ATP stimulates lysosomal sulphate transport at neutral pH: evidence for phosphorylation of the lysosomal sulphate carrier

Hsu-Fang CHOU, Merry PASSAGE and Adam J. JONAS¹

Division of Medical Genetics, Department of Pediatrics, Harbor-UCLA Medical Center, 1124 West Carson Street, Torrance, CA 90502, U.S.A.

ATP markedly stimulated sulphate uptake by rat liver lysosomes that had been treated with *N*-ethylmaleimide to block the effects of the lysosomal proton-translocating ATPase (H^+ -ATPase). Maximal stimulation required millimolar concentrations of ATP and neutral buffer pH. ATP-stimulated transport exhibited saturation kinetics with a K_m of 175 μ M, identical with the K_m for lysosomal sulphate uptake at pH 5.0, a process that does not require ATP. The requirement for ATP was specific: other nucleotides such as AMP, ADP, CTP, GTP, ITP and UTP failed to stimulate transport. Adenosine 5'-[β,γ -imido]triphosphate, the non-hydrolysable analogue of ATP, also failed to stimulate

sulphate uptake, suggesting a requirement for ATP hydrolysis. Lysosomal pH, membrane potential and glucose transport were unchanged by the presence of ATP under the experimental conditions, consistent with a direct effect of ATP on the sulphate transporter. Exposure of lysosomes to protein kinase A and protein kinase C inhibitors did not alter the stimulation of sulphate transport by ATP. The lysosomal sulphate transport protein might be subject to regulation by a phosphorylation pathway that is not dependent on protein kinase A or protein kinase C.

INTRODUCTION

Adenine nucleotides are recognized for their roles in the regulation of transport systems found in the plasma membrane and the membranes of intracellular organelles such as mitochondria. The role of adenine nucleotides is straightforward in some systems where the hydrolysis of ATP provides the energy necessary for active transport. Different mechanisms are responsible for the effects of adenine nucleotides on other systems. Some transport proteins have binding sites for ATP that when occupied result in conformational changes that alter the rate of flux. Such binding does not necessarily require ATP hydrolysis or phosphorylation of the carrier. Glucose transport by red cell ghosts serves as an example of this type of process in that the binding of intracellular ATP results in a decreased rate of transport [1,2]. Sometimes the effect of ATP is complex as in cystic fibrosis transmembrane regulator-mediated chloride transport, where both ATP binding and hydrolysis are essential for opening the chloride channel [3].

Although a number of distinct transport systems have been described in lysosomes from a variety of tissues including rat liver and human fibroblasts [4], relatively little is known about their regulation and structure. There is evidence to suggest that ATP has a role in the regulation of many of these systems, most often through the action of the membrane bound lysosomal H^+ -ATPase, which maintains the normally acidic lysosomal internal environment through the translocation of protons [5]. Proton transfer by this system has effects on the transport of sulphate [6], cystine [7], lysine [8], arginine [8] and taurine [9], either through maintenance of acidic pH or regulation of the electrochemical potential. Adenine nucleotides seem to be non-competitive inhibitors of phosphate transport by human fibroblast lysosomes. Phosphate transport is inhibited by many phosphate-containing compounds including the nucleotides ATP, ADP, CTP and non-hydrolysable ATP analogues [10]. Additional mechanisms of action might be involved in the effects of ATP on other lysosomal

membrane transport systems such as the Ca^{2+} uptake pump of human neutrophil lysosomes, which seems to be directly activated by $MgATP^{2-}$ [11]. There might be some tissue specificity to these systems because Na_2ATP^{2-} , $MgATP^{2-}$ and H_2ATP^{2-} all inhibit Ca^{2+} uptake by human fibroblast lysosomes [12]. A chloride channel has been described in rat liver lysosomes that is apparently activated not only by ATP but also by non-hydrolysable ATP analogues [13], consistent with a mechanism of allosteric alteration as a consequence of the binding of adenine nucleotides [13].

