Biochimica et Biophysica Acta 854 (1986) 102–108
Elsevier

BBA 72876

Similarity of lysosomal H^+-ATPase to mitochondrial F_0F_1-ATPase in sensitivity to anions and drugs as revealed by solubilization and reconstitution

Yoshinori Moriyama, Tatsuya Takano and Shoji Ohkuma *

Department of Microbiology and Molecular Pathology, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01 (Japan)

(Received July 23rd, 1985)

Key words: F_0F_1-ATPase; H^+-ATPase; Membrane reconstitution; Anion sensitivity; (Rat liver)

Lysosomal H^+-translocating ATPase (H^+-ATPase) was solubilized with lysophosphatidylcholine and reconstituted into liposomes (Moriyama, Y., Takano, T. and Ohkuma, S. (1984) J. Biochem. (Tokyo) 96, 927–930). In this study, the sensitivities of membrane-bound, solubilized and liposome-incorporated ATPase to various anions and drugs were measured in comparison with those of similar forms of mitochondrial H^+-ATPase (mitochondrial F_0F_1-ATPase) with the following results. (1) Bicarbonate and sulfite activated solubilized lysosomal H^+-ATPase, but not the membrane-bound ATPase or ATPase incorporated into liposomes. All three forms of mitochondrial F_0F_1-ATPase were activated by these anions. (2) All three forms of both lysosomal H^+-ATPase and mitochondrial F_0F_1-ATPase were strongly inhibited by SCN^-, NO_3^- and F^-, but scarcely affected by Cl^-, Br^- and SO_4^{2-}. (3) The solubilized lysosomal H^+-ATPase was strongly inhibited by azide, quercetin, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS), 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) and oligomycin. Its sensitivity was almost the same as that of mitochondrial F_0F_1-ATPase. Neither membrane-bound ATPase nor ATPase incorporated into liposomes was affected appreciably by these drugs. These results indicate that the sensitivity to anions and drugs of lysosomal H^+-ATPase depends on the form of the enzyme and that the sensitivity of the solubilized lysosomal H^+-ATPase is very similar to that of mitochondrial F_0F_1-ATPase. On the other hand, the two ATPases differ in their sensitivity to *N*-ethylmaleimide and pyridoxal phosphate: only the mitochondrial ATPase is inhibited by pyridoxal phosphate whereas only the lysosomal ATPase is inhibited by *N*-ethylmaleimide.

Introduction

H^+-translocating ATPase (H^+-ATPase) has been shown to be involved in the maintenance of the internal acidic pH of lysosomes (see reviews in Refs. 1 and 2). The ATPase has been characterized in normal (unmodified) lysosomes [3–5], tritosomes [3,6–8], and membrane ghosts derived from tritosomes [9]. The ATPase is insensitive to vanadate and sensitive to ADP and DCCD, indicating that the mechanism of H^+-translocation of lysosomal H^+-ATPase is fundamentally similar to that of F_0F_1-type H^+-ATPase. However, lysosomal H^+-ATPase differs from mitochondrial H^+-ATPase (mitochondrial F_0F_1-ATPase) in the following characteristics: it is not affected appreciably by bicarbonate, which strongly activates

* To whom correspondence should be addressed.

Abbreviations: Bicine, *N*,*N*-bis(2-hydroxyethyl)glycine; DCCD, *N*,*N*′-dicyclohexylcarbodiimide; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; NBD-Cl, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; SITS, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid; TMAH, tetramethylammonium hydroxide.

mitochondrial F_0F_1-ATPase [10,11], and it is resistant to NBD-Cl, quercetin and oligomycin, but sensitive to *N*-ethylmaleimide [9]. Thus, further characterization of lysosomal H^+-ATPase is required to determine its enzymatic properties.

Recently, we developed a procedure for solubilization and reconstitution of lysosomal H^+-ATPase [12]. In this work, using this technique, we investigated the behavior of lysosomal H^+-ATPase with various anions and drugs in comparison with that of mitochondrial F_0F_1-ATPase.

Part of this study was presented in abstract form at the 7th Symposium on the Interaction between Biological Membranes and Drugs, Tokyo (1984).

