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Influence of membrane physical state on lysosomal potassium ion permeability

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Abstract

Lysosomal permeability to potassium ions is an important property of the organelle. Influence of the membrane physical state on the potassium ion permeability of isolated lysosomes was assessed by measuring the membrane potential with bis(3-propyl-5-oxoisoxazol-4-yl)pentamethine oxonol and monitoring the lysosomal proton leakage with *p*-nitrophenol. The membrane fluidity of lysosomes was modulated by treatment with membrane fluidizer benzyl alcohol and rigidifier cholesteryl hemisuccinate. Changes in the membrane order were examined by steady-state fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene. The measurements of membrane potential and proton leakage demonstrated that the permeability of lysosomes to potassium ions increased with rigidification of their membranes by cholesteryl hemisuccinate treatment at 37 °C, and decreased with fluidization of their membranes by benzyl alcohol treatment at 2 °C. The changes in ion permeability could be recovered by fluidizing the rigidified membranes and rigidifying the fluidized membranes. The results suggest that the physical states of lysosomal membranes play an important role in the regulation of their K^+ permeability.

Keywords: Lysosomes; Membrane fluidity; Potassium ion permeability; Membrane potential

1. Introduction

Lysosomes in vivo are surrounded by a high concentration of cytoplasmic K^+ (140 mM) (Alberts et al., 1989). In the past, the effects of potassium ions on lysosomal activities have been actively investigated. A number of studies demonstrated that potassium ions play a regulatory role in lysosomal acidification (Reeves, 1984). An influx of K^+ into the lysosomes in exchange for their internal H^+ can raise intralysosomal pH (Ohkuma et al., 1982; Casey et al., 1978; Henning, 1975). On the other hand, the alkalization of lysosomal interiors by the influx of K^+ and an exchange of intralysosomal K^+ for extralysosomal H^+ favors lysosomal H^+-ATPase mediated proton translocation (Reeves, 1984; Dell'Antone, 1984).

As established previously, some lysosomal transport systems are sensitive to the movement of K^+ across their membranes. The lysosomal uptake of taurine (a sulfur amino acid derivative that has putative nutritional, osmoregulatory and neuroregulatory roles in a variety of cells) can be enhanced by the influx of K^+, which changes the cytoplasmic concentration of taurine (Vadgama et al., 1991). Cystinosis is a recessively inherited metabolic disorder characterized by intralysosomal cystine storage (due to a deficiency in

Abbreviations: BA, benzyl alchohol; CCCP, carbonyl cyanide mchlorophenylhydrazone; CHS, cholesteryl hemisuccinate; DPH, 1,6-diphenyl-1,3,5 hexatriene; MES, 2-[*N*-morpholino]ethanesulfonic acid; Oxonol VI, bis(3-propyl-5-oxoisoxazol-4-yl)pentamethine oxonol.

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lysosomal cystine transport) (Pisoni and Thoene, 1991). This disease can produce dwarfism, renal failure and eventual death before puberty (Kooistra et al., 1984). The inward permeation of K^+ can stimulate lysosomal cystine exodus for both normal and cystinotic cells (Bashan et al., 1984). It may have some biomedical significance. In addition to these pathophysiological effects, accumulation of K^+ in the lysosomes to some extent can osmotically destabilize the organelle (Yao and Zhang, 1997; Zhang and Yao, 1997; Ruth and Weglicki, 1983). The disruption of lysosomes may cause cell death (de Duve and Wattiaux, 1966).

As proposed previously (Zs-Nagy, 1979), some enzymes, such as DNA-dependent RNA polymerases, are extremely sensitive to the ionic strength of the medium. Increases in intracellular K^+ content can decrease the rate of enzymatic catalysis. Lysosomes contain at least 60 different hydrolases that are capable of digesting nucleic acids, proteins, lipids and carbohydrates. Whether the potassium ions within lysosomes affect their enzyme activities is still unknown. The evidence presented above indicates that lysosomal membrane permeability to K^+ is an important property. The lysosomal membrane shows only a limited permeability toward K^+ above 20 °C but becomes relatively permeable to the ions at 0 °C (Reijngoud and Tager, 1977) or after being photooxidized (Yao and Zhang, 1997; Zhong et al., 2000). Why the low temperature and photodamage can increase lysosomal K^+ permeability is unclear. Which properties of lysosomal membranes affect their permeability to K^+ is still unknown. To elucidate these issues is important in the study of lysosomes.