We have previously described a sulphate transport system that is distinct from other lysosomal anion transport systems and allows for the efflux of sulphate derived from the disassembly of glycosaminoglycans and sulpholipids. This system exhibits saturation kinetics, is inhibited by 4,4'-di-isothiocyanatostilbene-2,2'-disulphonate (DIDS) as well as many other known inhibitors of anion-transport systems and has a requirement for acidic pH for optimal function [6]. Because the action of the H^+ -ATPase maintains an acidic internal lysosomal environment with a more alkaline exterior, this should favour unidirectional transport and the egress of sulphate from lysosomes. Whether adenine nucleotides have a more direct role in the regulation of lysosomal sulphate transport was uncertain, prompting us to perform the current studies. Our results indicate that ATP has a significant effect on sulphate transport that is independent of the action of the lysosomal H^+ -ATPase.

MATERIALS AND METHODS

Materials

All chemicals were purchased from Sigma unless indicated. Dulbecco's PBS without Ca^{2+} was obtained from Irvine Scientific, acrylamide and bis-acrylamide from Boehringer Mannheim Biochemicals, 2-mercaptoethanol from Bio-Rad, 3,3'-dihexyloxycarbocyanine iodide (di-O-C₆) from Aldrich, Scintiverse II from Fisher, X-Omat AR-5 film from Kodak, and GFC/GFF glass

Abbreviations used: DIDS, 4,4'-di-isothiocyanatostilbene-2,2'-disulphonate; di-O-C₆, 3,3'-dihexyloxycarbocyanine iodide; NEM, *N*-ethylmaleimide; p[NH]ppA, adenosine 5'-[β,γ -imido]triphosphate.

¹ To whom correspondence should be addressed.

fibre filters from Whatman. $\text{Na}_2^{35}\text{SO}_4$ (1130 mCi/ml) and D-[U- ^{14}C]glucose (250 mCi/mmol) were from ICN Biochemicals, and [γ - ^{32}P]ATP (3000 mCi/mmol) was from NEN-Dupont. Female Sprague-Dawley rats weighing 190–250 g were obtained from Harlan.

Isolation of lysosomes

Rats were injected with 1000 i.u. of heparin into the tail vein and killed 15 min later by CO_2 narcosis. Livers were removed and flushed with ice-cold saline containing 1 mM EDTA to remove blood and then homogenized, with a Dounce homogenizer, in 0.25 M sucrose containing 1 mM EDTA. Purified lysosomes were obtained by differential centrifugation followed by Percoll density gradient centrifugation with 45% (v/v) buffered Percoll as previously described [14]. The dense lysosomal fraction was removed from the gradient, washed with ice-cold 0.25 M sucrose/2 mM Hepes (pH 7.0) and resuspended in the same buffer.

Formation of membrane vesicles

The lysosomal fraction (approx. 1 ml) collected from the Percoll gradient was incubated for 15 min at 25 °C in 0.25 M sucrose/20 mM Hepes/2 mM MgCl_2 (pH 7.0) containing 2 $\mu\text{g}/\text{ml}$ leupeptin, 1 $\mu\text{g}/\text{ml}$ pepstatin, 500 μM PMSF, 2 mg/ml BSA and 5 mM methionine methyl ester. Lysosomes rupture owing to the accumulation of methionine and spontaneously reseal to form membrane vesicles. The resulting membrane vesicles were mixed with an equal volume of ice-cold buffered Percoll and collected by centrifugation at 20000 *g* for 15 min. The banded vesicles were removed from the top of the gradient, washed and resuspended in 0.25 M sucrose/2 mM Hepes (pH 7.0) [6].