Materials and Methods

Solubilization and reconstitution of lysosomal H^+-ATPase. Lysosomal membrane ghosts were prepared from purified lysosomes (tritosomes) of rat liver as described previously [9]. The ATPase in the membrane fraction [9] is referred to as 'membrane-bound ATPase'. H^+-ATPase was solubilized from the membranes with lysophosphatidylcholine, and reconstituted into asolectin liposomes as described previously [12]. Briefly, membrane ghosts (about 1 mg protein/ml) were first treated with lysophosphatidylcholine (0.5 mg/ml) to solubilize membrane proteins other than ATPase. The ATPase was then extracted at 10°C for 15 min with a higher concentration (1.7 mg/ml) of lysophosphatidylcholine in 20 mM Bicine-TMAH (pH 8.5) containing 0.25 M sucrose, 0.5 mM EDTA, 1 mM dithiothreitol, 1 mM ATP and proteinase inhibitors (chymostatin, 5 μg/ml; leupeptin, 5 μg/ml; antipain, 2 μg/ml and pepstatin, 5 μg/ml). More than 50% of the ATPase of the original membranes was recovered in the supernatant fraction by centrifugation of the resultant mixture at 180000 × *g* for 40 min at 4°C. This fraction is referred to as 'solubilized ATPase'. Lysophosphatidylcholine was removed by passing this fraction through a column (1 × 4 cm) of Biobeads SM2 (Bio-Rad). A mixture of octyl-β-D-glucopyranoside and asolectin liposomes was added to the eluate (final concentration of octyl-β-D-glucopyranoside, 1.0%; liposomal lipid/protein ratio, about 20 by weight). Proteoliposomes were prepared by 20-fold dilution of the mixture, centrifuged at 130000 × *g* for 1 h at 4°C and suspended in 10 mM Bicine-TMAH (pH 8.5), 0.25 M sucrose and 1 mM dithiothreitol. The ATPase in this fraction is referred to as 'liposome-incorporated ATPase'.

Solubilization and reconstitution of mitochondrial F_0F_1-ATPase. Submitochondrial particles were prepared by the method of Spitsberg et al. [13] from rat liver mitochondria. The preparation was frozen in liquid nitrogen, and stored at −80°C until use. The ATPase in submitochondrial particles is referred to as 'membrane-bound mitochondrial F_0F_1-ATPase'. Solubilization of mitochondrial F_0F_1-ATPase from submitochondrial particles and its reconstitution into liposomes were carried out by the same procedure as described in the previous section and in Ref. 12 except that the first lysophosphatidylcholine (0.5 mg/ml) treatment was omitted. About 20% of the ATPase of submitochondrial particles was recovered in the solubilized fraction with some inactivation (about 30–60%). The ATPases in the solubilized and reconstituted fractions are referred to as 'solubilized mitochondrial F_0F_1-ATPase' and 'liposome-incorporated mitochondrial F_0F_1-ATPase'.

Assays. ATPase activity was assayed by measuring P_i released from ATP as described previously [9] with slight modifications. Details are given in the legends to figures. The standard assay medium consisted of 40 mM Bicine-Tris (pH 8.5), 4 mM Mg-ATP and enzyme samples. Protein was determined by the method of Schaffner and Weissmann [14] or Lowry et al. [15] with bovine serum albumin (fraction V) as a standard.

Chemicals. Lysophosphatidylcholine from egg yolk was kindly prepared and supplied from Nippon Shoji Co. (Osaka). L-α-Phosphatidylcholine (type II-S) and octyl-β-D-glucopyranoside were purchased from Sigma (St. Louis, MO). Other chemicals used in this study were commercial products of analytical grade.

Results

The procedure for solubilization and reconstitution of lysosomal H^+-ATPase, which is applicable to mitochondrial F_0F_1-ATPase, has been described

[12]. The sensitivities of lysosomal ATPase and mitochondrial F_0F_1-ATPase in the membrane-bound, solubilized and liposome-incorporated forms to anions and drugs were studied.

