Biochimica et Biophysica Acta, 777 (1984) 67–74
Elsevier

BBA 72264

BIOCHEMICAL CHARACTERIZATION OF (Ca^{2+} + Mg^{2+})-ATPase IN *TETRAHYMENA* MICROSOMES

YOSHINORI MUTO and YOSHINORI NOZAWA

Department of Biochemistry, Gifu University School of Medicine, Tsukasamachi-40, Gifu (Japan)

(Received April 24th, 1984)

Key words: (Ca^{2+} + Mg^{2+})-ATPase; Ca^{2+} transport; Microsome; (Tetrahymena)

The activity of calcium-stimulated and magnesium-dependent adenosinetriphosphatase ((Ca^{2+} + Mg^{2+})-ATPase, EC 3.6.1.3) was found to be high in *Tetrahymena* microsomes. The (Ca^{2+} + Mg^{2+})-ATPase has an apparent K_m of 0.17 μM for free calcium and a maximum reaction velocity of 74 nmol ATP hydrolysed/mg protein per min. The enzyme was dependent on 1–3 mM magnesium and the pH optimum was pH 7.2. Among the nucleoside triphosphates tested, ATP was the best substrate, with an apparent K_m of 63 μM. The (Ca^{2+} + Mg^{2+})-ATPase activity was unaffected by K^+, Na^+, dicyclohexylcarbodiimide, oligomycin, NaN_3 and ouabain, but was potently inhibited by orthovanadate. Although the calmodulin antagonist, trifluoperazine, inhibited the enzyme activity, addition of calmodulin isolated from *Tetrahymena* did not elicit any stimulatory effect on the activity. *Tetrahymena* microsomes also displayed ATP-dependent uptake of calcium which lead to the accumulation to a maximal level of 9.2 nmol/mg microsomal protein. And this activity was inhibited by vanadate at a similar concentration range to that required to inhibit (Ca^{2+} + Mg^{2+})-ATPase. These observations suggest that the (Ca^{2+} + Mg^{2+})-ATPase in microsomes may act as a calcium-pumping ATPase in this cell.

Introduction

It is well established that intracellular calcium plays a vital role in the control of many important aspects of cellular metabolism [1]. This is true with *Tetrahymena* and a related ciliate such as *Paramecium*, in which changes in the level of cytosolic calcium concentration regulate the direction of ciliary beating and thus the swimming direction [2]. Electrophysiological studies have shown that the ciliary reversal is associated with the influx of calcium ions down their electrochemical gradient into cilia through voltage-sensitive calcium channels [3,4]. This calcium influx raises the intracellular calcium concentration and triggers a temporary reversal of the direction of the power stroke of cilia [5]. In earlier reports, we described the presence of calmodulin in *Tetrahymena pyriformis* [6,7] and demonstrated that guanylate cyclase, which is entirely associated with plasma membrane, is activated by calmodulin in the presence of Ca^{2+} [8,9]. Furthermore, Schultz et al. [10,11] reported that guanylate cyclase and cyclic GMP-dependent protein kinase are present in *Paramecium* cilia [10,11]. These observations suggest the possibility that Ca^{2+}, calmodulin-dependent guanylate cyclase may play an essential role in the control of ciliary motility mediated by the change of intracellular Ca^{2+} concentration.

Since the free calcium concentration in the cytoplasm is 10^4-fold lower than the extracytoplasmic environment, the delicate regulation of the

Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*,-tetraacetic acid; Mops, 4-morpholinepropanesulfonic acid; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol.

low steady-state level of cytoplasmic calcium is a crucial aspect of the regulatory mechanism of ciliary motility in *Tetrahymena*. In particular, the renormalization period after ciliary reversal is considered to be associated with the removal of excess Ca^{2+} from cytoplasm [2]. One of the major systems responsible for regulating the cytoplasmic calcium level is Ca^{2+}-dependent ATPase. This enzyme has been found to be present in the mitochondria, endoplasmic reticulum and plasma membranes in higher organisms [12]. However, any system involving the regulation of cellular calcium concentration in *Tetrahymena* has not been clearly delineated, despite much information about Ca^{2+}-mediated functions in this cell.

In order to shed light on the calcium homeostasis in *Tetrahymena*, we need to study its calcium transport and related ATPase activity. To our knowledge, this is the first report to describe the presence and characterization of a $(Ca^{2+} + Mg^{2+})$-ATPase associated with microsomal fraction of *Tetrahymena pyriformis*.

