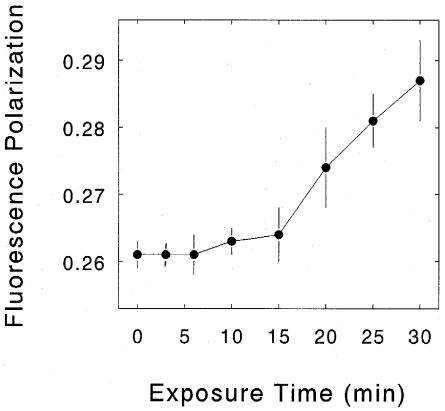

Fig. 2. Alteration of lysosomal membrane fluidity following MB-mediated photodamage. 50 μl lysosomal sample (1.125 mg protein) treated with MB was irradiated for indicated time, then added to 2 ml PBS (pH 7.4) containing 4 μM DPH. After incubation at 37°C for 30 min, fluorescence intensity of the dye was measured and the degree of fluorescence polarization was calculated as described in Section 2. Values are means ± S.D. of 6 measurements.

hibit a biphasic characteristic. At lower exposure, the lipid peroxidation increases slowly, showing a relatively light photodamage. When exposure time exceeds 10 min, the lipid peroxides accumulates rapidly. Compared with LOOHs, less TBARS were produced under the same conditions.

3.2. Photodamage decreases lysosomal membrane fluidity

Membrane lipid peroxidation is generally linked to the loss of membrane fluidity and increased ion permeability [34]. Lysosomal membrane fluidity was measured using DPH, one of the most efficient membrane fluidity probes. This dye can incorporate easily into the lipid bilayer of biomembrane [25]. The increases in its degree of fluorescence polarization stand for a loss of membrane fluidity. As shown in Fig. 2, the fluorescence polarization increases with light exposure. It indicates that MB-mediated photodamage causes a loss in lysosomal membrane fluidity. The loss of membrane fluidity and the production of lipid peroxides followed a similar exposure time course, implying a possible correlation between them.

3.3. Photodamage increases lysosomal permeability to the ions of K_2SO_4

Lysosomal solute permeability is usually assessed by the osmotic protection method [26,27]. As shown in Fig. 3, both control and photodamaged lysosomes are relatively stable in the isotonic sucrose medium during a 20-min period of incubation (curves 3 and 4), indicating that the photodamaged lysosomes still maintains its impermeability to sucrose. When the photodamaged lysosomes were incubated in the isotonic K_2SO_4 medium, the percentage free enzyme activity significantly increased with incubation com-

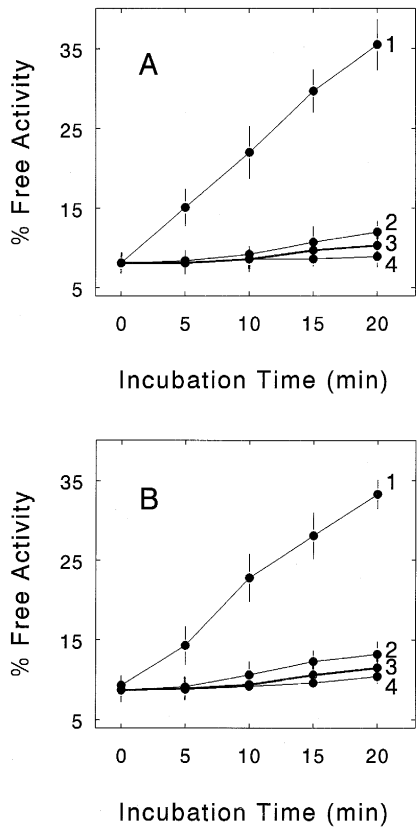

Fig. 3. Effects of MB-mediated photodamage on lysosomal permeability to sucrose and the ions of K_2SO_4. Incubation medium contained either 0.25 M sucrose (3,4) or 0.125 M K_2SO_4 (1,2), both buffered at pH 7.0 with 10 mM Hepes/KOH. Procedures of incubation and assay of enzyme activity as described in Section 2. (1,3) Lysosomes photodamaged for 20 min. (2,4) Control. (A) Percentage free activity of β-galactosidase. (B) Percentage free activity of β-hexosaminidase. Values are means ± S.D. of 4 measurements. The significance between the points of 20 min incubation in curve 3 and 4 was $P > 0.1$.

pared to control lysosomes (curves 1 and 2). It indicates that MB-mediated photodamage increases lysosomal permeability toward the ions of K_2SO_4. The osmotic destabilization of photodamaged lysosomes is presumably due to an increase in the uptake of K^+ via an electroneutral K^+/H^+ exchange and/or a K^+/anion co-uptake [12]. Similar results were also obtained when the lysosomes were incubated in an isotonic Na_2SO_4 medium for 20 min (data not shown).

