Cystine transport in purified rat liver lysosomes

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Amino acid efflux from highly purified rat liver lysosomes exposed to the methyl ester derivatives of leucine, methionine, tyrosine and cystine was examined. The lysosomal efflux of leucine, methionine and tyrosine was unaffected by the presence of MgATP, whereas cystine efflux was stimulated by MgATP. Exposure of lysosomes to 2 mM-MgATP resulted in lysosomal acidification and a 0.5 pH unit increase in the lysosomal pH gradient through the action of a proton-pumping ATPase. Cystine efflux was also stimulated when the lysosomal proton gradient was increased through changes in buffer pH. Decreasing the lysosomal proton gradient with ionophores resulted in diminished cystine efflux. Bivalent cations had no effect on the lysosomal efflux of leucine, methionine and tyrosine. However, cystine efflux was stimulated by the presence of bivalent cations even when the lysosomal proton gradient was minimized. Cation-stimulated cystine efflux was inhibited by the presence of the calcium ionophore A23187, which altered the lysosomal membrane potential. Cystine efflux from lysosomes appears to be uniquely dependent on pH gradients and cation concentrations.

INTRODUCTION

Lysosomal cystine transport is a carrier-mediated process in cell lines of human origin (Steinherz *et al.*, 1982a; Gahl *et al.*, 1982a,b, 1983, 1984a; Jonas *et al.*, 1982a,b, 1983). Impaired function of this process results in the human disorder cystinosis (Steinherz *et al.*, 1982a; Gahl *et al.*, 1982a,b, 1983, 1984a; Jonas *et al.*, 1982a,b, 1983). The lysosomal carrier appears to be quite specific for cystine, and the lysosomal efflux of other amino acids appears to be normal in individuals with cystinosis (Gahl *et al.*, 1982b, 1983, 1984b). In transformed human lymphoblasts, cystine transport has been found to be dependent on ATP-stimulated lysosomal acidification (Jonas *et al.*, 1982a,b). However, other studies using human leucocytes and fibroblasts have shown that cystine transport, although stimulated by Mg^{2+} , occurs in the absence of ATP (Gahl *et al.*, 1983, 1984a,b; Smith *et al.*, 1984). *N*-Ethylmaleimide and CCCP, which inhibit ATP-mediated lysosomal acidification, inhibit ATP-stimulated cystine transport in human lymphoblasts, but have had no effect on transport in other studies (Gahl *et al.*, 1983, 1984a,b; Jonas *et al.*, 1982b, 1983). In all of these studies, cystine transport was examined in only partially purified lysosomal preparations (Steinherz *et al.*, 1982a; Gahl *et al.*, 1982a,b, 1983, 1984a,b; Jonas *et al.*, 1982a,b, 1983; Smith *et al.*, 1984).

The lysosomal efflux of many other amino acids appears to proceed by a different mechanism. Most of the current information suggests that other amino acids exit from lysosomes by a diffusional process (Steinherz *et al.*, 1982b; Reeves, 1979). Efforts to inhibit leucine efflux in particular have failed to demonstrate any carrier-mediated transport (Reeves, 1979). More recently, carrier-mediated cationic amino acid transport has been described in human fibroblasts (Pisoni *et al.*, 1985).

We have examined amino acid transport in highly purified lysosomes from rat liver and have been able to demonstrate a cystine-transport system. Using this system, we have attempted to address some of the apparently contradictory information concerning lyso-

somal amino acid transport. Amino acid efflux has been studied during alteration of the lysosomal pH gradient and in the presence of various cations. Ionophores and the lysosomal alkalinizing agents, NH_4Cl and chloroquine, have been studied for their effects on amino acid efflux (Jonas *et al.*, 1983; Okhuma *et al.*, 1982). The actions of quercetin and *N*-ethylmaleimide, which inhibit the action of lysosomal proton-translocating ATPase (Jonas *et al.*, 1983; Okhuma *et al.*, 1982), have also been investigated.

METHODS

Materials

$U-^{14}C$ -labelled amino acids were obtained from New England Nuclear Corp. and ICN. $L-[^{35}S]$ Cystine was obtained from Amersham Corp. All chemicals and enzyme substrates, unless otherwise noted, were of the highest purity available from Sigma Chemical Co. Acetyl chloride was obtained from Aldrich. di- $O-C_5$ was obtained from Molecular Probes Inc. Sprague-Dawley rats were purchased from Timco Corp.