As described by Shinitzky (1984), a variety of membrane physiological and biochemical properties, such as membrane permeability and membrane-bound enzyme activity are influenced by the physical state of membranes. On the other hand, a wide range of physiological variables can modulate membrane lipid fluidity (Zhang et al., 2000). As demonstrated previously, the potassium ion permeability of some membranes is affected by changes in their fluidity (Boonstra et al., 1982; Medow and Lipkowitz, 1994). Since lysosomal membrane fluidity is liable to change under various physiological and pathological conditions (Zhang et al., 2000), it may be significant to clarify whether the physical state of lysosomal membranes affects their K^+ permeability. In a recent study (Zhang et al., 2000), we investigated the influence of membrane fluidity on lysosomal proton permeability by the treatment of lysosomes with membrane fluidity-modulating agents benzyl alcohol (BA) (Gordon et al., 1980) and cholesteryl hemisuccinate (CHS) (Yuli et al., 1981). Using a similar approach, we examined the effects of membrane physical state on lysosomal permeability to K^+. The results show that lysosomal K^+ permeability can be increased and decreased by decreasing and increasing their membrane fluidity, respectively.

2. Materials and methods

2.1. Chemicals

Oxonol VI was purchased from Molecular Probes (Eugene, OR, USA); CCCP, Valinomycin, DPH, CHS and MES were from Sigma (St. Louis, MO, USA). The other chemicals were made by The Beijing Chemical Factory and were of analytical grade.

2.2. Preparation of lysosomes

Male Wistar rats were starved for 24 h and killed by decapitation. Rat liver lysosomes were isolated by the differential centrifugation method of Ohkuma et al. (1982). All procedures were carried out at 0–4 °C. Lysosomes were resuspended in 0.25 M sucrose medium at a final protein concentration of 11.8 mg/ml.

2.3. Modulation of lysosomal membrane fluidity

Lysosomal membrane fluidity was modulated by treatment with BA and CHS (Zhang et al., 2000). To fluidize the membranes, isolated lysosomes were incubated in the presence of 0.2 mM BA at 2 °C for the indicated time. To decrease the fluidity of the BA-treated membranes, the above incubation was continued for the indicated time upon addition of 2 mM (final concentration) CHS to the suspension. Rigidification of the membranes was accomplished by incubating the isolated lysosomes in the presence of 2 mM CHS at 37 °C for the indicated time. To increase the fluidity of the CHS-treated membranes, the above incubation was continued for the indicated time upon addition of 2.5 mM (final concentration) BA to the incubation medium. Control lysosomal samples were incubated similarly but in the absence of BA or CHS (BA or CHS was added to the control sample at the same final concentration as that of the treated sample just before DPH labeling or the measurements of membrane potential and proton leakage).

2.4. Steady-state fluorescence anisotropy measurement

DPH labeling solution (4 μM) was prepared by diluting the tetrahydrofuran-dissolved DPH stock solution (2 mM) with 0.1 M PBS buffer (pH 7.4) containing 0.1 M sucrose and stirring vigorously. For labeling, lysosomal samples were incubated in the labeling solution (0.295 mg protein/ml) at 37 °C for 90 min. Fluorescence (excitation wavelength at 350 nm, emission

wavelength at 452 nm) of the BA and CHS-treated samples was measured on a Hitachi 850 fluorescence spectrophotometer at 2 °C and 37 °C, respectively. Steady-state fluorescence anisotropy (r) was calculated according to the equation (Gimpl et al., 1997)

$$r=(I_{VV}-GI_{VH})/(I_{VV}+2GI_{VH}) \quad (1)$$

where I_{VV} and I_{VH} are the fluorescence intensities measured with the excitation polarizer in the vertical position and the analyzing emission polarizer mounted vertically and horizontally, respectively. $G = I_{HV}/I_{HH}$ is the correction factor. Correction for light scattering was carried out as described previously (Zhang et al., 2000). High levels of fluorescence anisotropy indicate high levels of membrane order and low levels of membrane fluidity, and vice-versa (Van Blitterswijk et al., 1981).

2.5. Measurement of lysosomal membrane potential

Lysosomal K^+ permeability was assessed by the measurement of their membrane potential with oxonol VI (Zhong et al., 2000; Van Walraven et al., 1985). The assay medium contained 0.25 M sucrose, pH adjusted to 6.7 with imidazole. Oxonol VI (1 μM), CCCP (2 μM) and lysosomal sample (0.593 mg protein/ml) were added to 2 ml assay medium. K_2SO_4 (100 μl of 0.75 M) was added at the indicated time. Membrane potential was registered by the absorbance difference $A_{625-587}$ on a Hitachi U-3200 spectrophotometer (Loh et al., 1984). Measurements of the BA- and CHS-treated samples were carried out at 2 °C and 37 °C, respectively.