The inward K^+ permeation can elevate internal membrane potential of lysosomes. To establish if MB-mediated photodamage increases lysosomal K^+ permeability, lysosomal membrane potential was measured using $DiOC_6(3)$. As shown in Fig. 4, the addition of K_2SO_4 to photodamaged lysosomal sample causes a larger increase in fluorescence (Fig. 4B) compare to control lysosomal sample (Fig. 4A). 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 [31,32]. The results indicate that the larger magnitude of elevated internal potential of photodamaged lysosomes is due to an increased permeability to K^+. The addition of valinomycin, an ionophore of K^+, caused additional fluorescence increase, indicating a further enhanced K^+ permeability.

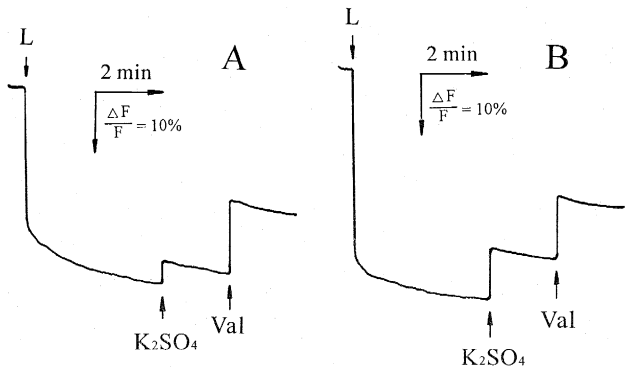

Fig. 4. Effects of K_2SO_4 on the membrane potential of photodamaged lysosomes. Assay medium contained 0.25 M sucrose and 3 μM $DiOC_6(3)$, buffered at pH 6.0 with 10 mM MES/Tris. At indicated time, 4 μl lysosomal sample (0.09 mg protein), 50 μl 0.75 M K_2SO_4 and 10 μl 1 mM valinomycin were added to 2 ml medium, respectively. 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 percentage of its intensity just before addition of the lysosomes ($\Delta F/F$). (A) Control. (B) Lysosomes photodamaged for 20 min. A typical result out of three experiments is shown.

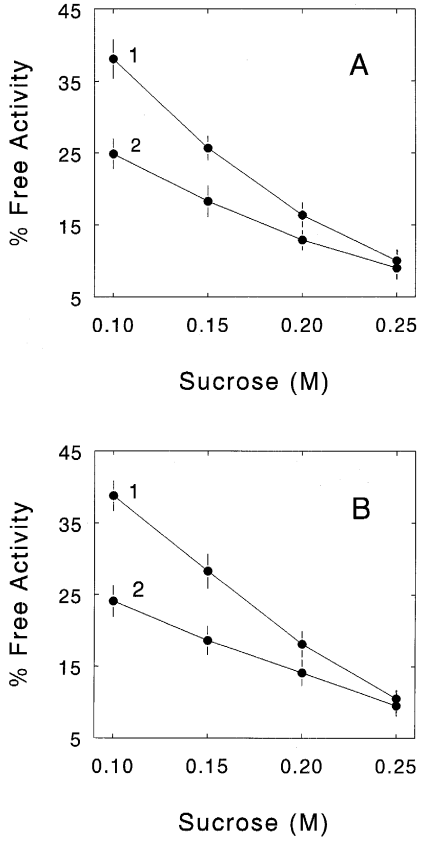

Fig. 5. Effects of MB-mediated photodamage on lysosomal osmotic sensitivity. Incubation medium contained sucrose with different concentration, buffered at pH 7.0 with 10 mM Hepes/KOH. 30 μl lysosomal samples (0.675 mg protein) were incubated in 1 ml medium at 37°C for 20 min. After incubation, 50 μl of the suspension was used to determine the enzyme free activity. (1) lysosomes photodamaged for 20 min. (2) Control. (A) Percentage free activity of β-galactosidase. (B) Percentage free activity of β-hexosaminidase. Values are means $\pm$ S.D. of 4 measurements.