Anion sensitivity

The effects of bicarbonate on lysosomal ATPase in the three forms are shown in Fig. 1A. Bicarbonate had little effect on the membrane-bound enzyme, consistent with our previous finding [9]. It also had little effect on the liposome-incorporated ATPase, but, at 50 mM it activated the solubilized ATPase about 1.7-fold. It also activated mitochondrial F_0F_1-ATPase about 2-fold under the same assay conditions as for lysosomal ATPase (Fig. 1B), a result that was consistent with reported findings [10,11]. Activation of mitochondrial F_0F_1-ATPase by bicarbonate was, however, independent of the form of the enzyme unlike in the case of lysosomal ATPase.

The difference in the effects of bicarbonate on lysosomal ATPase and mitochondrial F_0F_1-ATPase might be due to difference caused by the procedures used for their solubilization and reconstitution. This possibility was excluded by the following experiments. First, lysosomal membrane ghosts and submitochondrial particles were mixed in ratios of 1/0, 1/1, 1/2 and 0/1 of the ATPases, and solubilized as described in Materials and Methods. The ATPases in all these solubilized fractions were found to be activated by bicarbonate to nearly the same extent (about 2-fold) (data not shown). Second, the ATPases were solubilized separately, and then combined and reconstituted. Fig. 2 shows the effect of bicarbonate

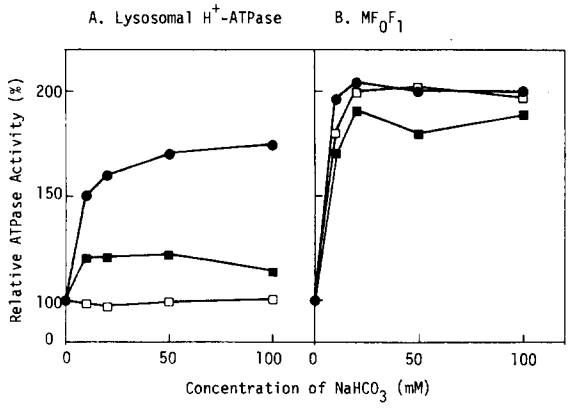

Fig. 1. Effect of bicarbonate on lysosomal and mitochondrial ATPases in different enzyme forms. ATPase activity was measured in the standard assay mixture with the indicated concentration of bicarbonate, and activity is shown as a percentage of that in the absence of bicarbonate. The specific activities of membrane-bound, solubilized and liposome-incorporated ATPases, respectively, in the absence of salt were 0.50, 0.64 and 0.92 units/mg protein for lysosomal ATPase and 3.5, 0.60 and 0.90 units/mg protein for mitochondrial F_0F_1-ATPase. □———□, Membrane-bound enzyme; ●———●, solubilized enzyme; ■———■, liposome-incorporated enzyme.

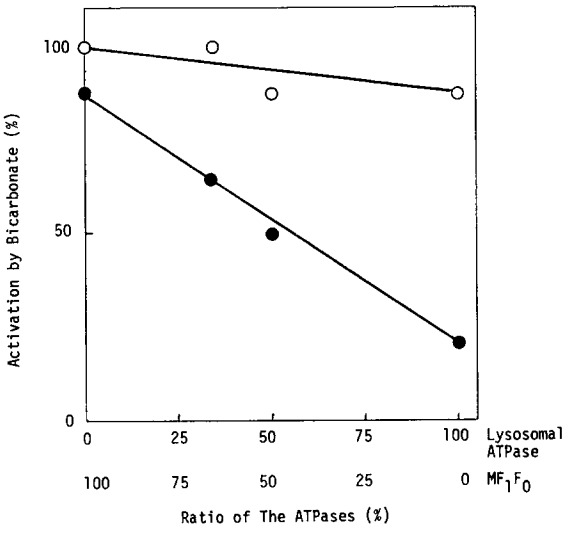

Fig. 2. Effect of bicarbonate on reconstituted ATPases prepared from mixtures of solubilized lysosomal ATPase and mitochondrial F_0F_1-ATPase. Both lysosomal ATPase and mitochondrial F_0F_1-ATPase were solubilized and passed through a Bio-beads SM2 column. The eluates were mixed in the indicated ratios to give the same total units of ATPase, reconstituted into proteoliposomes as described in Materials and Methods and suspended in 0.5 ml of buffer. The ATPase activities in the solubilized mixtures and the proteoliposomes were measured in the absence and in the presence of 20 mM bicarbonate as described in the legend of Fig. 1. The liposome-incorporated ATPase activities in all the proteoliposome preparations were nearly equal. Activation of ATPases by bicarbonate is shown as % activation calculated by the following equation:

$$\%\ \text{activation} = \frac{[\text{ATPase}\ (+\text{bicarbonate})] - [\text{ATPase}\ (-\text{bicarbonate})]}{[\text{ATPase}\ (-\text{bicarbonate})]} \times 100$$

○———○, Solubilized enzyme mixture; ●———●, liposome-incorporated enzyme.

TABLE I

EFFECT OF ANIONS ON THE THREE DIFFERENT FORMS OF LYSOSOMAL ATPase

ATPase activities in the presence of listed salts at 50 and 100 mM are expressed as percentage of activity as described in the legend of Fig. 1. The ranges of specific activities of membrane-bound, solubilized and liposome-incorporated enzymes in the absence of salt (control) were 0.50–0.59, 0.64–0.67 and 0.92–0.94 units/mg protein, respectively.

Salt	ATPase activity (% of control)					
	membrane-bound enzyme		solubilized enzyme		liposome-incorporated enzyme	
Salt concn. (mM):	50	100	50	100	50	100
$NaHCO_3$	98	100	170	174	122	113
Na_2SO_3	125	114	220	210	132	122
Na_2SO_4	87	81	127	131	97	78
NaBr	99	95	116	105	87	76
NaCl	95	85	99	88	93	79
NaI	102	96	85	69	71	45
$NaNO_3$	82	79	73	73	47	38
NaSCN	74	67	57	57	22	28
NaF	72	6	70	22	69	29

on the combined ATPases in proteoliposomes. Activation of the ATPase decreased linearly with increase in the relative amount of lysosomal ATPase. These results suggested that the dependence of the effect of bicarbonate on the enzyme form was a unique property of lysosomal ATPase.

Table I shows the effects of several anions on the lysosomal ATPase. From the results, these anions can be classified into three groups: stimulatory, with little effect and inhibitory. Sulfite and bicarbonate activated the solubilized ATPase, but had little effect on the membrane-bound or liposome-incorporated ATPase. SO_4^{2-} and Br^- slightly activated the solubilized ATPase, but had little effect on the membrane-bound or the liposome-incorporated enzyme. Cl^- had scarcely any effect. I^-, NO_3^-, SCN^- and F^- at 0.1 M inhibited all three forms of ATPase. Thus, the effects of only certain oxyanions (bicarbonate and sulfite) depend on the form of lysosomal ATPase.

We investigated the kinetics of the activation by bicarbonate and sulfite. Double-reciprocal analysis with ATP as a substrate gave non-linear convex plots for Cl^- (40 mM) but linear plots for bi-

TABLE II

EFFECTS OF ANIONS ON THE THREE FORMS OF MITOCHONDRIAL F_0F_1-ATPase

ATPase activities are expressed as for Table I. The ranges of specific activities of membrane-bound, solubilized and liposome-incorporated enzymes in the absence of salts (control) were 3.5–3.8, 0.58–60, 0.88–0.90 units/mg protein, respectively.

Salt	ATPase activity (% of control)					
	membrane-bound enzyme		solubilized enzyme		liposome-incorporated enzyme	
Salt concn. (mM):	50	100	50	100	50	100
$NaHCO_3$	202	197	200	200	179	188
Na_2SO_3	260	254	238	200	210	183
Na_2SO_4	119	119	100	90	100	100
NaBr	130	129	92	100	120	91
NaCl	92	85	95	87	95	89
NaI	105	105	88	76	85	75
$NaNO_3$	71	64	60	58	58	53
NaSCN	23	23	23	19	10	6
NaF	60	28	78	14	24	0

carbonate and sulfite (40 mM each). The K_m values for ATP and the V_{max} values were 0.36 mM and 1.18 units/mg protein, respectively, in the presence of bicarbonate and 0.26 mM and 1.25 units/mg protein, respectively, in the presence of sulfite. These kinetic properties were similar to those for mitochondrial F_0F_1-ATPase [16].