2.6. Measurement of lysosomal proton leakage

The influx of K^+ into lysosomes can elevate the intralysosomal pH and cause an efflux of the internal H^+ via a K^+/H^+ exchange (Ohkuma et al., 1982; Casey et al., 1978; Henning, 1975). By measuring the inward K^+ permeation-induced efflux of their protons, we assessed lysosomal K^+ permeability (Zhong et al., 2000; Henning, 1975). As described previously, the acidification of assay medium induced by the lysosomal proton leakage can be measured by monitoring the decrease in *p*-nitrophenol absorbance at 400 nm, 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 and 0.1 mM *p*-nitrophenol. A lysosomal sample was added to 2 ml assay medium at 0.593 mg protein/ml, followed by the addition of 200 μl 0.75 M K_2SO_4. The absorbance (400 nm) of the pH sensitive dye *p*-nitrophenol was measured immediately after adding 4 μl 1 mM CCCP to the medium. Measurements of the BA- and CHS-treated samples were carried out at 2 °C and 37 °C, respectively, on a Hitachi U-3200 spectrophotometer.

3. Results

3.1. Modulation of lysosomal membrane fluidity

Lysosomal membrane fluidity was modulated by treatment with membrane fluidizer BA and rigidifier CHS. As shown in Table 1, treatment of the lysosomes with BA (at 2 °C) for 15 and 30 min decreased the fluorescence anisotropy (r) from 0.201 to 0.191 and 0.180, respectively. It indicates that membrane order decreased (or membrane fluidity increased) upon treatment with BA. The fluorescence anisotropy (r) of the BA-treated (30 min) lysosomes increased to 0.214 after the sample was treated subsequently with CHS for an additional 15 min, showing that BA-induced membrane fluidization was reversed by treatment with CHS. In contrast to the effects of BA on membrane fluidity, treatment of the lysosomes with CHS (at 37 °C) for 15 and 30 min increased the fluorescence anisotropy (r) from 0.165 to 0.177 and 0.186, respectively. The increase in anisotropy (r) could be mostly reversed by subsequent treatment with BA (reduced to 0.170). These results indicate that membrane order increased (or membrane fluidity decreased) upon treatment with CHS and that CHS-treated lysosomal membranes can be fluidized by BA treatment.

Table 1
Effects of BA and CHS treatments on the fluorescence anisotropy of DPH-labeled lysosomes

Treatment of lysosomes	Anisotropy (r)	P
Control[a]	0.201 ± 0.002	–
0.2 mM BA 15 min[aa]	0.191 ± 0.001	<0.01
0.2 mM BA 30 min[aa,b]	0.180 ± 0.002	<0.001
0.2 mM BA 30 min then 2 mM CHS 15 min[bb]	0.214 ± 0.002	<0.001
Control[c]	0.165 ± 0.002	–
2 mM CHS 15 min[cc]	0.177 ± 0.002	<0.01
2 mM CHS 30 min[cc,d]	0.186 ± 0.002	<0.001
2 mM CHS 30 min then 2.5 mM BA 15 min[dd]	0.170 ± 0.001	<0.001

Lysosomal membranes were fluidized by treatment with 0.2 mM BA at 2 °C for the indicated time. To decrease the fluidity of the BA-treated membranes (treatment for 30 min), the sample was treated subsequently with 2 mM CHS for 15 min. To rigidify the membranes, the lysosomes were incubated in the presence of 2 mM CHS at 37 °C for the indicated time. To increase the fluidity of the CHS-treated membranes (treatment for 30 min), the sample was treated subsequently with 2.5 mM BA for 15 min. All procedures and calculations of fluorescence anisotropy (r) are described in Section 2. Values are means ± S.D., $n = 6$. Statistical analysis was performed using Student's t test. Note: aa vs a, bb vs b, cc vs c and dd vs d.

3.2. Effects of lysosomal membrane fluidity on membrane potential

Lysosomal K^+ permeability can be assessed by measurement of their membrane potential with oxonol VI (Zhong et al., 2000). An increase in the differential absorbance ($A_{625-587}$) of the dye, indicating a more positive interior potential of the membrane, is observed when potassium ions are allowed to enter the lysosomes (Zhong et al., 2000; Van Walraven et al., 1985). Using this method, we measured lysosomal permeability to K^+. As shown in Fig. 1, the dye absorbance of control sample (at 2 °C) increased markedly upon addition of K^+ to the measuring medium (line 1). The magnitude of the absorbance increase decreased after the lysosomes were treated with BA for 15 min (line 2) and 30 min (line 3). This indicates that lysosomal K^+ permeability decreases after BA treatment.

This conclusion was supported by experiments in which the potassium ionophore valinomycin was used to permeablize lysosomes to K^+. As shown in Fig. 2, the absorbance of control (line 1) and BA-treated lysosomes (line 2) increased by different magnitudes upon addition of K^+ to the measuring medium. The absorbance of the two samples increased further upon the subsequent addition of valinomycin, and reached the same level.