3.4. Photodamage increases lysosomal osmotic sensitivity

To clarify if MB-mediated photodamage increases lysosomal osmotic fragility, lysosomal osmotic sensitivity was assessed [28]. As shown in Fig. 5, the percentage free enzymes activity increases with de-

creasing sucrose concentration from 0.25 M to 0.1 M. Compared with control lysosomes (curve 2), the percentage free activity of photodamaged lysosomes increased more greatly in the hypotonic sucrose medium (curve 1), demonstrating that the photodamaged lysosomes lost its normal ability to resist hypotonic pressure. Since the photodamaged lysosomes still maintained impermeability to sucrose (Fig. 3), the largely increased free activity in the hypotonic sucrose was completely due to a photodamage-promoted osmotic sensitivity or increased osmotic fragility. The results indicate that MB-mediated photodamage causes lysosomes to become more susceptible to osmotic shock.

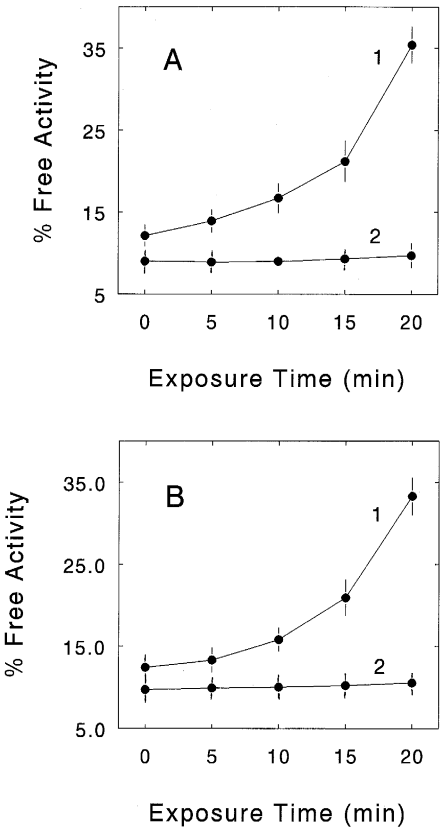

Fig. 6. Effects of MB-mediated photodamage on lysosomal integrity. 30 μl lysosomes (0.675 mg protein) treated with MB were irradiated for indicated time, then incubated at 37°C for 20 min in 1 ml medium containing: (1) 0.125 M K_2SO_4, buffered at pH 7.0 with 10 mM Hepes/KOH; (2) 0.25 M sucrose, buffered at pH 7.0 with 10 mM Hepes/KOH. After incubation, lysosomal integrity was assessed as 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 4 measurements.

3.5. Stability of photodamaged lysosomes depends on solute permeability

K^+ is the most abundant monovalent cation in cytoplasm. It is of interest to investigate the osmotic effects of K^+ on the photodamaged lysosomes. As shown in Fig. 6, the photodamaged lysosomes remain stable in the isotonic sucrose medium (curve 2), while they lose latency in an exposure time-dependent fashion in the isotonic K_2SO_4 medium (curve 1). The results indicate that the osmotic stability of photodamaged lysosomes depends on solute permeability, and an osmotic stress induced by the increased uptake of K^+ destabilizes the photodamaged lysosomes.

4. Discussion

Lysosomes are a main intracellular photodamage site [2–10]. Although photoinduced lysosomal disruption has been extensively investigated, exact mechanism of the lysis is still unclear. In the past years, most studies focused on lysosomal membrane lipid peroxidation. Some investigators proposed that membrane lipid peroxidation is the immediate cause of lysosomal destabilization [13,14]; others questioned this point [18,19]. The difference is whether lysosomal membrane lipid peroxidation definitely results in its destabilization. As shown in this study, MB-mediated photodamage caused lysosomal membrane lipid peroxidation, loss of membrane fluidity and increases in both K^+ permeability and osmotic sensitivity. Since the photodamaged lysosomes maintained impermeability to sucrose (Fig. 3), the lysosomes were relatively stable in isotonic sucrose (Fig. 6) even though its osmotic sensitivity increased and membrane lipid peroxidation occurred. It indicates that membrane lipid peroxidation does not definitely decrease lysosomal stability or at least not immediately cause its destabilization. In contrast, the photodamage increased lysosomal K^+ permeability (Fig. 3) and promoted its destabilization in an exposure time-dependent fashion in the isotonic K^+-containing solution (Fig. 6). It thus suggests that the lysosomal destabilization is caused directly by an osmotic imbalance across its membrane via the increased K^+ uptake, while the photoinduced increase in lysosomal osmotic sensitivity favors its destabilization. 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 [35]. In an earlier study, we demonstrated that MB-mediated photodamage increases lysosomal permeability to both K^+ and H^+. The enhanced K^+ uptake is mainly through an increased K^+/H^+ exchange [12]. Lysosomal permeability to the solutes of suspending medium is critical for its osmotic stability [29,36], but previous studies neglected the effects of photodamage on lysosomal osmotic properties. Apparently, the photodamage-induced increase in lysosomal ion permeability is detrimental to lysosomal stability.