Assay of sulphate and glucose transport

Sulphate transport was assayed as described previously [15]. Briefly, 15 μl of lysosomes (40–80 μg of protein) or membrane vesicles (20–40 μg of protein) were incubated at 25 °C in a total volume of 45 μl of buffered 0.25 M sucrose/100 μM Na_2SO_4 containing 2–4 μCi of $\text{Na}_2^{35}\text{SO}_4$ for 30–60 s. The assay mixture was buffered with either 20 mM Mes, pH 5.0, or 20 mM Hepes, pH 7.0, as indicated. For kinetic studies the Na_2SO_4 concentration in each sample was varied from 25 to 1000 μM and the initial rate of sulphate uptake was measured for 30 s. After incubation, transport was stopped by the addition of 1 ml of ice-cold PBS without Ca^{2+} . Lysosomes and membrane vesicles were collected by filtration through GFC or GFF glass fibre filters respectively, which were immediately washed with 10 ml of ice-cold stop solution. Filters were dried and subjected to scintillation counting. Glucose uptake was determined with an assay mixture containing 0.25 sucrose, 20 mM Hepes, pH 7.0, 10 mM D-glucose and 2 μCi of D-[U- ^{14}C]glucose. Incubation times were varied from 15 s to 5 min as indicated. Sulphate uptake was expressed as pmol of sulphate per incubation time per unit of β -hexosaminidase for lysosomes and pmol of sulphate per incubation time per mg of protein for vesicles. Glucose uptake was expressed as nanomoles glucose/unit β -hexosaminidase/incubation time. β -Hexosaminidase activity was determined fluorometrically [16]. A unit of activity was defined as 1 μmol of product formed/min at 37 °C.

Measurement of membrane potential

Lysosomal membrane potential was determined with the carbo-cyanine dye di-O-C₆. Density gradient-purified lysosomes (1 ml)

were washed with 10 ml of ice-cold 0.25 M sucrose/20 mM Hepes (pH 7.0) and collected by centrifugation at 20000 *g* for 10 min to remove the Percoll. The pellet was resuspended in this buffer at a protein concentration of approx. 10 mg/ml. Lysosomes (10 μl) were added to a cuvette containing 2 ml of buffer with 25 nM di-O-C₆ at 25 °C. After a period of incubation to allow the fluorescence to reach equilibrium, various chemicals were added as indicated. Fluorescence was recorded in a spectrofluorometer with an excitation wavelength of 460 nm and an emission wavelength of 510 nm [17].

RESULTS

Lysosomal sulphate transport was examined in the presence of various nucleotides. Although sulphate uptake by lysosomes is optimal at a buffer pH of 5.0, studies were performed at a buffer pH of 7.0 because this is more favourable for the metabolism of nucleotides. Under these conditions the unstimulated rate of sulphate uptake is approx. 10% of that observed at an optimal acidic pH. Most nucleotides had little or no effect on sulphate transport at neutral pH with the exception of MgATP^{2-} , which increased transport approx. 2-fold (Table 1). To exclude the possibility that the effects of MgATP^{2-} were mediated by the lysosomal H^+ -ATPase, lysosomes were incubated with *N*-ethylmaleimide (NEM) before the addition of ATP. NEM, which covalently binds thiol groups, is a recognized inhibitor of proton translocation by the lysosomal H^+ -ATPase [18,19]. Treatment with NEM had no effect on baseline sulphate uptake at neutral pH. However, sulphate uptake increased by 3–4-fold in this experiment when NEM-treated lysosomes were exposed to either MgATP^{2-} or $\text{Na}_2\text{ATP}^{2-}$ (Table 2). Stimulation of transport was dependent on ATP concentration, with a maximum effect achieved at approx. 1 mM ATP (Figure 1). All subsequent studies were therefore performed with this concentration of nucleotide.

Because the stimulation of lysosomal sulphate transport by ATP was observed only under specific conditions, a series of studies were performed to determine whether this represented a real biological effect as opposed to an artifact. The first study was to determine whether the observed stimulation of transport

Table 1 Nucleotides and lysosomal sulphate uptake at pH 7.0

Isolated lysosomes were incubated for 1 min at 25 °C in 0.25 M sucrose/20 mM Hepes (pH 7.0) containing 100 μM Na_2SO_4 , 2 μCi of $\text{Na}_2^{35}\text{SO}_4$ and 1 mM nucleotides as indicated. Lysosomes were collected by filtration on glass fibre filters as described in the Materials and methods section. Results are means $\pm$ S.D. for triplicate samples. Abbreviation: ATP[S], adenosine 5' [γ -thio]triphosphate.

