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POTASSIUM ION DEPENDENT PROTON EFFLUX AND DEPOLARIZATION FROM SPLEEN LYSOSOMES

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SUMMARY: Lysosomes, isolated from various organs, exhibited an acidic interior (≅ pH 5.2) when incubated in a buffer at neutral pH. K^+-induced proton efflux was observed in spleen lysosomes, but not in liver or kidney lysosomes. The initial velocity of the proton efflux showed saturation kinetics with Km value of about 15 mM K^+. Rb^+ and Cs^+ have an effect similar to K^+, while Na^+, Li^+ or divalent cations have little or no effect. The properties of the K^+ induced proton efflux correlated with the K^+-induced depolarization of the lysosomes, suggesting the presence of K^+-transport system(s) in lysosomal membranes.

INTRODUCTION: Lysosomes, one of the vacuolar organelles in mammalian cells, play an important role in intracellular digestion (1). Since nutrients are hydrolyzed inside and reutilized outside of the organelles, transport systems for the metabolites have been postulated in lysosomal membranes (2). Some sugars and amino acids are extruded from lysosomes via specific transport systems (2). Amino acid efflux might be coupled with proton motive force which is formed by lysosomal H^+-ATPase (3,4). Moreover, it has been proposed that some genetic disorders are due to defective lysosomal transport systems (3,5). Lysosomal ion transport systems may function in processes such as regulation of electrochemical potential, osmotic pressure, and volume changes. Although such ion transport systems were found in various vacuolar systems (6,7,8), little is known about ion transport in lysosomes.

Fluorescein isothiocyanate dextran (FITC-dextran) has been used to measure the internal pH of lysosomes (9). FITC-dextran is taken up via endocytosis and trapped in lysosomes, allowing measurement of intralysosomal pH specifically, even in the presence of other organelles. Using this method, the effect of various salts on internal pH of lysosomes prepared from various organs was studied. It was found that K^+ induced proton efflux and depolarization in spleen lysosomes.

MATERIALS AND METHODS

Materials. FITC-dextran (FD-70S) and Triton WR 1339 (tyroxapol) were purchased from Sigma. 3,3'dipropylthiadicarbocyanine iodide (diS-C3(5)) was obtained

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from Molecular Probes. Animals (rats, Wistar male, 200-250 g body weight, mouse, ddY male, about 20 g body weight) were supplied from Charles River Co.

Analytical procedures. Fluorescence change of FITC-dextran and diS-C3(5) were measured with Perkin Elmer MPF66 fluorescence spectrometer as described previously (9,10,11). The assay solution consisted of 20 mM MOPS-tris pH 7.0, 0.3 M sucrose and lysosomes. Lysosomal latency and marker enzyme activities for lysosomes, mitochondria and plasma membrane were measured as described previously (12). Protein concentration was estimated by the method of Lowry et al. (13).

Preparations. FITC-dextran (20 mg/ml) was introduced into lysosomes as described previously (9). After overnight starvation, animals were decapitated and various organs were isolated. Crude lysosomes from liver or kidney were prepared by differential centrifugation according to the published procedures (9,10). Crude lysosomes from spleen were prepared as follows: Isolated spleen was homogenized in 3 vol of 0.3 M sucrose containing 1 mM EDTA and 0.1% v/v ethanol pH 7.0 (SVE). The homogenate was centrifuged at 270 g for 10 min and the supernatant was diluted twice with SVE and centrifuged at 500 g for 5 min to remove erythrocytes. This step was repeated a few times if necessary. The supernatant was then centrifuged at 12000 g for 10 min, and the pellets (crude spleen lysosomes) were washed twice with SVE, suspended in the same solution, and kept on ice until use.

Highly purified lysosomes from liver or kidney were prepared by the published procedures (9,10). Highly purified lysosomes from rat spleen were prepared as follows: Triton WR 1339 (0.85 mg/g of body weight) was injected intraperitoneally into rats 1 week before preparation. After overnight starvation, rats were killed and spleens were isolated. Crude spleen lysosomes were prepared as described above and suspended in 45% (w/w) sucrose and layered onto 60% (w/w) sucrose. A discontinuous sucrose density gradient was made by adding 12 ml of 34.5% (w/w) sucrose and 8 ml of 14.3% (w/w) sucrose. The gradient was centrifuged at 90000 g for 2 h. Lysosomes which were recovered from the interface between 34.5% and 14.3% sucrose were taken by pipetting and kept on ice until use. Lysosomal purity was estimated from marker enzymes to be about 30-35 fold over the homogenate, which is the highest purity achieved for lysosomes from lymphoid tissues (14). In addition, the latency of the purified lysosomes was greater than 80%.