In mammalian cells, cytoplasmic $[K^+]$ (140 mM) is generally more than 10-fold higher than $[Na^+]$ (5–15 mM) [37]. The danger of lysosomal osmotic disruption induced by abnormal entry and accumulation of K^+ in lysosomes has been emphasized for a long time [31,38–41]. The limited lysosomal permeability toward K^+ is a barrier for the ion entry [29]. The destructions of the barrier may cause lysosomal osmotic lysis. Since membrane lipid peroxidation is generally linked to the increased ion permeability [34], it is likely that lipid photoperoxidation of lysosomal membrane may increase its permeability to K^+, therefore causing an osmotic imbalance across the membrane and lysosomal destabilization. As shown in this study, the photodamaged lysosomes mainly accumulated LOOHs, the lipid hydroperoxides derived from phospholipids and cholesterol [34]. In addition, the photodamage increased lysosomal K^+ permeability and promoted lysosomal destabilization in the K^+-containing solution. Since the membrane structures concerning lysosomal ion permeability have not been identified to date, it is extremely difficult to determine which photomodifiable component of lysosomal membrane is responsible for the increased K^+ permeation. Just as Valenzeno described, biological membranes are prone to photomodification, but in most cases, which membrane component is the critical target is not known [42]. It has been demonstrated that cholesterol can decrease the permeability of phospholipid vesicles to K^+ [43,44]. It appears likely that an increase in K^+ permeability of photodamaged lysosomes might correlate to its membrane lipid peroxidation, but correlations with other structural photomodifications also possibly exist.

MB-mediated photodamage caused lysosomal membrane lipid peroxidation (Fig. 1). Compared with LOOHs, less TBARS were produced under the same conditions. As Girotti pointed out, if Type II (singlet oxygen, $^1O_2$) photoperoxidation is monitored by iodometric assay, steadily increasing LOOH values should be observed during the course of irradiation. TBARS are generally considered to be end-products of Type I (free radical) photoperoxidation. On theoretical grounds, no TBARS should be generated in a pure Type II photoreaction [34]. Since singlet oxygen is the major reactive oxygen species generated in MB-mediated photosensitization [34,45], more LOOHs and less TBARS were measured by the methods of this study. It is consistent with the description of Girotti.

Acknowledgements

We would like to thank professors Wang, Zhi-Xin and Zhang, Zhi-Yi for their many helpful suggestions and assistance.

References

[1] De Duve, C. and Wattiaux, R. (1966) Ann. Rev. Physiol. 28, 435–492.
[2] Gomer, C.J. (1991) Photochem. Photobiol. 54, 1093–1107.
[3] Moan, J. and Berg, K. (1992) Photochem. Photobiol. 55, 931–948.
[4] Henderson, B.W. and Dougherty, T.J. (1992) Photochem. Photobiol. 55, 145–157.
[5] Santus, R., Kohen, C., Kohen, E., Reyftmann, J.P., Morliere, P., Dubertret, L. and Tocci, P.M. (1983) Photochem. Photobiol. 38, 71–77.
[6] Lin, C.-W., Shulok, J.R., Kirley, S.D., Cincotta, L. and Foley, J.W. (1991) Cancer Res. 51, 2710–2719.
[7] Allison, A.C., Magnus, I.A. and Young, M.R. (1966) Nature 209, 874–878.
[8] Zdolsek, J.M., Olsson, G.M. and Brunk, U.T. (1990) Photochem. Photobiol. 51, 67–76.
[9] Bachor, R., Shea, C.R., Gillies, R. and Hasan, T. (1991) Proc. Natl. Acad. Sci. USA 88, 1580–1584.
[10] Lin, C.-W., Shulok, J.R., Kirley, S.D., Bachelder, C.M., Flotte, T.J., Sherwood, M.E., Cincotta, L. and Foley, J.W. (1993) Photochem. Photobiol. 58, 81–91.
[11] Yao, J. and Zhang, G.-J. (1996) Biochim. Biophys. Acta 1284, 35–40.