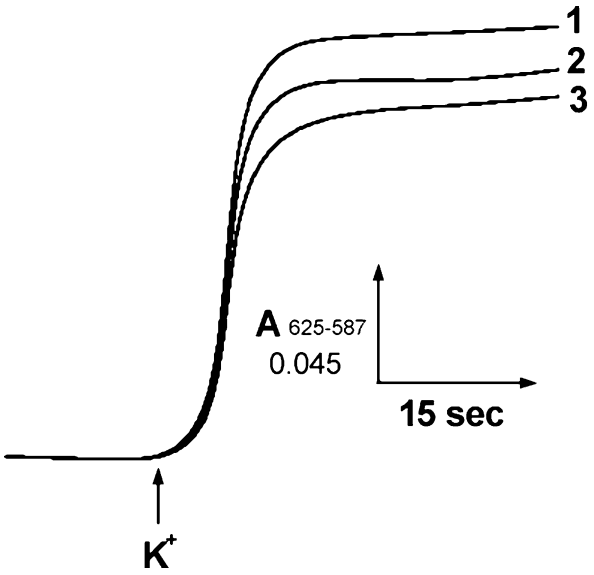

Fig. 1. Effects of BA treatment on lysosomal membrane potential. Measurement of lysosomal membrane potential and treatment of lysosomes with BA are described in Section 2. Assay medium contained 0.25 M sucrose, pH was adjusted to 6.7 with imidazole. Oxonol VI, CCCP and lysosomal sample were added to 2 ml assay medium sequentially at 1 μM, 2 μM and 0.593 mg protein/ml, respectively. K_2SO_4 (100 μl, 0.75 M) was added at the indicated time. Treatment of lysosomes with 0.2 mM BA was at 2 °C for: (1) 0 min, (2) 15 min, (3) 30 min. BA was added to the samples of (1) at 0.2 mM just before the absorbance measurement ($A_{625-587}$) of oxonol VI. A typical result out of three measurements is shown.

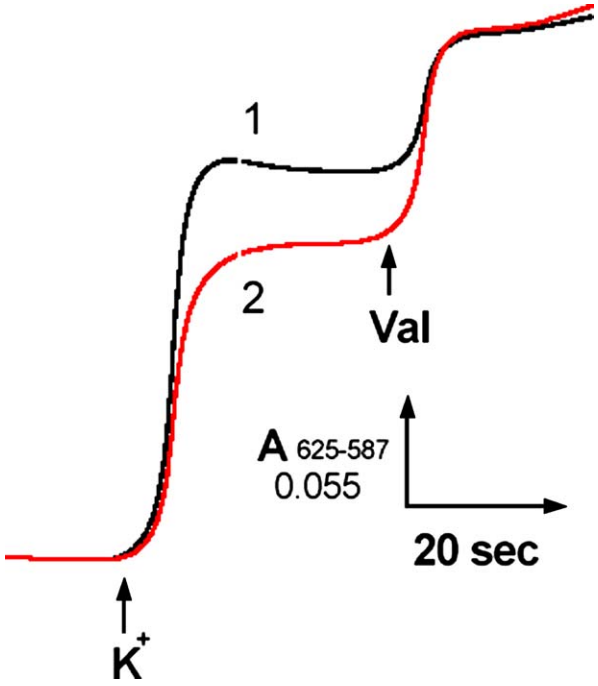

Fig. 2. Effects of valinomycin on the membrane potential of normal and BA-treated lysosomes. Assay medium and other conditions were as described in Fig. 1. Valinomycin was added at the indicated time at a final concentration of 5 μM. Treatment of lysosomes with 0.2 mM BA at 2 °C for: (1) 0 min, (2) 30 min. BA was added to the samples of (1) at 0.2 mM just before the absorbance measurement ($A_{625-587}$) of oxonol VI. A typical result out of three measurements is shown.

The degree of ionophore-produced absorbance increase in control lysosomes (line 1) is smaller than that of BA-treated lysosomes (line 2). This indicates that the K^+ permeability of the former is closer to valinomycin-induced permeability than that of the latter, i.e. the potassium ion permeability of the control lysosomes is greater than that of the BA-treated lysosomes.

Based on the results shown in Table 1, the reduction in the K^+ permeability of the BA-treated lysosomes at low temperature is presumably due to BA-produced membrane fluidization. To confirm this conclusion, we examined whether the decrease in K^+ permeability of the BA-treated lysosomes could be reversed by treatment with CHS (to rigidify the BA-treated lysosomal membranes). As shown in Fig. 3, fluidizing the lysosomal membranes with BA decreased the increase in dye absorbance (compare line 2 with line 1), while treating the BA-treated lysosomes with CHS recovered the absorbance increase (compare the top of line 3 with line 1). The results further establish that fluidizing the lysosomal membranes at low temperatures can decrease their K^+ permeability.