Synthesis of methyl esters

Radiolabelled amino acids were freeze-dried and then placed in a solution of 1 ml of methanol and 240 μ l of acetyl chloride for 24 h at 25 °C. The material was dried under vacuum, washed with methanol, and dried again by rotary evaporation under vacuum. The methyl esters were resuspended in methanol and stored at -20 °C. Purity was assessed by t.l.c. on cellulose plates with methanol/water/formic acid (35:14:1, by vol.) as solvent. The solvent for leucine methyl ester was methanol/water/formic acid (485:14:1, by vol.)

Assays

The lysosomal enzyme β -hexosaminidase, the mitochondrial marker succinate:2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium ('INT') reductase and the endoplasmic-reticulum marker neutral α -glucosidase were assayed as previously described (Jonas *et al.*, 1983;

Abbreviation used: CCCP, carbonyl cyanide *m*-chlorophenylhydrazine; di- $O-C_5$, 3',3'-dipentyloxycarbocyanine iodide.

Barrett, 1972; Pennington, 1961; Burns & Touster, (1982). Lysosomal latency was determined by assay of α -galactosidase at pH 4.4 by using the p-nitrophenyl derivative in the presence and absence of 0.1% Triton X-100. Protein was measured colorimetrically as previously described (Bradford, 1976; Sedmak & Grossberg, 1977). Lysosomal samples were diluted 10-fold in buffer and collected by centrifugation before protein determination in order to remove Percoll.

Lysosomal preparation

Sprague-Dawley female rats (150–200 g) were killed by decapitation, and their livers homogenized in 0.25 M-sucrose/1 mM-EDTA, adjusted to pH 7.0 with Tris base, with a Dounce homogenizer. The liver homogenate was suspended in 40 ml of the buffer and centrifuged for 10 min at 750 g. The nuclear pellet was discarded and the centrifugation was repeated. The supernatant was centrifuged for 10 min at 20000 g, yielding a pellet which was resuspended in 40 ml of the buffer and subjected to a second centrifugation. The final pellet containing the cellular organelles was suspended in 5.5 ml of 0.25 M-sucrose/20 mM-Hepes, pH 7.0, and mixed with 4.5 ml of Percoll/0.25 M-sucrose/20 mM-Hepes, pH 7.0. The organelle/Percoll mixture was centrifuged for 90 min at 40000 g. The dense lysosomal band near the bottom of the gradient, of approx. 600 μ l, was removed and diluted to 2 ml with Hepes/sucrose buffer. All steps after removal of the livers were performed at 4 °C.

Lysosomal amino acid loading

A sample (1 ml) of lysosomes was incubated with 20 μ M amino acid methyl ester derivative for 10 min at 25 °C. At the end of the incubation, 9 ml of ice-cold Hepes/sucrose buffer was added, and the lysosomes were collected by centrifugation for 10 min at 20000 g. The lysosomal pellet was immediately resuspended in Hepes/sucrose buffer containing 400 μ g of human serum albumin/ml. Lysosomes were mixed with cold methanol and assayed for conversion of the methyl ester derivative into the native amino acid, by t.l.c. before use in experiments.

Lysosomal efflux

Amino acid-loaded lysosomes (50 μ l) were mixed with 1 ml of buffered sucrose solution containing 400 μ g of human serum albumin/ml and incubated at room temperature in polypropylene micro-centrifuge tubes. Lysosomal material was collected by centrifugation for 2 min in an Eppendorf micro-centrifuge. The supernatant was removed by aspiration and the pellet broken by sonication in 500 μ l of 40 mM-sodium phosphate/citrate buffer, pH 4.4, with 200 μ g of human serum albumin/ml and 0.1% Triton X-100. Then 250 μ l of the sample was removed and counted for radioactivity in a Tracor scintillation counter by using Aquasol II (New England Nuclear). The remainder was used for assay of β -hexosaminidase activity. Lysosomal amino acid content was expressed as nmol/unit of β -hexosaminidase activity.