Nucleotide	Sulphate uptake	
	(pmol/min per unit of β -hexosaminidase)	(% of control)
None	6.1 $\pm$ 1.1	100
MgATP^{2-}	12.6 $\pm$ 1.0	207 $\pm$ 15
$\text{Na}_2\text{ATP}^{2-}$	8.4 $\pm$ 1.0	137 $\pm$ 11
Na_2ADP^-	5.6 $\pm$ 1.0	92 $\pm$ 17
AMP	5.9 $\pm$ 0.1	97 $\pm$ 1
p[NH]ppA	4.6 $\pm$ 0.4	76 $\pm$ 7
ATP[S]	5.4 $\pm$ 1.0	89 $\pm$ 14
CTP	5.0 $\pm$ 0.1	83 $\pm$ 2
GTP	5.7 $\pm$ 1.0	93 $\pm$ 17
ITP	4.7 $\pm$ 0.1	78 $\pm$ 6
UTP	5.5 $\pm$ 1.0	90 $\pm$ 10

Table 2 Effect of nucleotides and nucleotide analogues on sulphate transport by lysosomes treated with NEM

Isolated lysosomes were incubated with 5 mM NEM for 10 min at 25 °C, washed and resuspended in 0.25 M sucrose/20 mM Hepes (pH 7.0). Transport was assayed as previously described except that each assay tube contained 4 μ Ci of $\text{Na}_2^{35}\text{SO}_4$. Values are means $\pm$ S.D. for triplicate determinations. Statistical analysis was performed with Student's *t* test; *P* > 0.05 is taken as not significant (n.s.). The actual rate of uptake was 4.1 ± 0.4 pmol/min per unit of β -hexosaminidase.

Addition	Sulphate uptake (% control)	<i>n</i>	<i>P</i>
None	100	4	
1 mM MgATP ²⁻	178 $\pm$ 38	3	< 0.02
1 mM Na ₂ ATP ²⁻	117 $\pm$ 3	3	n.s.
5 mM NEM	90 $\pm$ 17	4	n.s.
+ 1 mM MgATP ²⁻	322 $\pm$ 125	3	< 0.02
+ 1 mM Na ₂ ATP ²⁻	424 $\pm$ 136	3	< 0.005
+ 1 mM Na ₂ ADP ⁻	80 $\pm$ 6		< 0.05
+ 1 mM AMP	70 $\pm$ 8		< 0.02
+ 1 mM p[NH]ppA	85 $\pm$ 30		n.s.
+ 1 mM CTP	68 $\pm$ 7		< 0.05
+ 1 mM GTP	115 $\pm$ 34		n.s.
+ 1 mM ITP	99 $\pm$ 29		n.s.
+ 1 mM UTP	66 $\pm$ 20		n.s.
+ 20 μ M Cibacron Blue 3GA	29 $\pm$ 8		< 0.001
+ 20 μ M Erythrosine B	44 $\pm$ 15		< 0.005

Figure 1 Effect of ATP concentration on sulphate uptake by lysosomes treated with NEM

Lysosomes were incubated with 5 mM NEM for 10 min at 25 °C in 0.25 M sucrose/20 mM Hepes (pH 7.0). Sulphate uptake over a 1 min interval was determined as described in the Materials and methods section. Uptake was expressed as pmol/min per unit of β -hexosaminidase. Values are means $\pm$ S.D. for triplicate determinations.

could be reproduced with other nucleotides. Only ATP had a stimulatory effect on sulphate transport by NEM-treated lysosomes, whereas all other nucleotides tested had either no effect or were slightly inhibitory (Table 2). This was true for other adenine nucleotides as well as the triphosphate nucleotides CTP, GTP, ITP and UTP. Stimulation of transport is thus a specific effect of ATP. Because the non-hydrolysable ATP analogue adenosine 5'-[β , γ -imido]triphosphate (p[NH]ppA) failed to stimulate sulphate uptake, it seems that hydrolysis is required for stimulation by ATP. Cibacron Blue 3GA and erythrosin B, which are known to compete for ATP binding sites and can stimulate systems