Using the above methods, we studied the effect of membrane rigidification on lysosomal K^+ permeability. The dye absorbance of the CHS-treated lysosomes increased more than the control sample (at 37 °C) upon addition of K^+ to the measuring medium (Fig. 4,

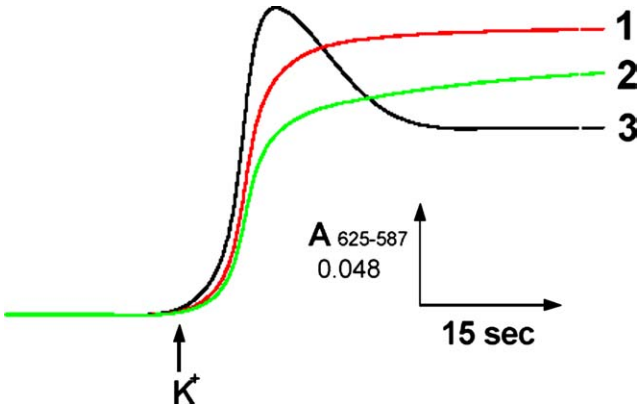

Fig. 3. Effect of CHS treatment on the membrane potential of normal and BA-treated lysosomes. Assay medium and other conditions were as described in Fig. 1. Treatment of lysosomes with 0.2 mM BA at 2 °C for: (1) 0 min, (2) 30 min, (3) 30 min. For (3), the BA-treated lysosomes were subsequently treated with 2 mM CHS at 2 °C for 15 min. 0.2 mM BA and 2 mM CHS were added to the sample of (1), and 2 mM CHS was added to the sample of (2) just before the absorbance measurement ($A_{625-587}$) of oxonol VI. A typical result out of three measurements is shown.

compare lines 1 and 2 with line 3). It indicates that the CHS treatment increased the lysosomal K^+ permeability at 37 °C. This conclusion was supported by additional experiments. As shown in Fig. 5, the K^+-induced absorbance increase of the CHS-treated lysosomes (line 1) is greater than that of the control lysosomes (line 2), while the valinomycin-induced additional absorbance increase of the former is less than that of the latter. It suggests that the K^+ permeability of the CHS-treated lysosomes was closer to the valinomycin-induced permeability than that of the

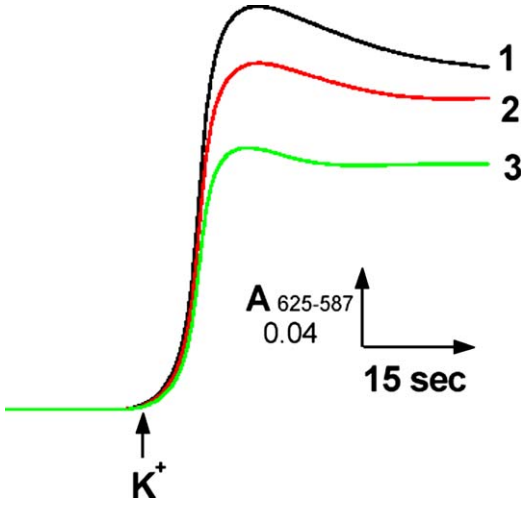

Fig. 4. Effects of CHS treatment on lysosomal membrane potential. Assay medium and other conditions were as described in Fig. 1. Treatment of lysosomes with 2 mM CHS at 37 °C for: (1) 30 min, (2) 15 min, (3) 0 min. CHS was added to the samples of (3) at 2 mM just before the absorbance measurement ($A_{625-587}$) of oxonol VI. A typical result out of three measurements is shown.

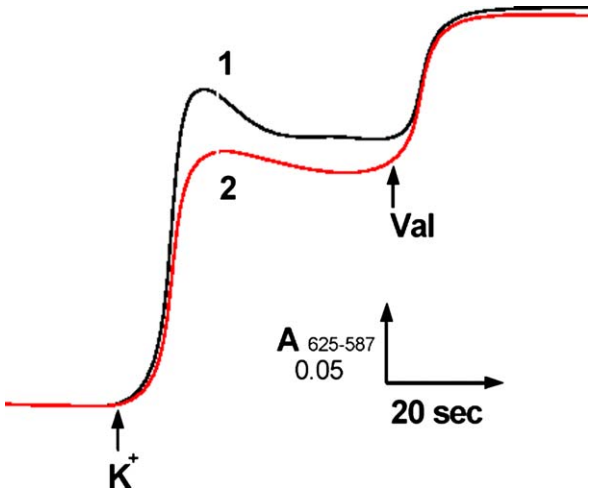

Fig. 5. Effects of valinomycin on the membrane potential of normal and CHS-treated lysosomes. Assay medium and other conditions were the same as that of Fig. 4. Valinomycin was added at the indicated time at a final concentration of 5 μM. Treatment of lysosomes with 2 mM CHS at 37 °C for: (1) 30 min, (2) 0 min. CHS was added to the sample of (2) at 2 mM just before the absorbance measurement ($A_{625-587}$) of oxonol VI. A typical result out of three measurements is shown.

control lysosomes. In other words, the K^+ permeability of the former is greater than that of the latter. Using BA to fluidize the CHS-treated lysosomal membranes, we studied the effect of membrane rigidification on lysosomal K^+ permeability. The results show that the increase in absorbance of CHS-treated lysosomes (Fig. 6, line 1) was reduced by BA treatment (line 3). It indicates that the increase in lysosomal K^+ permeability produced by CHS treatment was decreased by the fluidization of the membrane. These results support the conclusion that rigidifying the lysosomal membranes at physiological temperatures can increase their K^+ permeability.