Lysosomal acidification

Lysosomal pH was determined as previously described by using lysosomes isolated from rats injected intraperitoneally with fluoresceinated dextran (M_r 70000) 24 h previously (Okhuma *et al.*, 1982). Measurements were performed with lysosomes directly obtained from Percoll

Fig. 1. Lysosomal amino acid efflux

Loss of leucine (●), cystine (▲), methionine (■) and tyrosine (▼) by rat liver lysosomes at 25 °C suspended in 0.25 M-sucrose/5 mM-MgCl₂/20 mM-Hepes, pH 7.0, containing 0.4 mg of human serum albumin/ml was measured without (a) or with (b) 2 mM-NaATP. Error bars indicate S.D.

gradients. The pH of cystine-loaded lysosomes was determined by exposing Percoll-purified lysosomes to 20 μ M-cystine dimethyl ester for 10 min at 25 °C, diluting them with cold buffer, and collecting them by centrifuging on a second Percoll gradient. Measurements of pH were performed with a Hitachi 650-10S fluorescence spectrophotometer.

Lysosomal membrane potential

Lysosomes were incubated in 0.25 M-sucrose/20 mM-Hepes, pH 7.0, at 25 °C in the presence of 25 nM-di-O-C₈ (Harikumar & Reeves, 1983; Seliyaman & Gallin, 1983). Membrane potential was monitored fluorimetrically by using an excitation wavelength of 460 nm and an emission wavelength of 510 nm. The presence of Percoll interfered with measurements on the addition of salts. In order to avoid artifacts, purified lysosomes were diluted 10-fold with buffer and collected by centrifugation to free them of Percoll before measurements.

RESULTS

Purification

Methyl ester derivatives of amino acids were at least 95% pure as assessed by t.l.c. Specific activity of β -hexosaminidase in lysosomal fractions revealed a purification of 89 ± 28 -fold (mean $\pm$ S.D., $n = 27$). Purification of neutral α -glucosidase was 0.24 ± 0.24 -fold and

Table 1. Nucleotides and cystine efflux

Lysosomes were incubated for 10 min at 25 °C in 0.25 M-sucrose/20 mM-Hepes, pH 7.0, with 0.4 mg of human serum albumin/ml and 5 mM-MgCl₂. Lysosomes contained 748 ± 72 (*n* = 6) pmol of cystine/unit of β-hexosaminidase at the start of experiments. Values represent means ± s.d. Data were evaluated by using Student's *t* test. Efflux was not stimulated by ATP in the absence of Mg²⁺.

Nucleotide	MgCl ₂	Efflux (pmol/unit per min)	<i>P</i>
None	5 mM	61 ± 4	—
2 mM-ATP	5 mM	108 ± 8	< 0.002
2 mM-ADP	5 mM	46 ± 12	> 0.1
2 mM-AMP	5 mM	52 ± 11	> 0.2
None	None	12 ± 4	< 0.001

that of succinate:INT reductase was 1.1 ± 1.7-fold in the lysosomal fractions. Lysosomes contained 1.8 ± 0.4 nmol of cystine/unit of β-hexosaminidase, 1.6 ± 0.6 nmol of leucine/unit of enzyme, 1.2 ± 0.3 nmol of methionine/unit of enzyme or 1.7 ± 0.9 nmol of tyrosine/unit of enzyme at the start of experiments. Over 95% of the radiolabelled lysosomal amino acid was in its unmethylated form at the start of experiments, as determined by t.l.c. Mg²⁺ caused lysosomes to bind to the polypropylene tubes, although even 5 mM-Mg²⁺ had no effect on lysosomal latency. Recovery of a visible lysosomal pellet was markedly enhanced by the presence of albumin when Mg²⁺ was used. Amino acid efflux was unchanged when experiments were performed either with or without albumin.

Amino acid efflux

Efflux of amino acids from lysosomes was determined by using lysosomes suspended in 0.25 M-sucrose/5 mM-MgCl₂/20 mM-Hepes, pH 7.0, with 400 μg of albumin/ml (Fig. 1a). The effluxes of cystine, methionine, tyrosine and leucine were similar under these circumstances. When 2 mM-NaATP was added to the buffer mixture, the efflux of cystine increased dramatically by 9 min, as analysed by Student's *t* test (*P* < 0.001), whereas the efflux of leucine (*P* > 0.2), methionine (*P* > 0.1) and tyrosine (*P* > 0.1)

Fig. 2. Effect of Mg²⁺ on cystine efflux

Loss of cystine at 25 °C by lysosomes suspended in 0.25 M-sucrose containing 0.4 mg of human serum albumin/ml and 40 mM-Hepes at pH 6.0 (▲), 7.0 (●) and 8.0 (■) was measured in the presence of Mg²⁺. Mg²⁺-independent efflux was subtracted from Mg²⁺-dependent efflux to give the values shown.

did not change significantly (Fig. 1b). In a similar study, basal cystine efflux was not enhanced when either ADP or AMP was substituted for ATP (Table 1).