Materials and Methods

Materials. Nucleotides (Tris salt) and various inhibitors were purchased from Sigma Chemical Co., St. Louis, MO. $^{45}CaCl_2$ was obtained from the Radiochemical Centre, Amersham, Ionophore A23187 was from Calbiochem and calmodulin was purified from *Tetrahymena* as previously described [8]. All other reagents were purchased from standard sources and were of the highest quality available commercially.

Cell growth and isolation of microsomes. A thermotolerant strain of *T. pyriformis*, strain NT-1, was grown at 39.5°C in an enriched proteose-peptone medium [13]. Cells were harvested during the early stationary phase. Microsomes were isolated according to the procedure of Nozawa and Thompson [14]. All subsequent procedures were performed at 4°C. The harvested cells were washed in a high potassium phosphate buffer (0.2 M K_2HPO_4/0.2 M KH_2PO_4/3 mM EDTA/0.1 M NaCl, pH 7.2) by centrifugation at $1000 \times g$ for 5 min. The washed cells were resuspended in the same high phosphate buffer. After removal of cilia by mild homogenization by hand, deciliated cells were homogenized vigorously (80–100 twisting strokes) in a tight-fitting glass homogenizer (Arthur H. Thomas Co., Philadelphia, PA) until almost all the cells were ruptured but intact pellicle ghosts (plasma membranes). After pellicle ghosts were isolated by centrifugation on a discontinuous sucrose gradient, the resulting supernatant was centrifuged at $19\,600 \times g$ for 20 min to spin down mitochondria in the HB-4 rotor of a Sorvall RC2-B centrifuge (Ivan Sorvall), and the resulting supernatant was centrifuged further at $105\,000 \times g$ for 60 min to sediment microsomes. The pellet was resuspended and washed with 10 mM Mops-Tris (pH 7.2) containing 100 mM sucrose. The final pellet was resuspended in the same medium, divided into aliquots and stored at −80°C.

When glucose-6-phosphatase of the endoplasmic reticulum marker enzyme and adenylate cyclase of the plasma membrane marker were assayed as described earlier [9,15], the microsomal preparation showed a 15-fold enrichment of glucose-6-phosphatase and less than 4% of total adenylate cyclase of whole cell homogenate. The electronmicrographs showed that the microsomal preparation was composed of homogeneous vesicular structures and free of mitochondria [14]. Thus, the preparation is highly enriched in endoplasmic reticulum.

Protein was determined by the method of Lowry et al. [16], with bovine serum albumin as standard.

$(Ca^{2+} + Mg^{2+})$-ATPase assay. ATPase was measured in a 0.5 ml medium containing 5–40 μg protein, 20 mM Mops-Tris (pH 7.2), 1 mM Tris-ATP, 1 mM $MgCl_2$, 1 mM EGTA and $CaCl_2$ to yield the desired free calcium. The free calcium concentration was calculated according to the method of Pershadsingh and McDonald [17]. To minimize endogenous free calcium, distilled water used for all the experiments was passed through a column of Chelex 100 (Bio-Rad). The reaction was started by adding ATP, and carried out at 37°C. After 5–20 min, depending on the activity, the reaction was stopped by adding 5% trichloroacetic acid. The released inorganic phosphate was determined colorimetrically [18]. The activity of $(Ca^{2+} + Mg^{2+})$-ATPase was determined by subtracting values obtained with EGTA plus magnesium (Mg^{2+}-ATPase activity) from those with calcium-EGTA buffer plus magnesium.

Ca^{2+} uptake measurement. $^{45}Ca^{2+}$ uptake was

measured by the Millipore filtration technique. Approx. 0.1–1 mg protein/ml was incubated at 37°C in 1 ml medium containing 20 mM Mops-Tris (pH 7.2), 200 mM sucrose, 1 mM $MgCl_2$, 1 mM ATP, 0.2 mM EGTA, 5–10 μCi $^{45}Ca^{2+}$ and the required concentration of $CaCl_2$ to yield the low free Ca^{2+}. The reaction was started by the addition of microsomal membranes after preincubation for 5 min at 37°C. At the required time-intervals after addition of microsomes, aliquots (0.1-ml) were removed and filtered rapidly through 0.45 μm HAWP Millipore filters prewetted with 20 mM Mops-Tris (pH 7.2), 5 mM $MgCl_2$, 5 mM EGTA, 200 mM sucrose. The filters were washed rapidly with 10 ml of the same buffer at 4°C, and transferred to a scintillation vial and counted for radioactivity.