Figure 2 pH dependence of ATP stimulation of sulphate transport

The pH dependence of sulphate transport was determined for control lysosomes (A) and lysosomes that had been exposed to 5 mM NEM (B). After incubation for 10 min at 25 °C in 0.25 M sucrose/20 mM Hepes in the presence or absence of 5 mM NEM, lysosomes were washed with 0.25 M sucrose and collected by centrifugation. Lysosomes were resuspended in 0.25 M sucrose/20 mM Mes/Tris at the indicated pH and preincubated with (●, ▼) or without (○, ▽) 1 mM Na₂ATP²⁻ for 30 s at 25 °C. Sulphate uptake was initiated by the addition of a cocktail to bring the final assay mix to 0.25 M sucrose/20 mM Mes/Tris/100 μ M Na₂SO₄ with 4 μ Ci of Na₂³⁵SO₄. Sulphate transport was assayed as described for Figure 1. Values are means of triplicate measurements.

dependent on such binding [20], caused a 60–70% inhibition of sulphate transport. However, because the structures of these inhibitors include anionic groups, it is possible that a different mechanism of action accounts for their inhibitory effects on transport.

The effect of pH was examined because pH sensitivity would be another characteristic of a biological process. As previously reported, sulphate transport by control lysosomes is dependent on pH and is optimal at acidic pH, whereas transport is decreased at more alkaline pH. Transport was also optimal at acidic pH for lysosomes that were exposed to either ATP or NEM alone (Figures 2A and 2B). However, sulphate transport was optimal at neutral pH for lysosomes that had been exposed to NEM and then incubated with ATP (Figure 2B). Stimulation of transport by ATP was decreased at more acidic or alkaline buffer pH. Interestingly, the rate of ATP-stimulated transport at pH 7.0 was similar to the rate found in control lysosomes at pH 5.0.

Time-course studies were performed to contrast the effects of

Figure 3 Time course of sulphate (A) and glucose (B) uptake by lysosomes treated with NEM and ATP

Control lysosomes (○, ●) and lysosomes that had been exposed to 5 mM NEM (▽, ▼) were incubated in 0.25 M sucrose/20 mM Hepes (pH 7.0) at 25 °C. Incubations were performed in the absence (○, ▽) or presence (●, ▼) of 1 mM $\text{Na}_2\text{ATP}^{2-}$. Sulphate uptake (A) was determined as described in the Materials and methods section and was expressed as pmol/unit β -hexosaminidase. Values are the means of triplicate determinations. Glucose uptake (B) was determined as described in the Materials and methods section and was expressed as nmol/unit β -hexosaminidase. Values are the means of triplicate determinations.

ATP on the uptake of sulphate (Figure 3A) and glucose (Figure 3B), which are mediated by different lysosomal transport systems. Sulphate uptake by control lysosomes, lysosomes treated with NEM alone and lysosomes treated with $\text{Na}_2\text{ATP}^{2-}$ alone all exhibited similar slow rates of sulphate uptake at pH 7.0. The only condition that resulted in increased transport was when NEM-treated lysosomes were exposed to ATP with continued accumulation of sulphate over the 5 min incubation period. In contrast, lysosomal glucose uptake was virtually identical under the different experimental conditions and was largely complete by 1 min of incubation. Thus the stimulatory effect of ATP is not the consequence of a generalized phenomenon such as lysosomal swelling but has specificity for sulphate transport.

An analysis of the kinetics of ATP-stimulated sulphate uptake was undertaken to allow comparison with the previously reported characteristics of the lysosomal sulphate-transport system. Sulphate uptake by NEM-treated lysosomes that were exposed to ATP exhibited saturated kinetics consistent with transport by a single system with an estimated K_m of 175 μM (Figures 4A and

Figure 4 Kinetics of ATP-stimulated sulphate uptake

Lysosomes were incubated with 5 mM NEM for 10 min at 25 °C in 0.25 M sucrose/20 mM Mes/Tris (pH 7.0) before the addition of 1 mM $\text{Na}_2\text{ATP}^{2-}$. After a 30 s incubation with ATP, a cocktail was added to bring the incubation mixture to the indicated sulphate concentration (S) with 4 μCi of $\text{Na}_2^{35}\text{SO}_4$ per assay point. Transport was determined over a 30 s interval as described in the Materials and methods section. Values are means of triplicate measurements. Michaelis-Menten (A) and Hanes plots (B) of the data are presented. V_{max} = 28 pmol/30 s per unit of β -hexosaminidase; K_m = 175 μM .