RESULTS AND DISCUSSION

Internal pHs of lysosomes from various organs. FITC-dextran was found to be present in various organs following intraperitoneal injection. Appreciable amounts of FITC-dextran were found in liver, spleen, kidney, adrenal and testis as shown in Table 1. Subcellular fractionation studies revealed that almost all FITC-dextran was present in the particulate fraction corresponding to lysosomes (not shown). Thus, the lysosomal pH of these organs can be measured using the FITC-dextran fluorescence technique developed for liver lysosomes (9). All lysosomes prepared from these organs maintained an acidic pH ($\cong$ pH 5.2) when suspended in the buffer at neutral pH. In lysosomes from all organs addition of MgATP (1 mM) resulted in decreased FITC-dextran fluorescence, indicating acidification by H^+-ATPase. The properties of the MgATP-induced acidification (divalent cation requirement, substrate specificity, sensitivity to inhibitors) were essentially the same as those from previous reports (9-12), suggesting that the same type of H^+-ATPase operates in lysosomes from various organs.

K^+-induced H^+ efflux in spleen lysosomes. It is well known that plant vacuoles contain cation/proton antiporters (6-8), which take up cations at the expense of proton motive force established by the H^+-ATPase. The effect of cations on lysosomal pH was measured to identify similar systems in lysosomes.

Fig. 1 shows the effect of KCl on rat lysosomal pH. Addition of KCl significantly enhanced the fluorescence intensity of FITC-dextran (Fig. 1-a), without changing the

TABLE I

Distribution of FITC-dextran into Various Organs from Rat and Mouse

Organ	Content of FITC-dextran (relative fluorescence intensity per weight of organ)	
	rat	mouse
liver	100	100
spleen	30	24
kidney	15	7
adrenal	5	5
testis	4	7
brain	< 0.5	< 0.5
lung	< 0.5	< 0.5
heart	ND	3

ND: not detected.

FITC-dextran was injected into animals and the organs were isolated and homogenized as described in "Materials and Methods". After removing nucleous and unbroken cells by centrifugation, content of FITC-dextran in the post-nuclear supernatant was estimated by its fluorescence in 20 mM MOPS-tris pH 7.0 containing 0.05% Triton X-100. Content of FITC-dextran was expressed as relative fluorescence intensity per wet weight of organ with that of liver taken as 100%.

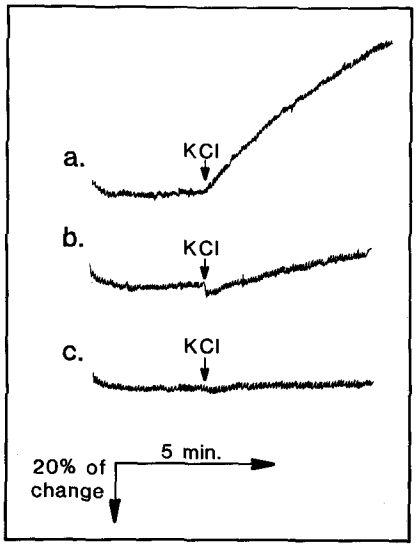

Figure 1. KCl-induced proton efflux from lysosomes. Changes in intralysosomal pH were measured by FITC-dextran fluorescence as described in "Materials and Methods". KCl was added at final concentration of 25 mM as indicated. a, lysosomes from rat spleen, 40 μg protein; b, lysosomes from rat liver, 80 μg protein; c, lysosomes from rat kidney, 40 μg protein.

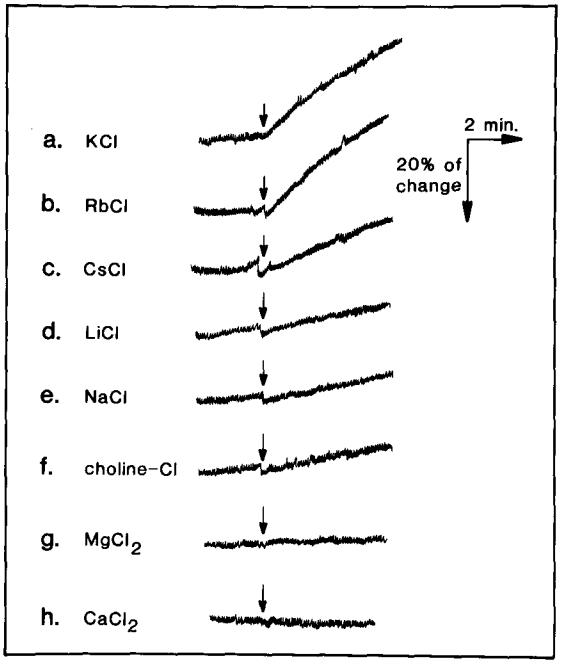

Figure 2. Cation specificity of proton efflux. Changes in the internal pH of spleen lysosomes following the addition of various salts were measured as described in "Materials and Methods". Fifty µl of 1 M salt were added to give a final concentration of 25 mM as indicated. Fifty µg protein of lysosomes were used per assay.

latency of the lysosomes, indicating alkalization of intralysosomal space. The KCl induced proton efflux was predominantly observed in spleen lysosomes. However, KCl had little effect on lysosomes from liver or kidney (Fig. 1-b,c). Essentially the same phenomena were observed in lysosomes prepared from mouse organs. The KCl induced proton efflux showed typical saturation kinetics with a half maximum effect at 15 mM KCl giving maximum change of initial velocity (ΔpH 0.02/min). Fig. 2 shows the effect of various salts on spleen lysosomal pH. Rb^+ and Cs^+ were also effective, however, other monovalent or divalent cations have no significant effect. Anions (Cl^-,Br^-, SO_4^{2-}, I^-, NO_3^-, F^- and SCN^-) have essentially no effect on K^+ induced proton efflux (data not shown). These results indicated that K^+ induced efflux of protons from spleen lysosomes and suggested the presence of K^+-transport system(s) in lysosomal membranes, most probably K^+/H^+ exchange system.