Results

Activity of a Ca^{2+}-stimulated ATPase in Tetrahymena microsomes

The variations of the Ca^{2+}-stimulated ATPase activity of *Tetrahymena* microsomes over a range of calcium concentrations are shown in Fig. 1. The addition of increasing concentrations of Ca^{2+} in the presence of 1 mM $MgCl_2$ enhanced the basal Mg^{2+}-ATPase activity and the activity reached a maximum at a free Ca^{2+} concentration of approx. 4 μM. Kinetic analysis (Fig. 1 inset) revealed an apparent K_m for Ca^{2+} of 0.17 μM and a V_{max} of 74 nmol/mg protein per min. By contrast, in the absence of added $MgCl_2$, Ca^{2+} exerted a stimulation of the ATPase activity, but the optimal concentration was 1–3 mM with a K_m of 160 μM (data not shown). This low-affinity Ca^{2+}-ATPase was not investigated further, since the activity was evident only at higher Ca^{2+} concentrations by two to three orders of magnitude of the physiological cytoplasmic levels [5].

Fig. 2 shows the pH profile of microsomal ATPase activities in the absence or presence of calcium. In the presence of Ca^{2+}, ATPase showed an optimal pH of 7.2 and gradually decreased as the pH shifted to more acidic or alkaline values. In contrast, the Mg^{2+}-ATPase in the absence of Ca^{2+} showed a weak pH-dependence with an optimal value of approx. pH 6.7.

Next, Mg^{2+} requirement of the Ca^{2+}-stimu-

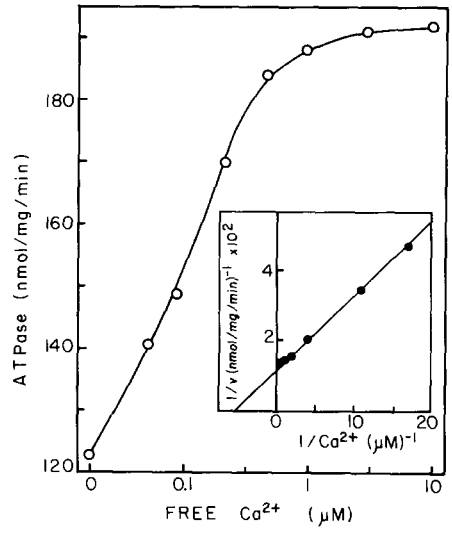

Fig. 1. Effects of Ca^{2+} concentration on ATPase activity of *Tetrahymena* microsomes. ATPase activity was assayed under standard conditions described in Materials and Methods in the presence of various concentrations of Ca^{2+}. Ca^{2+} concentrations in the reaction medium were determined also as described. The inset represents the double-reciprocal plot of (Ca^{2+} + Mg^{2+})-ATPase activity. The ordinate represents the reciprocal of net Ca^{2+}-stimulated ATPase activity, i.e., the difference between ATPase activity in the presence and absence of Ca^{2+}. Data are from a typical experiment.

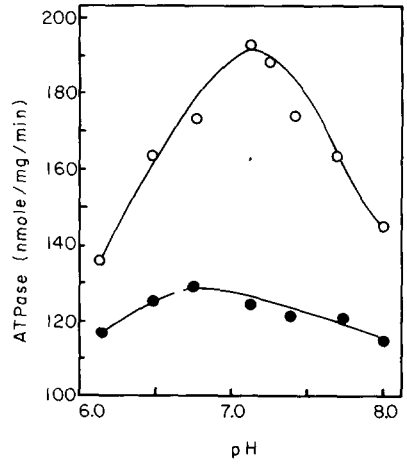

Fig. 2. pH dependency of (Ca^{2+} + Mg^{2+})- and Mg^{2+}-ATPase. EGTA was omitted from the standard incubation medium of (Ca^{2+} + Mg^{2+})-ATPase because its ability to complex calcium ions is highly pH-dependent. The (Ca^{2+} + Mg^{2+})-ATPase (○———○) was assayed at a total calcium concentration of 20 μM, and Mg^{2+}-ATPase (●———●) in the presence of 1 mM EGTA.