Lysosomal amino acid efflux was examined over a 10 min interval in the presence of a variety of substances known to interfere with ATP-mediated lysosomal acidification (Okhuma *et al.*, 1982) (Table 2). The protonophore CCCP had no effect on amino acid efflux, except for ATP-stimulated cystine efflux. Quercetin was inhibitory for the efflux of all four amino acids. *N*-Ethylmaleimide, a thiol-modifying agent, had little effect on basal cystine efflux, although it was found to be an irreversible inhibitor of ATP-stimulated cystine efflux. Although the presence of *N*-ethylmaleimide inhibited the efflux of other amino acids, this inhibition was reversible. Basal cystine efflux was relatively insensitive to the presence of chloroquine, and was completely insensitive to the presence of NH₄Cl. However, ATP-stimulated cystine efflux was extremely sensitive to these lysosomal alkalinizing agents. Chloroquine and NH₄Cl caused

Table 2. Amino acid efflux and inhibitors

Lysosomes were incubated for 15 min at 25 °C in the same buffer as in Table 1. Cystine efflux was determined both with and without 2 mM-NaATP. Efflux without inhibitors was considered maximal efflux. Initial values (pmol/unit of β-hexosaminidase) were: cystine 2182 ± 81, leucine 2447 ± 159, methionine 915 ± 31 and tyrosine 2265 ± 48 (values are means ± s.d.).

Amino acid	Inhibitor...	Efflux (% of maximal)				
		10 μM-CCCP	100 μM-Quercetin	1 mM- <i>N</i> -Ethylmaleimide	250 μM-Chloroquine	10 mM-NH ₄ Cl
Leucine		99	53	69	57	85
Methionine		93	0	35	52	72
Tyrosine		92	1	49	35	57
Cystine		100	69	90	79	100
Cystine (MgATP-stimulated)		34	0	41	18	22

Fig. 3. Effect of pH on cystine efflux

Loss of cystine at 25 °C by lysosomes suspended in 0.25 M-sucrose containing 0.4 mg of human serum albumin/ml and 40 mM-Hepes at pH 6.0 (▲), 7.0 (●) and 8.0 (■) was measured. Error bars indicate s.d.

Table 3. Amino acid efflux versus pH

Efflux was determined over 15 min at 25 °C in 0.25 M-sucrose/40 mM-Hepes, with 0.4 mg of human serum albumin/ml adjusted with NaOH to the appropriate pH. Efflux is expressed as pmol/unit of β -hexosaminidase; values are means $\pm$ s.d.

pH	Efflux		
	Leucine	Methionine	Tyrosine
6.0	77 $\pm$ 2	55 $\pm$ 2	46 $\pm$ 4
7.0	63 $\pm$ 1	55 $\pm$ 2	36 $\pm$ 5
8.0	67 $\pm$ 5	60 $\pm$ 1	34 $\pm$ 4

moderate decreases in the efflux of tyrosine, leucine and methionine.

As indicated in Table 1, lysosomal cystine efflux was stimulated by the presence of Mg^{2+} . Lysosomes incubated at pH 7.0 in 0.25 M-sucrose/20 mM-Hepes with 400 μ g of human serum albumin/ml showed an increase in cystine efflux with increasing amounts of $MgCl_2$. In a

separate experiment, this effect was also seen when the buffer pH was 6.0, but was abolished when buffer pH was raised to 8.0. The results of these studies are shown in Fig. 2. The presence of Mg^{2+} had no effect on the efflux of leucine, methionine or tyrosine at pH 7.0 (results not shown).