3.3. Effects of lysosomal membrane fluidity on K^+/H^+ exchange-induced proton leakage

Lysosomal K^+ permeability can be semi-quantitatively assessed by the measurement of K^+/H^+ exchange-induced proton leakage (Zhong et al., 2000). As described by Reeves (1984), the pH gradient across lysosomal membranes provides a driving force for their proton efflux. The proton leakage by an electroneutral exchange of intralysosomal H^+ for external K^+ depends on lysosomal permeability to both K^+ and H^+. As demonstrated recently (Zhang et al., 2000), lysosomal proton permeability changes with alterations in their membrane fluidity. To compare K^+ permeability of lysosomes exhibiting different membrane physical states by measuring their proton leakage, the lysosomes should be permeablized to H^+. Thus, the extent of proton leakage of the lysosomes that exhibit different

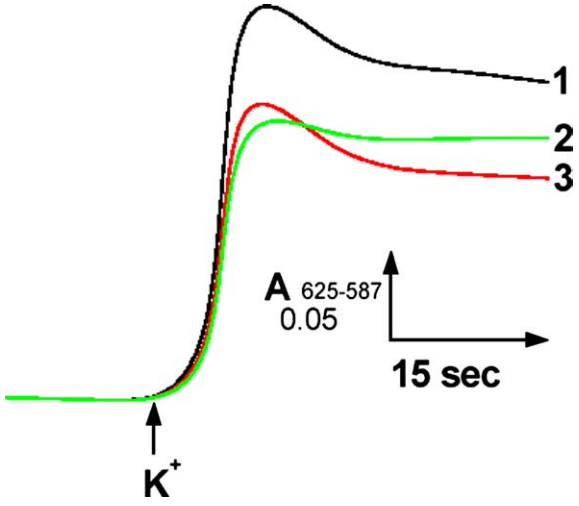

Fig. 6. Effect of BA treatment on the membrane potential of CHS-treated lysosomes. Assay medium and other conditions were the same as Fig. 4. Treatment of lysosomes with 2 mM CHS at 37 °C for: (1) 30 min, (2) 0 min, (3) 30 min. For (3), the CHS-treated lysosomes were then treated with 2.5 mM BA at 37 °C for 15 min. 2.5 mM BA and 2 mM CHS were added to the sample of (2), and 2.5 mM BA was added to the sample of (1) just before the absorbance measurement ($A_{625-587}$) of oxonol VI. A typical result out of three measurements is shown.

membrane physical states will depend solely on their own K^+ permeability.

In this work, we used protonophore CCCP to permeablize the lysosomes to H^+. The lysosomal proton leakage was measured by monitoring the absorbance (400 nm) decrease of *p*-nitrophenol. As shown in Fig. 7, the dye absorbance decreased upon addition of K^+ and CCCP to the control lysosomal sample at 2 °C (line 3). It is presumably due to a K^+/H^+ exchange-induced proton leakage. Under the same conditions, the absorbance reduction was abolished when the measuring medium was buffered (line 1). It indicates that the absorbance decrease (line 3) is due to acidification of the measuring medium. Whether fluidizing the lysosomal membranes affects their permeability to K^+ was examined. Treatment of the lysosomes with BA (at 2 °C) reduced the absorbance decrease (compare line 2 with line 3), reflecting a decrease in K^+/H^+ exchange-induced proton leakage. Since these lysosomal samples were permeablized to H^+, the reduction in proton leakage was due to a decrease in lysosomal K^+ permeability. The results support the above conclusion that fluidizing the lysosomal membranes at low temperatures can decrease their K^+ permeability.

Using a similar approach, we re-examined the effect of membrane rigidification on lysosomal K^+ permeability. The results show that the dye absorbance of the CHS-treated lysosomes decreased more markedly than that of the control lysosomes at 37 °C (Fig. 8, compare line 3 with line 2). It reinforces the conclusion that rigidifying the lysosomal membranes at physiological temperatures can increase their K^+ permeability. Taken together, the above results indicate that the physical state of lysosomal membranes affects their permeability to K^+.