4B). These results are similar to those obtained from a study of sulphate transport by control lysosomes (K_m 215 μM) at a buffer pH of 5.0 [15]. Similarly to its effects on sulphate transport at pH 5.0, ATP-stimulated sulphate transport was inhibited by 100 μM DIDS. Sulphate uptake decreased by more than 70 % from 14.4 ± 1.8 to 4.2 ± 1.9 pmol/30 s per unit of β -hexosaminidase.

Previous studies have indicated that changes in membrane potential might play a role in sulphate transport by lysosomal membrane vesicles [6]. To rule out changes in membrane potential under the experimental conditions, studies were performed with the cyanine dye di-O-C₆. MgATP^{2-} -induced changes in lysosomal membrane potential as seen in Figure 5(A) were completely ablated when lysosomes were first exposed to 5 mM NEM before the addition of MgATP^{2-} (Figure 5B). Thus the stimulation of sulphate transport by ATP was not mediated by altering membrane potential. $\text{Na}_2\text{ATP}^{2-}$ had no effect on membrane potential without (Figure 5C) or with (Figure 5D) NEM. Sulphate itself had no measurable effect on membrane potential. Treatment with NEM had no effect on valinomycin/KCl-induced

Figure 5 Lysosomal membrane potential

Lysosomes were incubated at 25 °C in a cuvette containing 2 ml of 0.2 M sucrose, 20 mM Hepes, pH 7.0, and 25 nM di-O-C₆. Fluorescence was determined as described in the Materials and methods section. An equilibration period was allowed before measurements were taken. Additions are indicated by vertical arrows. Final concentrations were 1 mM MgATP²⁻, 1 mM Na₂ATP²⁻, 5 mM NEM, 100 μM Na₂SO₄, 2.5 μM valinomycin and 25 mM KCl. The horizontal arrow indicates a 2 min interval. (A) Effect of MgATP²⁻; (B) effect of MgATP²⁻ with NEM; (C) effect of Na₂ATP²⁻; (D) effect of Na₂ATP²⁻ with NEM.

Table 3 Effect of protein kinase inhibitors on ATP-stimulated sulphate uptake in NEM-treated lysosomes

Isolated lysosomes were treated with 5 mM NEM in the presence or absence of protein kinase inhibitors for 5 min at 25 °C. Lysosomes were then incubated in the presence or absence of ATP for 30 s. Sulphate transport was determined as described previously. Values are means ± S.D. for triplicate determinations.

Condition	Sulphate uptake (pmol/30 s per unit of β-hexosaminidase)
Control	3.8 ± 0.1
1 μM protein kinase C inhibitor	4.9 ± 2.8
20 μg of protein kinase A inhibitor	3.7 ± 0.9
1 mM ATP	8.1 ± 0.4
+ 1 μM protein kinase C inhibitor	8.4 ± 0.8
+ 20 μg of protein kinase A inhibitor	7.3 ± 0.4

membrane potential changes, indicating the viability of the lysosomal preparation and serving as an internal control for the measurement of membrane potential (Figures 5B, 5C and 5D). Studies by us and others with lysosomes filled with fluoresceinated dextran have demonstrated that NEM inhibits ATP-mediated changes in lysosomal pH [5,21].

The mechanism of action of ATP seems to involve hydrolysis as suggested by the failure of non-hydrolysable ATP derivatives to stimulate transport. Studies were therefore performed to determine whether phosphorylation is involved in the stimulation of transport. Incubation of lysosomes with inhibitors of protein kinase A or protein kinase C had no consistent effects on stimulation of transport by ATP (Table 3) but this does not rule

out the possible involvement of other kinases or autophosphorylation.