Efflux of cystine was markedly affected by the extralysosomal pH. Lysosomes were placed in buffer containing 0.25 M-sucrose, 40 mM-Hepes and 400 μ g of human serum albumin/ml which had been adjusted to different pH values with NaOH. Cystine efflux was stimulated when lysosomes were placed in alkaline buffer and was inhibited as the buffer pH was lowered (Fig. 3). Similar results were obtained with Na^+ -free Tris-buffered solutions. When the buffer pH was 8.0, cystine efflux was inhibited by $50 \pm 15\%$ (mean $\pm$ s.d., $n = 9$) by 10 μ M-CCCP. Lysosomal efflux of leucine, methionine and tyrosine was not stimulated by increasing the extralysosomal pH (Table 3). Efflux of leucine and tyrosine was actually decreased as buffer pH was raised. Lysosomes pretreated at pH 7.0 with either valinomycin/CCCP/KCl or nigericin/KCl to abolish the proton gradient had diminished cystine efflux at pH 8.0 (Table 4).

Cystine efflux was determined in the presence of a variety of cations. At concentrations of 1 mM, efflux at pH 7.0 was stimulated approx. 1.5-fold by the presence of bivalent cations (Table 5); all cations were used as chloride salts. NaCl was not stimulatory at a concentration of 2 mM. Mg^{2+} - and Ca^{2+} -stimulated efflux was completely inhibited by the calcium ionophore A23187 (Table 6). A23187 had minimal effects on transport in the absence of added cations.

Measurement of lysosomal pH was performed with fluoresceinated-dextran-loaded lysosomes. The pH of isolated lysosomes was 5.4 ± 0.1 (mean $\pm$ s.d., $n = 4$), and decreased by 0.6 unit to 4.8 ± 0.1 (mean $\pm$ s.d., $n = 4$; $P < 0.001$) in the presence of 2 mM- $MgCl_2$ /ATP over 4 min at 25 °C in 0.25 M-sucrose/20 mM-Hepes, pH 7.0. The pH of cystine-loaded lysosomes was 5.6 ± 0.0 (mean $\pm$ s.d., $n = 4$) and decreased by 0.5 unit to 5.1 ± 0.1 (mean $\pm$ s.d., $n = 4$; $P < 0.001$) over 4 min in the presence of MgATP. ATP-stimulated acidification was blocked completely by the presence of 10 μ M CCCP. Ca^{2+} could not substitute for Mg^{2+} in supporting ATP-dependent lysosomal acidification. The pH of lysosomes was unaffected by either 5 mM- $MgCl_2$ or 5 mM- $CaCl_2$ in the absence of ATP. Nigericin and valinomycin had rapid

Table 4. Ionophores and cystine efflux

Cystine-loaded lysosomes were incubated for 5 min at 25 °C in 0.25 M-sucrose/20 mM-Hepes, pH 7.0, with the noted additions. After this incubation, lysosomes were collected by centrifugation and resuspended in Hepes/sucrose buffer containing human serum albumin at pH 8.0. Efflux was measured over a 15 min period. Cystine loading was 10–20% higher in lysosomes exposed to either valinomycin or nigericin compared with the other conditions. The efflux rate in KCl was 90% of the rate without KCl. Values represent means $\pm$ s.d. Data analysis was performed by Student's *t* test.

Pretreatment	Efflux (pmol/unit of β -hexosaminidase per min)	<i>P</i>
50 mM-KCl	27 $\pm$ 3	–
50 mM-KCl, 10 μ M-CCCP	28 $\pm$ 2	> 0.5
50 mM-KCl, 10 μ M-CCCP, 2.5 μ M-valinomycin	14 $\pm$ 2	< 0.001
50 mM-KCl, 2.5 μ M-nigericin	17 $\pm$ 5	< 0.002

Table 5. Cation-stimulated cystine efflux

Cystine-loaded lysosomes were incubated for 15 min at 25 °C in 0.25 M-sucrose/20 mM-Hepes, pH 7.0, containing 0.4 mg of human serum albumin/ml. Values represent means $\pm$ S.D. Data analysis was performed by Student's *t* test.