For more precise comparison of lysosomal ATPase and mitochondrial F_0F_1-ATPase, the effects of these anions on mitochondrial F_0F_1-ATPase were also investigated (Table II). The effects of the anions were essentially independent of the form of mitochondrial F_0F_1-ATPase, and both bicarbonate and sulfite were stimulatory. Br^-, SO_4^{2-} and Cl^- were less effective and I^-, NO_3^-, SCN^- and F^- were inhibitory. The effects of these anions on mitochondrial F_0F_1-ATPase were thus nearly the same as those on solubilized lysosomal ATPase.

Drug sensitivity

Table III summarizes the sensitivities of the three enzyme forms of lysosomal ATPase to various drugs. The membrane-bound ATPase was scarcely affected by any of the drugs except DCCD, consistent with previous results [9]. On the other hand, the solubilized enzyme was inhibited by NaN_3, NBD-Cl, quercetin, SITS, DIDS and oligomycin. The sensitivities of liposome-incorporated ATPase to these drugs were reduced to the level of membrane-bound ATPase. DCCD inhibited the solubilized ATPase only 26%, although it inhibited both the membrane-bound and the liposome-incorporated ATPase almost 50%. *N*-Ethylmaleimide, an inhibitor of the lysosomal H^+-pump [3,9], inhibited all three enzyme forms of ATPase. Pyridoxal 5-phosphate, an inhibitor of mitochondrial F_0F_1-ATPase [17], had little effect on the three forms of lysosomal ATPase.

The effects of the same inhibitors on mitochondrial F_0F_1-ATPase were also investigated (Table IV). All these drugs except *N*-ethylmaleimide strongly inhibited mitochondrial F_0F_1-ATPase, and except in the case of DCCD and quercetin their inhibitory effects were not influenced appreciably by the form of the enzyme. The three forms of mitochondrial F_0F_1-ATPase were insensitive to *N*-ethylmaleimide and were inhibited by pyridoxal 5-phosphate, unlike lysosomal ATPase (Table III).

Discussion

In this study, we compared the effects of anions and drugs on lysosomal H^+-ATPase and mitochondrial F_0F_1-ATPase.

TABLE III

EFFECTS OF DRUGS ON THE THREE FORMS OF LYSOSOMAL ATPase

The assay medium consisted of 40 mM Bicine-Tris (pH 8.5), 40 mM NaCl, 4 mM Na-ATP, 2 mM $MgCl_2$, the indicated drug and the enzyme sample. ATPase activities are expressed as percentages of the activity in the absence of drugs. The drugs were dissolved in distilled water (*N*-ethylmaleimide, NaN_3), 0.1 M Bicine-Tris (pH 8.5) (pyridoxal 5-phosphate), ethanol (oligomycin, NBD-Cl, quercetin, DCCD) or dimethylsulfoxide (SITS, DIDS). When present in the medium, organic solvents were at concentrations of less than 1%, and did not affect the ATPase activity. The ranges of specific activities of membrane-bound, solubilized and liposome-incorporated enzymes in the absence of drugs (control) were 0.40–0.55, 0.55–0.64 and 0.92–0.95 units/mg protein, respectively.

Drug	Concn.	ATPase activity (% of control)		
		membrane-bound enzyme	solubilized enzyme	liposome-incorporated enzyme
Oligomycin	5 μM	90	50	71
NaN_3	5 mM	78	27	77
NBD-Cl	0.1 mM	80	38	44
SITS	0.5 mM	59	10	40
DIDS	0.5 mM	61	7	36
Quercetin	0.1 mM	76	45	62
DCCD	0.1 mM	49	74	50
N-Ethylmaleimide	1 mM	86	81	66
Pyridoxal 5-phosphate	5 mM	98	96	89

TABLE IV

EFFECTS OF DRUGS ON THE THREE FORMS OF MITOCHONDRIAL F_0F_1-ATPase

ATPase activities were measured and expressed as for Table III. The ranges of specific activities of membrane-bound, solubilized and liposome-incorporated enzymes in the absence of drugs (control) were 3.0–3.8, 0.54–0.60 and 0.77–0.90 units/mg protein, respectively.