4. Discussion

Membrane lipid fluidity can be modulated by a wide range of physiological variables, such as fatty acid composition, aging, alcohol, phosphatidylethanol, sterols, insecticides, diacylglycerols, drug-induced cytochrome P-450 activity and ether lipids (Zhang et al., 2000). A number of studies have demonstrated that the physical state of lysosomal membranes is liable to change under various conditions, such as the onset of apoptosis (Singh et al., 1996) and membrane lipid peroxidation (Zhang and Yao, 1997). In addition, polyanions (Kielian and Cohn, 1982) and some bioactive compounds, such as bilirubin, farmorubicin and chelerythrine (Mozhenok et al., 1998), can modulate lysosomal membrane fluidity. Although the effects of

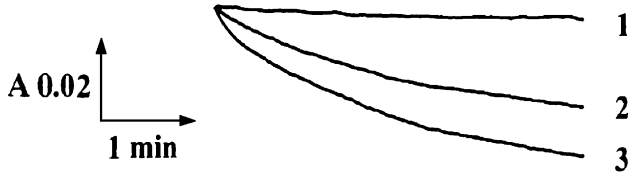

Fig. 7. Effect of BA treatment on lysosomal proton leakage. All assay media (1–3) contained 0.25 M sucrose and 0.1 mM *p*-nitrophenol, the medium was buffered at pH 6.0 with 10 mM MES/Tris only for (1). Lysosomal sample was added to 2 ml assay medium at 0.593 mg protein/ml, followed by the addition of 200 μl 0.75 M K_2SO_4. Absorbance (400 nm) of the pH sensitive dye *p*-nitrophenol was measured immediately after adding 4 μl 1 mM CCCP to the medium. Treatment of lysosomes with 0.2 mM BA at 2 °C for: (1) 0 min, (2) 30 min, (3) 0 min. BA was added to the samples of (1) and (3) at 0.2 mM just before the measurement. A typical result out of three measurements is shown.

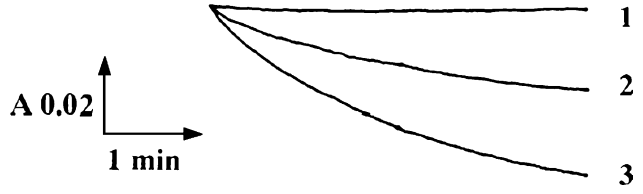

Fig. 8. Effect of CHS treatment on lysosomal proton leakage. All assay media contained 0.25 M sucrose and 0.1 mM *p*-nitrophenol, the medium was buffered at pH 6.0 with 10 mM MES/Tris only for (1). Lysosomal sample was added to 2 ml assay medium at 0.593 mg protein/ml, followed by the addition of 200 μl 0.75 M K_2SO_4. Absorbance (400 nm) of the pH sensitive dye *p*-nitrophenol was measured immediately after adding 4 μl 1 mM CCCP to the medium. Treatment of lysosomes with 2 mM CHS at 37 °C for: (1) 0 min, (2) 0 min, (3) 30 min. CHS was added to the samples of (1) and (2) at 2 mM just before the measurement. A typical result out of three measurements is shown.

various factors on lysosomal membrane fluidity are being actively studied, little information is available concerning the influence of membrane fluidity on lysosomal membrane permeability.

Lysosomal permeability to K^+ plays an important role in the organelle's activities, but which properties of lysosomal membranes affect their K^+ permeability is still unclear. For some membranes, ion permeability can be regulated by their physical state (Shinitzky, 1984; Boonstra et al., 1982; Medow and Lipkowitz, 1994). The elucidation of the effects of membrane fluidity on lysosomal K^+ permeability is important for the study of lysosomal pathophysiology. We have established that the physical state of lysosomal membranes can modulate their permeability to protons (Zhang et al., 2000). The results of this work show that lysosomal K^+ permeability increases and decreases with decreasing and increasing membrane fluidity, respectively. It provides new insights into the properties of lysosomal membranes that affect K^+ permeability.

As demonstrated previously, lysosomal ion permeability can be affected by a variety of factors, such as temperature (Reeves, 1984; Reijngoud and Tager, 1977), photooxidation (Zhang and Yao, 1997; Yao and Zhang, 1997) and the antineoplastic drug lonidamine (Dell'Antone and Piergallini, 1997). However, the mechanism responsible for changing lysosomal ion permeability has yet to be elucidated. The evidence presented by this study will remind investigators to note the physical state of membranes when lysosomal potassium ion permeability changes under certain conditions. In other words, it is likely that some pathophysiological factors may affect lysosomal ion permeability by modulating the membrane physical state.

Over the last 25 years, a number of studies have presented various lines of evidence that membrane permeability to K^+ can be increased and decreased by decreasing and increasing membrane fluidity, respectively (Medow and Lipkowitz, 1994; Gordon et al., 1980; Singelmann et al., 1984; Shinozawa et al., 1980). In this study, we reached a similar conclusion. At present, the precise mechanism for the effect of membrane fluidity on the permeability to K^+ is unclear. Two theories concerning membrane permeability have been proposed. As for the permeation of solutes by the solubility–diffusion mechanism (Finkelstein, 1987), Born energy is required to transfer charged particles from the high dielectric aqueous phase to the low dielectric membrane interior. Based on this theory, lipid bilayers are virtually impermeable to most ions, because the electrostatic energy of ions is much lower in a water medium with high dielectric constant (about 80) than in a typical bilayer with low dielectric constant (about 2) (Orme et al., 1988). As proposed previously (Kimura and Ikegami, 1985), the dielectric constant of membranes increases when their fluidity is increased. It suggests that increasing membrane fluidity may decrease the electrostatic energy of ions in the membranes, and vice-versa. This may explain why increasing and decreasing membrane fluidity can increase and decrease membrane permeability to most ions, respectively.