K^+-induced depolarization of lysosomes. Lysosomes have been shown to be polarized inside negative when suspended in K^+-free buffer (10,11,15). Addition of K^+ depolarized the membrane potential. To see the correlation between the K^+-induced depolarization and proton efflux, changes in membrane potential of spleen lysosomes was measured. DiS-C3(5) fluorescence can be used to measure lysosomal membrane potential semiquantitatively (10,11). As shown in Fig. 3, similar amounts of fluorescence quenching was attained after several minutes incubation following the addition of

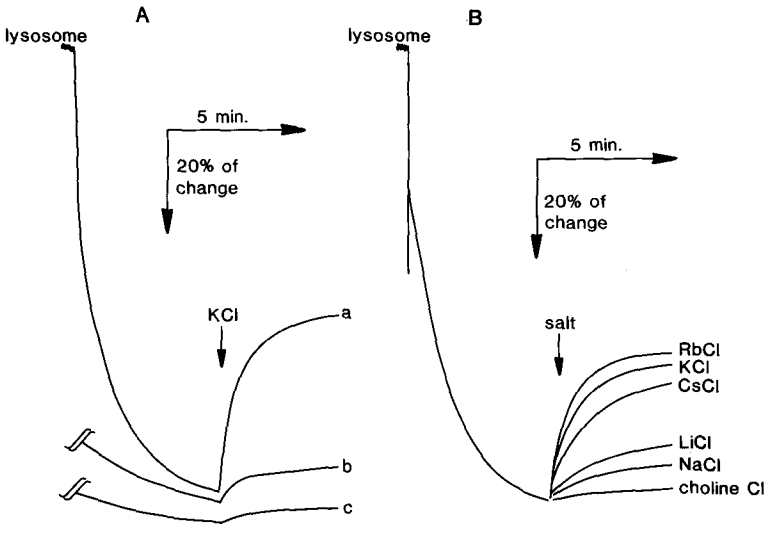

Figure 3. K^+-induced depolarization of lysosomes. The membrane potential of lysosomes was measured as described previously (11) in buffer containing 20 mM MOPS-tris pH 7.0, 0.3 M sucrose and 1 μM diS-C3(5). (A) Depolarization by K^+ in various lysosomes. KCl was added at 25 mM. Lysosomes from rat liver, kidney and spleen were purified as described in "Materials and Methods". (a) Spleen lysosomes (20 μg), (b) liver lysosomes (35 μg) and (c) kidney lysosomes (20 μg) were used for assay. (B) Cation specificity. Various salt solutions (25 mM) were added as indicated. Spleen lysosomes (20 μg) were used per assay.

lysosomes from all sources. Therefore, all lysosomes tested maintained nearly the same membrane potential (about 100 mV to 120 mV (10,11)). Addition of KCl decreased quenching, indicating depolarization of membrane potential. As expected, the initial velocity of depolarization in spleen lysosomes was about 5-fold and 20-fold faster than those of liver and kidney lysosomes, respectively (Fig. 3-A). Depolarization exhibited the same cation specificity as that of proton efflux (Fig. 3-B) and the same order of Km (10 mM, K^+) (not shown). These results indicated the close relationship between K^+-induced proton efflux and depolarization, and suggest that both activities are catalyzed by the same enzyme. It is concluded that an electrogenic K^+/H^+antiport may operate in lysosomal membrane. Of course, the possibility that influx of K^+ results in secondary movement of protons cannot be excluded. These activities are more pronounced in spleen in comparison with lysosomes from other sources (Fig. 1 and 3-A). This may be due to different activities or number of carriers in lysosomes from various organs. Thus, spleen lysosomes may be useful to study the K^+ transport system.

The physiological role of K^+-induced proton efflux and depolarization is not known. One possible role is in the regulation of intralysosomal pH. Chromaffin granule H^+-ATPase, which is one of the vacuolar H^+-ATPases, is regulated by anions and membrane potential (16,17). Similar mechanisms may be present to regulate lysosomal H^+-ATPase. *In vivo*, electrogenic K^+/H^+ transport or K^+ transport presented in this paper may cancel part of the membrane potential formed by H^+-ATPase, which helps to form a

larger ΔpH across the lysosomal membrane. In fact, the initial velocity of MgATP dependent acidification was accelerated about twice in the presence of K^+ (not shown). Recently, a similar K^+ conductance has been reported in various endomembrane systems where vacuolar type H^+-ATPase operates (18-21). It will be interesting to see the structural and functional relationship among these activities.

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