Cation	Efflux (pmol/unit of β -hexosamini- dase per min)	<i>P</i>
None	21 $\pm$ 1	—
1 mM-BaCl ₂	33 $\pm$ 1	< 0.001
1 mM-CaCl ₂	32 $\pm$ 1	< 0.001
1 mM-MgCl ₂	31 $\pm$ 0	< 0.001
1 mM-MnCl ₂	31 $\pm$ 0	< 0.001
1 mM-SrCl ₂	31 $\pm$ 1	< 0.001
2 mM-NaCl	21 $\pm$ 2	> 0.5

Table 6. Cation-stimulated cystine efflux and A23187

Cystine-loaded lysosomes were incubated in buffer as described in Table 5. Efflux in the presence of A23187 alone was approx. 85% of the unstimulated value. Values represent means $\pm$ S.D. Data analysis was performed by Student's *t* test.

Cation	Efflux (pmol/unit of β -hexosamini- dase per min)	<i>P</i>
None	19 $\pm$ 1	—
5 mM-MgCl ₂	38 $\pm$ 1	—
5 mM-CaCl ₂	35 $\pm$ 2	—
5 mM-MgCl ₂ /5 μ M-A23187	13 $\pm$ 5	< 0.001
5 mM-CaCl ₂ /5 μ M-A23187	6 $\pm$ 4	< 0.001

Table 7. Effects of A23187 on lysosomal pH

Fluoresceinated-dextran-loaded lysosomes were incubated in 0.25 M-sucrose/20 mM-Hepes, pH 7.0, at 25 °C as described in the Methods section. Additions comprised less than 1% of the total volume. Values represent means $\pm$ S.D. Data analysis was performed by Student's *t* test.

Addition	pH	<i>P</i>
None	5.7 $\pm$ 0.1	—
5 μ M-A23187	6.0 $\pm$ 0.0	< 0.001
5 μ M-A23187/1 mM-CaCl ₂	6.1 $\pm$ 0.0	< 0.001
5 μ M-A23187/5 mM-CaCl ₂	6.1 $\pm$ 0.1	< 0.001
5 μ M-A23187/1 mM-MgCl ₂	6.1 $\pm$ 0.1	< 0.001
5 μ M-A23187/5 mM-MgCl ₂	6.1 $\pm$ 0.0	< 0.001

alkalinizing effects that were dependent on the presence of KCl. The magnitude of alkalinization varied with KCl concentration (+0.4 pH unit with 20 mM-KCl). Lysosomes in buffer with KCl underwent slow alkalinization, which was accelerated by the presence of CCCP. These

Fig. 4. Effect of cations on the fluorescence quenching of di-O-C₆ by lysosomes

Lysosomes were added to cuvettes containing 1 ml of 0.25 M-sucrose/20 mM-Hepes, pH 7.0, with 25 nM-di-O-C₆. CaCl₂ or MgCl₂ were added as 10 μ l of a 500 mM solution. Final concentrations were: valinomycin, 2.5 μ M; KCl, 10 mM; A23187, 5 μ M.

findings are consistent with previous reports (Okhuma *et al.*, 1982). The presence of A23187 resulted in lysosomal alkalinization, an effect which was blocked by the presence of 1 mM-EGTA. This alkalinization was probably secondary to the presence of contaminating cation. Prompt lysosomal alkalinization was observed with A23187 in the presence of either 1 mM- or 5 mM-CaCl₂. Similar results were obtained with this ionophore and either 1 mM- or 5 mM-MgCl₂, although the rate of alkalinization was approximately half that observed with Ca²⁺ (Table 7). A23187 had no effects on lysosomal stability or the fluorescence spectrum of fluoresceinated dextran.

Lysosomal membrane-potential measurements performed using the cyanine dye di-O-C₆ were similar in cystine-filled and in unloaded lysosomes. Administration of either Ca²⁺ or Mg²⁺ resulted in a decreased membrane potential. This potential was increased by the presence of either 2.5 μ M-valinomycin/10 mM-KCl or 5 μ M-A23187 (Fig. 4).

DISCUSSION

Cystine transport in rat liver lysosomes is stimulated both by Δ pH and by the presence of bivalent cations. In fact, what was first identified as basal cystine efflux from lysosomes actually represented cation-stimulated efflux, as this work was performed in the presence of Mg²⁺. Transport can be maximally driven by one condition without an additive effect by the second component. Such is the case when Δ pH is very large. Under these conditions, the addition of Mg²⁺ does not further stimulate cystine efflux. This observation serves as an argument against the existence of two lysosomal cystine-transport systems, one sensitive to cations and the other dependent on lysosomal ATPase. Artificially imposed lysosomal pH gradients mimic the effects of ATP on transport, suggesting that lysosomal ATPase is only secondarily involved in cystine transport. Predictably,

alkalinization of lysosomes through the use of ionophores or weak bases results in decreased cystine transport.