In addition to the solubility–diffusion mechanism, transient defects and/or pores are the other permeation pathway for various solutes (Deamer and Bramhall, 1986). Lateral lipid density fluctuation of membranes can induce the formation of short-lived defects and/or pores in the surface region of the bilayer. Ions and other solutes first enter the defects and pores. When the pore closes, the ions are then trapped in the hydrocarbon region of the bilayer, across which they can pass through the membrane (Nagle and Scott, 1978). By passing through transient defects, the permeating particles can avoid the Born energy barrier. As described previously (Nagle and Scott, 1978; Scarlata, 1991; Träuble, 1971), the higher the membrane fluidity, the greater the lateral lipid density fluctuation and the more defects and/or pores are formed. This accounts for the effects of membrane fluidity on ion permeation through transient defects. According to the mechanisms of either solubility–diffusion or transient defects, the fluidization and rigidification of membranes can increase and decrease their ion permeability, respectively. It is consistent with the correlation between the membrane fluidity of lysosomes and their permeability to either protons or water (Zhang et al., 2000).

Interestingly, the results of this and other studies (Medow and Lipkowitz, 1994; Gordon et al., 1980; Singelmann et al., 1984; Shinozawa et al., 1980) demonstrated an inverse correlation between membrane fluidity and membrane permeability to K^+, i.e. increasing and decreasing membrane fluidity can decrease and increase permeability to K^+. It suggests that there must be other mechanisms underlying the effect of membrane fluidity on the permeability to K^+. It has been established that the increase in membrane fluidity is associated with closure of the potassium ion channels of rat liver cell membranes (Thalhammer et al., 1993). Whether the lysosomal membranes of rat liver also contain potassium ion channels is unclear. One possibility is that there are potassium ion channels in the lysosomal membranes and that membrane fluidity affects the channels in a similar way to those in the cell membranes. Further studies are needed to clarify this issue.

The lysosomal membrane shows only a limited permeability to K^+ at 37 °C (Reeves, 1984; Reijngoud and Tager, 1977). Generally, biomembranes are at a relatively higher fluidized state above their phase transition temperature than at lower temperatures (Shinitzky, 1984). The phase transition temperature of lysosomal membranes is about 15 °C (Ruth and

Weglicki, 1982). In this study, we increased lysosomal membrane fluidity (at 37 °C) by BA treatment, but the membrane permeability to K^+ did not decrease further (data not shown). However, membrane K^+ permeability increased when membrane fluidity (at 37 °C) was decreased by CHS treatment. The results indicate that the rigidification of lysosomal membranes can increase their permeability to K^+ at physiological temperatures. The cytoplasm contains abundant K^+ (Alberts et al., 1989). Since uptake of K^+ by lysosomes is detrimental to their osmotic stability and simultaneously affects organelle acidification by K^+/H^+ exchange, the rigidification of lysosomal membranes may have some pathophysiological significance. Previous studies have demonstrated that the lysosomal permeability to K^+ increased markedly at low temperatures, but the reason is still unclear (Reeves, 1984; Reijngoud and Tager, 1977). As shown in this study, ion permeability decreased markedly at low temperatures when the membranes were fluidized by treatment with BA. Therefore, we propose that the rigidification of lysosomal membranes might be a major cause for the low temperature-induced increase in their K^+ permeability. In conclusion, membrane physical state plays an important role in the regulation of lysosomal K^+ permeability.

The lysosomal membrane potential is negative inside due to the pH gradient across the membrane. In mammalian cells, lysosomes are surrounded by a high concentration of cytoplasmic K^+ (140 mM) (Alberts et al., 1989). Generally, lysosomal membranes show only a limited permeability to K^+, so the ions do not markedly affect lysosomal membrane potential (Harikumar and Reeves, 1983). However, the membrane potential depolarizes when potassium ions are allowed to enter the lysosomes. In studies of lysosomes, lysosomal membrane potential can be measured using fluorescent dyes, such as $DiSC_3(5)$ (Harikumar and Reeves, 1983) and Oxonol VI (Zhong et al., 2000). These measurements often provide semi-quantitative indications of changes in the membrane potential (Harikumar and Reeves, 1983). In this study, we found that Oxonol VI was more sensitive to K^+-entry induced changes in the membrane potential than $DiSC_3(5)$. At present, we are not able to explain why this should be.

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