Drug	Concn.	ATPase activity (% of control)		
		membrane-bound enzyme	solubilized enzyme	liposome-incorporated enzyme
Oligomycin	5 μM	4	5	15
NaN_3	5 mM	17	19	28
NBD-Cl	0.1 mM	16	17	24
SITS	0.5 mM	5	8	3
DIDS	0.5 mM	10	11	7
Quercetin	0.1 mM	12	57	49
DCCD	0.1 mM	15	62	23
N-Ethylmaleimide	1 mM	100	95	98
Pyridoxal 5-phosphate	5 mM	64	51	76

We found that the sensitivity of lysosomal ATPase to anions and drugs depended on the form of the enzyme: ATPase solubilized with lysophosphatidylcholine was activated by bicarbonate and sulfite and strongly inhibited by drugs such as oligomycin, SITS, azide and quercetin, most of which did not have any significant effects on the ATPase in the original membranes or in proteoliposomes (Figs. 1 and 2 and Tables I and III). ATPase solubilized with 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (Chaps) was also activated by bicarbonate, suggesting that the dependence of effects on the enzyme form was not influenced by the type of detergent used (data not shown). The reason for this form-dependence of effects is not clear at present. These oxyanions and some of the inhibitors have been shown to interact with the catalytic or regulatory sites of mitochondrial F_0F_1-ATPase resulting in activation or inhibition of its ATPase activity. For example, it has been suggested that bicarbonate binds to the ADP-binding site [16] or a regulatory site for the binding of Mg-ATP [18]. NBD-Cl has been shown to bind covalently to the β-subunit of F_1 [19,20]. Oligomycin is known to interact with the oligomycin sensitivity conferring protein and azide has been shown to bind non-covalently to the F_1 moiety [19,20]. Conceivably, the binding sites on the lysosomal ATPase for interaction with these substances are exposed in solubilized ATPase but buried in the surrounding lipid bilayer in membrane-bound ATPase. Thus, part of this effect may be because most of lysosomal H^+-ATPase is embedded in membrane lipids. In support of this idea, the lysosomal ATPase activity in proteoliposomes was also not affected appreciably by these anions and drugs (Tables I and III). This effect seems to be unique to lysosomal H^+-ATPase, since it was scarcely observed with mitochondrial F_0F_1-ATPase (Tables II and IV).

We also found that solubilized lysosomal ATPase was very similar to mitochondrial F_0F_1-ATPase in its sensitivities to anions and drugs: it was (1) inhibited by SCN^-, NO_3^-, F^-, (2) activated by some oxyanions (bicarbonate and sulfite), (3) hardly affected by Cl^-, Br^- and SO_4^{2-}, (4) strongly inhibited by azide, SITS, quercetin and oligomycin, and (5) less sensitive than the membrane-bound enzyme to DCCD. As some of these anions and drugs have been shown to react with binding sites in mitochondrial F_0F_1-ATPase (as discussed above), there may be similar binding sites in lysosomal ATPase. The observed effects were not those of contaminating mitochondrial F_0F_1-ATPase, because the original lysosomal membranes were contaminated less than 3% by mitochondria as judged by marker enzyme studies and measurement of the sensitivities of the ATPase activity to anions and drugs, as discussed in the previous paper [9]. Furthermore, even after solubilization, lysosomal

ATPase, unlike mitochondrial F_0F_1-ATPase, was insensitive to pyridoxal 5-phosphate and sensitive to *N*-ethylmaleimide (Tables III and IV). Moreover, different forms of mitochondrial F_0F_1-ATPase show similar sensitivities to anions and drugs (Tables II and IV). Consequently, there are some similarities as well as distinct differences between lysosomal ATPase and mitochondrial F_0F_1-ATPase. These results suggest that lysosomal H^+-ATPase is a unique F_0F_1-type ATPase or a new type of ATPase.