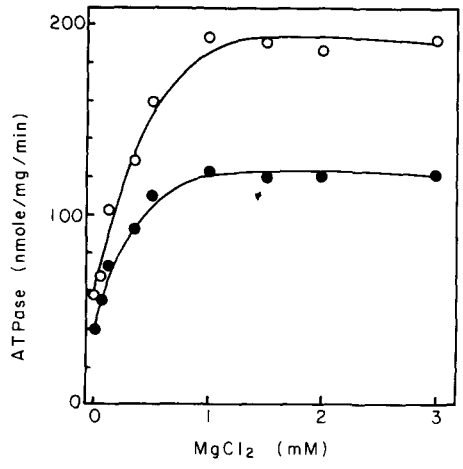

Fig. 3. Effects of Mg^{2+} on the $(Ca^{2+} + Mg^{2+})$- and Mg^{2+}-ATPase activities in *Tetrahymena* microsomes. ATPase activity was assayed in the presence (O——O) or absence (●——●) of 10 μM free Ca^{2+} with varying concentrations of $MgCl_2$ as indicated.

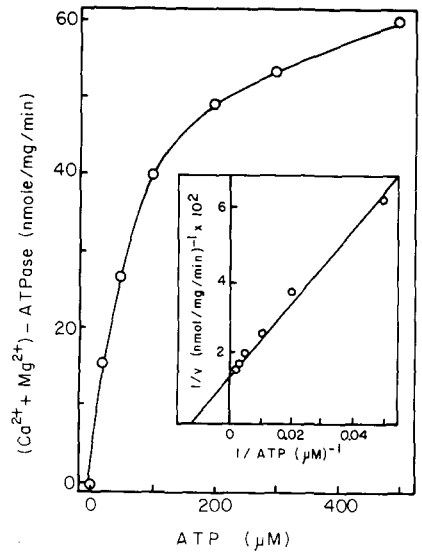

Fig. 4. Dependence on ATP concentration of the $(Ca^{2+} + Mg^{2+})$-ATPase. ATPase activity was assayed as described in Materials and Methods, except that ATP concentration was varied as shown. The free Ca^{2+} concentration was maintained at 10 μM. The ordinate represents the net Ca^{2+}-stimulated activity, i.e., the difference between ATPase activity in the presence and absence of Ca^{2+}. The inset shows the double-reciprocal plot, which yields an apparent K_m of 66 μM.

lated ATPase was examined. The effects of Mg^{2+} concentration on ATPase activities in the absence or presence of Ca^{2+} are shown in Fig. 3. In the presence of 1 mM ATP, both ATPase activities were enhanced by the addition of increasing concentrations of magnesium and reached a maximum at approx. 1 mM $MgCl_2$. Although a low activity of Ca^{2+}-stimulated ATPase could be measured in the absence of added $MgCl_2$ as described above, a requirement of magnesium for the Ca^{2+}-dependent ATP hydrolysis was obvious. Thus, the Ca^{2+}-stimulated ATPase activity in *Tetrahymena* microsomes was referred to as $(Ca^{2+} + Mg^{2+})$-ATPase, like other Ca^{2+}-transport ATPases [19].

Substrate kinetics and specificity

The dependence of $(Ca^{2+} + Mg^{2+})$-ATPase activity on ATP concentration is shown in Fig. 4. The total ATP concentration was varied from 20 to 500 μM and the total $MgCl_2$ concentration was kept constant at 1 mM. The K_m for ATP of the $(Ca^{2+} + Mg^{2+})$-ATPase, derived from the Lineweaver-Burk plot (Fig. 4 inset), was approx. 66 μM and the V_{max} was about 71 nmol/mg protein per min. On the other hand, the K_m for Mg^{2+}-ATPase (in the absence of Ca^{2+}) was about 142 μM (data not shown), significantly different from the activity of $(Ca^{2+} + Mg^{2+})$-ATPase.

To determine the nucleotide specificity of the enzyme, various nucleotides were substituted for ATP in the assay mixture. The ability of the microsomal enzyme to hydrolyse the different nucleotides is shown in Table I. Among nucleotides tested at 1 mM, the $(Ca^{2+} + Mg^{2+})$-ATPase

TABLE I

SUBSTRATE SPECIFICITY OF MICROSOMAL ATPase

The rate of substrate hydrolysis was determined under standard conditions at a nucleotide concentration of 1 mM. Results are presented as the means ± S.E. of triplicate determinations. The $(Ca^{2+} + Mg^{2+})$-ATPase activity is net stimulation of the Mg^{2+}-ATPase by 4 μM free Ca^{2+}. The Mg^{2+}-ATPase activity was assayed in the presence of 1 mM EGTA.