DISCUSSION

A number of our findings suggest that under proper circumstances, lysosomal sulphate transport is regulated by an ATP-dependent process. The stimulatory effect of ATP is dependent on time and is optimal at neutral pH, as might be expected for a process involving the cytoplasmic side of the lysosomal membrane. Several observations point to the specificity of the ATP effect. The first of these is that lysosomal glucose transport, which requires a completely different carrier from that involved in sulphate transport, is unaffected by conditions that lead to stimulation of sulphate transport. Secondly, under the experimental conditions employed in our studies, lysosomal pH and membrane potential have been eliminated as environmental factors that might lead to alteration of transport. Finally, ATP is the only nucleotide capable of stimulating transport. Two mechanisms of action that have been observed in other transport systems are phosphorylation of the carrier and/or occupation of a specific binding site with ATP. However, the fact that the non-hydrolysable derivative p[NH]ppA cannot substitute for ATP argues against a mechanism of action dependent on simple interaction with an ATP-binding site. Rather, the results are more suggestive of a process that involves ATP hydrolysis, although this could also be coupled to interaction with a binding site as occurs with cystic fibrosis transmembrane regulator-mediated chloride transport.

Although it is possible that ATP-dependent sulphate transport involves a different carrier from that involved in the previously

described pH-dependent sulphate transport system, the evidence suggests that this is not so. The K_m values for sulphate transport are identical for ATP-stimulated and pH-dependent transport, as are the optimal rates of transport, consistent with a single transport system that is operable under different conditions. In addition, there is no indication of the presence of more than one transport system on analysis of the kinetic data for ATP-stimulated transport. Finally, transport is inhibited by DIDS under both circumstances. Because sulphate transport is not stimulated by non-hydrolysable ATP analogues, it seems unlikely that activation of the lysosomal chloride channel is responsible for ATP-stimulated sulphate transport. The physiological role of sulphate transport into lysosomes is uncertain. There are no known intra-lysosomal synthetic processes requiring free sulphate; significant amounts of free sulphate are already produced by normal degradation of macromolecules. Finally, ATP-stimulated uptake of sulphate requires thiol blockade for maximal effect, an artificial condition. It remains possible that such transport permits the lysosomal storage of sulphate under oxidizing conditions. However, it is likely that such transport does not have a true physiological role.

The reason that NEM is required for observation of the ATP effect is uncertain. It is interesting to note that an interaction between NEM and ATP has been demonstrated for other membrane transport systems. For instance, ATP stimulates K^+ flux in low- K^+ sheep erythrocytes [22] and human red cell ghosts [23] that have been treated with NEM. It has also been shown that ATP-dependent inhibition of potassium uniport by rat liver mitochondria is enhanced when mitochondria have been treated with NEM [24]. However, the mechanisms responsible for these interactions are unknown. In lysosomal sulphate transport it is possible that activity of the H^+ -ATPase with subsequent changes in lysosomal membrane potential and the lysosomal proton gradient masks the effect of ATP on transport. Thus exposure to NEM would inhibit the H^+ -ATPase and permit the observation of stimulation of transport by ATP. However, it seems that the story is more complex, as sulphate transport is not stimulated when lysosomes are exposed to Na_2ATP^{2-} even though the H^+ -ATPase, which has a requirement for Mg^{2+} ion, is inactive. Although NEM by itself seems to have little effect on sulphate transport at pH 7.0, we have found that it and other thiol-binding reagents have an inhibitory effect on transport at pH 5.0 by causing a marked alteration of K_m (H. F. Chou, M. Passage and A. J. Jonas, unpublished work). Thus NEM not only inhibits the H^+ -ATPase but also interacts directly with the sulphate transporter. It is possible that thiol binding by this reagent results in conformational changes that make the carrier susceptible to phosphorylation either through autophosphorylation or the action of a phosphorylase in the lysosomal membrane. Many other possibilities exist, including the activation of a nearby phosphorylase by NEM.

The experimental evidence points towards a mechanism of phosphorylation of the sulphate carrier. Not only are many

lysosomal membrane proteins phosphorylated under conditions similar to those used for the assay of ATP-stimulated sulphate transport, but the exposure of lysosomal membrane vesicles to NEM results in a change in protein phosphorylation patterns (results not shown). This would be consistent with our observations on sulphate transport. Although alteration of the pattern of phosphorylation could be due to a phenomenon such as the inhibition of thiol-sensitive proteases, it is also possibly due to allosteric changes that alter susceptibility to phosphorylation. Although ATP-stimulated sulphate transport is unaffected by the presence of inhibitors of either protein kinase A or protein kinase C, this does not rule out the involvement of other kinases or a mechanism of autophosphorylation.

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