H^+-ATPases that are similar to lysosomal H^+-ATPase have been found in plant vacuoles [21,22], secretory granules [23–29], endosomes [30], coated vesicles [31] and the Golgi apparatus [32]. However, little is known about their anion and drug sensitivities, especially in their solubilized forms. The present results on lysosomal H^+-ATPase were obtained after solubilization and reconstitution of the enzyme. Similar form-dependence of the sensitivities of other vacuolar ATPases to anions and drugs may be expected.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

References

1 Reeves, J.P. (1984) in Lysosomes in Biology and Pathology (Dingle, J.T., Dean, R.T. and Sly, W., eds.), Vol. 7, pp. 175–199, Elsevier Biomedical Press, Amsterdam
2 Ohkuma, S. (1985) in Lysosomes: Their Role in Protein Breakdown (Glaumann, H. and Ballard, F.J., eds.), Academic Press, New York, in the press
3 Ohkuma, S., Moriyama, Y. and Takano, T. (1982) Proc. Natl. Acad. Sci. USA 79, 2758–2762
4 Harikumar, P. and Reeves, J.P. (1983) J. Biol. Chem. 258, 10403–10410
5 Dell'Antone, P. (1979) Biochem. Biophys. Res. Commun. 86, 180–189
6 Schneider, D.L. (1981) J. Biol. Chem. 256, 3858–3864
7 Moriyama, Y., Takano, T. and Ohkuma, S. (1982) J. Biochem. (Tokyo) 92, 1333–1336
8 Ohkuma, S., Moriyama, Y. and Takano, T. (1983) J. Biochem. (Tokyo) 94, 1935–1943
9 Moriyama, Y., Takano, T. and Ohkuma, S. (1984) J. Biochem. (Tokyo) 95, 995–1007
10 Mitchell, P. and Moyle, J. (1971) Bioenergetics 2, 1–11
11 Lambeth, D.O. and Lardy, H.A. (1971) Eur. J. Biochem. 22, 355–363
12 Moriyama, Y., Takano, T. and Ohkuma, S. (1984) J. Biochem. (Tokyo) 96, 927–930
13 Spitsberg, V.L., Morris, H.P. and Chan, S.H.P. (1979) Arch. Biochem. Biophys. 195, 136–144
14 Schaffner, W. and Weissmann, S. (1973) Anal. Biochem. 56, 502–514
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
16 Ebel, R.E. and Lardy, H.A. (1975) J. Biol. Chem. 247, 191–196
17 Sugiyama, Y. and Mukohata, Y. (1979) FEBS Lett. 98, 276–280
18 Baubichon, H., Di Pietro, A., Godinot, C. and Gautheron, D.C. (1982) FEBS Lett. 137, 261–264
19 Futai, M. and Kanazawa, H. (1980) Curr. Top. Bioenerg. 10, 181–215
20 Pedersen, P.L. (1975) Bioenergetics 6, 243–275
21 Marin, B. (1983) Plant Physiol. 73, 973–977
22 Uchida, E., Ohsumi, Y. and Anraku, Y. (1985) J. Biol. Chem. 260, 1090–1095
23 Peerce, B.E., Smolka, A. and Sachs, G. (1984) J. Biol. Chem. 259, 9255–9262
24 Apps, D.K. and Schatz, G. (1979) Eur. J. Biochem. 100, 411–419
25 Cidon, S., Ben-David, H. and Nelson, N. (1983) J. Biol. Chem. 258, 11684–11688
26 Hutton, J.C. and Peshavaria, M. (1982) Biochem. J. 204, 161–170
27 Scherman, D., Nordmann, J. and Henry, J.-P. (1982) Biochemistry 21, 687–694
28 Toll, L. and Howard, B.D. (1978) Biochemistry 17, 2517–2523
29 Gristein, S. and Furuya, W. (1983) J. Biol. Chem. 258, 7876–7882
30 Galloway, C.J., Dean, G.E., Marsh, M., Rudnick, G. and Mellman, I. (1983) Proc. Natl. Acad. Sci. USA 80, 3334–3338
31 Stone, D.K., Xie, X.-S. and Racker, E. (1983) J. Biol. Chem. 258, 4059–4062
32 Glickman, J., Croen, K., Kelly, S. and Al-Awqati, Q. (1983) J. Cell Biol. 97, 1303–1308