[12] Yao, J. and Zhang, G.-J. (1997) Biochim. Biophys. Acta 1323, 334–342.
[13] Torinuki, W., Miura, T. and Seiji, M. (1980) Br. J. Dermatol. 102, 17–27.
[14] Mak, I.T., Misra, H.P. and Weglicki, W.B. (1983) J. Biol. Chem. 258, 13733–13737.
[15] Fong, K.-L., McCay, P.B. and Poyer, J.L. (1973) J. Biol. Chem. 248, 7792–7797.
[16] Abok, K., Hirth, T., Ericsson, J.L.E. and Brunk, U. (1983) Virchows Arch. 43, 85–101.
[17] Zdolsek, J.M. and Svensson, I. (1993) Virchows Arch. B 64, 401–406.
[18] Olsson, G.M., Brunmark, A. and Brunk, U.T. (1989) Virchows Arch. B 56, 247–257.
[19] Olsson, G.M., Svensson, I., Zdolsek, J.M. and Brunk, U.T. (1989) Virchows Arch. B 56, 385–391.
[20] Ohkuma, S., Moriyama, Y. and Takano, T. (1982) Proc. Natl. Acad. Sci. USA 79, 2758–2762.
[21] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[22] Girotti, A.W. and Thomas, J.P. (1984) Biochem. Biophys. Res. Commun. 118, 474–480.
[23] Girotti, A.W., Thomas, J.P. and Jordan, J.E. (1985) Arch. Biochem. Biophys. 236, 238–251.
[24] Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Anal. Biochem. 95, 351–358
[25] Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367–394.
[26] Lloyd, J.B. and Forster, S. (1986) TIBS 11, 365–368.
[27] Casey, R.P., Hollemans, M. and Tager, J.M. (1978) Biochim. Biophys. Acta 508, 15–26.
[28] Neely, A.N., Nelson, P.B. and Mortimore, G.E. (1974) Biochim. Biophys. Acta 338, 458–472.
[29] Reijngoud, D.-J. and Tager, J.M. (1977) Biochim. Biophys. Acta 472, 419–449.
[30] Bird, S.J., Forster, S. and Lloyd, J.B. (1987) Biochem. J. 245, 929–931.
[31] Harikumar, P. and Reeves, J.P. (1983) J. Biol. Chem. 258, 10403–10410.
[32] Greene, A.A. and Schneider, J.A. (1992) in Pathophysiology of Lysosomal Transport (Thoene, J.G. ed.), pp. 7–44, CRC Press, Boca Raton, FL.
[33] Jonas, A.J. and Jobe, H. (1990) J. Biol. Chem. 265, 17545–17549.
[34] Girotti, A.W. (1990) Photochem. Photobiol. 51, 497–509.
[35] Reeves, J.P. (1984) in Lysosomes In Biology and Pathology (Dingle, J.T., Dean, R.T. and Sly, W. eds), Vol. 7, pp. 175–199, Elsevier, Amsterdam.
[36] Forster, S. and Lloyd, J.B. (1988) Biochim. Biophys. Acta 947, 465–491.
[37] Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J.D. (1989) in Molecular Biology of the Cell, 2nd edn., pp. 300–301, Garland Publishing, New York.
[38] Ruth, R.C. and Weglicki, W.B. (1978) Biochem. J. 172, 163–173.
[39] Ruth, R.C. and Weglicki, W.B. (1980) Biochem. J. 186, 243–256.
[40] Ruth, R.C. and Weglicki, W.B. (1982) Am. J. Physiol. 242, C192–C199.
[41] Ruth, R.C. and Weglicki, W.B. (1983) Am. J. Physiol. 245, C68–C73.
[42] Valenzeno, D.P. (1987) Photochem. Photobiol. 46, 147–160.
[43] Papahadjopoulos, D., Nir, S. and Ohki, S. (1972) Biochim. Biophys. Acta 266, 561–583.
[44] Yeagle, P.L. (1985) Biochim. Biophys. Acta 822, 267–287.
[45] Foote, C.S. (1976) in Free Radicals in Biology (Pryor, W.A. ed.), Vol.2, pp. 85–134, Academic Press, New York.