The cationophore A23187 permits exchange of bivalent cations for H^+ , resulting in equilibration of cation concentrations across the lysosomal membrane. Interestingly, this lessens all driving forces for cystine transport. A23187 has minimal effects on transport in the absence of added bivalent cations, suggesting that this ionophore does not directly inhibit the cystine carrier. The effect of A23187 on cation-stimulated cystine transport was not as dramatic when Mg^{2+} was substituted for Ca^{2+} . A23187 is more effective as an ionophore for Ca^{2+} than for other cations, which is consistent with the experimental findings. The particular effectiveness of A23187 in decreasing lysosomal cystine efflux may be related not only to its effects on lysosomal pH but to its ability to permeabilize the lysosome to bivalent cations.

Since cystine transport can be driven by either ΔpH or by extralysosomal bivalent-cation concentration, it is attractive to assume that cystine transport is linked to the electrochemical potential. However, the effects of bivalent-cation concentration are difficult to interpret, as Ca^{2+} and Mg^{2+} have no effect on ΔpH and cause a decrease in membrane potential. These results can be explained if bivalent cations decrease the binding of cystine to the cystine transporter. Thus cystine would be released by the transporter in response to external cation concentrations. When lysosomes are made permeable to cations, as with the ionophore A23187, cystine cannot be effectively bound and transported out of the lysosome. Alternatively, it is possible that cystine binding to the transporter is modulated by both pH and bivalent cations, or that small amounts of cation diffuse into the lysosome and exchange with bound protons, freeing them for transport processes.

In many respects, lysosomal cystine transport is similar to the transport of amines by another vesicular structure, the chromaffin granule. Amine transport in chromaffin granules responds to either membrane potentials or proton gradients (Knoth *et al.*, 1982). Proton-gradient-stimulated transport in these structures may be elicited by manipulation of external buffer pH or via internal acidification through the action of an ATPase (Johnson *et al.*, 1982). It is interesting to speculate on similar transport mechanisms in other cellular vesicular structures such as peroxisomes.

The effects of ΔpH and cations must be considered when evaluating cystine transport in different tissues. Earlier studies of cystine countertransport have been performed in the presence of *N*-ethylmaleimide to decrease thiol/disulphide interactions. This agent irreversibly blocks proton translocation mediated by lysosomal ATPase (Okhuma *et al.*, 1982). Thus, under these conditions, Mg^{2+} would potentiate transport, whereas ATP would not have any effect on transport. In studies of lymphoblast lysosomes, *N*-ethylmaleimide was not used, and cystine efflux was stimulated by ATP through changes in ΔpH .

Differences in purification procedures may play a role in producing disparate observations. Extensive purification procedures would more effectively remove bivalent cations from the preparations, especially if chelating agents are employed. In addition, separation of lysosomes from mitochondria would decrease possible contamination of the preparation with adenine nucleotides. The

effects of ΔpH and cations may be more easily differentiated in highly purified lysosomal preparations.

An additional consideration in comparing studies of lysosomes from different tissues is that lysosomes may have different tissue-specific proton permeabilities. This, in part, may have a bearing on the observations noted by Gahl & Tietze (1985) in studies of cystine efflux from leucocytes. Lysosomes from some sources may become more readily alkalinized during purification procedures, resulting in decreased ΔpH . This is one possible explanation for the finding that highly purified lymphoblast lysosomes have a higher pH than purified rat liver lysosomes, despite similar isolation procedures. Isolation and reconstitution of the cystine carrier may ultimately be necessary to resolve some of these tissue specific differences in transport.

The lysosomal efflux of leucine, methionine and tyrosine is not stimulated by large lysosomal proton gradients or bivalent cations, and most probably takes place via a different mechanism. Tyrosine efflux actually decreased by 25% when ΔpH was changed from 0.6 to 1.6. It is noteworthy that the efflux of these amino acids may be inhibited under a variety of conditions, suggesting that efflux of these amino acids involves more than simple diffusion. Further work will be required both to establish their means of lysosomal egress and to establish whether substances other than cystine are transported in response to ΔpH and bivalent cations.

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