Nucleoside triphosphate	ATPase activity (nmol/mg per min)	
	$(Ca^{2+} + Mg^{2+})$-ATPase	Mg^{2+}-ATPase
ATP	67.2 ± 8.5	118 ± 10
GTP	21.3 ± 4.3	106 ± 8
UTP	18.5 ± 2.9	66 ± 5
CTP	10.1 ± 5.8	84 ± 3
ITP	9.8 ± 5.2	92 ± 5

was found to utilize ATP as the best substrate. The rate of hydrolysis for other nucleotides corresponded to at most 15–32% of the rate for ATP. Mg^{2+}-ATPase showed little substrate specificity (Table I).

Effects of various agents on the activity of (Ca^{2+} + Mg^{2+})-ATPase

As shown in Table II, mitochondrial ATPase inhibitors such as dicyclohexylcarbodiimide, oligomycin and NaN_3 gave no effect on the (Ca^{2+} + Mg^{2+})-ATPase activity. These compounds were also ineffective for the Mg^{2+}-ATPase activity (data not shown), indicating the absence of contaminated mitochondrial ATPase activity in this microsomal preparation. NaCl and KCl had no significant effect, unlike Ca^{2+}-pumping ATPases of heart [20] and skeletal muscle [21] sarcoplasmic reticulum which are stimulated by these ions. Also, ouabain, a specific (Na^+ + K^+)-ATPase inhibitor [19], did not alter the microsomal enzyme activity in *Tetrahymena*. Orthovanadate, a potent inhibitor of ATPases in many cell types [19], inhibited the activity of *Tetrahymena* microsomal (Ca^{2+} + Mg^{2+})-ATPase with IC_{50} (the concentration needed for half-maximal inhibition) of 30 μM (Fig. 6). This IC_{50} value is similar to that observed with (Ca^{2+} + Mg^{2+})-ATPase of sarcoplasmic reticulum [22], but higher than those reported for plasma membrane [23].

The phenothiazine drug, trifluoperazine, had a substantial inhibitory effect on (Ca^{2+} + Mg^{2+})-ATPase (Table II), with little influence on basal Mg^{2+}-ATPase. This inhibitor is known to act in many cases by interferring with the action of calmodulin. However, the concentration required for the inhibition of *Tetrahymena* microsomal (Ca^{2+} + Mg^{2+})-ATPase was at a rather non-specific concentration region for calmodulin [24]. Furthermore, no consistent and significant stimulation by added *Tetrahymena* calmodulin was able to be demonstrated, even after the microsomal preparation was washed extensively with 5 mM EGTA to remove endogenous calmodulin. Therefore, (Ca^{2+} + Mg^{2+})-ATPase of *Tetrahymena* microsomes may be calmodulin-independent and the inhibition by trifluoperazine is probably due to interaction of the drug with components other than calmodulin [24].

Ca^{2+} uptake by Tetrahymena microsomes

The uptake of Ca^{2+} by *Tetrahymena* microsomes in the presence and absence of ATP is

TABLE II

EFFECTS OF VARIOUS AGENTS ON THE ACTIVITY OF (Ca^{2+} + Mg^{2+})-ATPase

(Ca^{2+} + Mg^{2+})-ATPase was assayed in the presence of 4 μM free Ca^{2+} under standard conditions. Data represent the means ± S.E. of triplicate determinations. The (Ca^{2+} + Mg^{2+})-ATPase activity is net stimulation of the Mg^{2+}-ATPase by 4 μM free Ca^{2+}. DCCD, dicyclohexylcarbodiimide, TFP, trifluoperazine.

Additions	Concentration	(Ca^{2+} + Mg^{2+})-ATPase activity	
		(nmol/mg per min)	(%) [a]
None		68.5 ± 5.7	100
DCCD	100 μM	67.8 ± 3.1	99
Oligomycin	5 μg/ml	70.1 ± 7.0	102
NaN_3	10 mM	73.0 ± 3.5	107
NaCl	10 mM	68.7 ± 4.2	100
KCl	100 mM	64.3 ± 2.3	94
Ouabain	1 mM	69.3 ± 5.4	101
TFP	10 μM	61.3 ± 5.6	89
TFP	100 μM	36.7 ± 3.8	54
Calmodulin	20 μg/ml	69.0 ± 3.1	100

[a] Percentage of control activity.

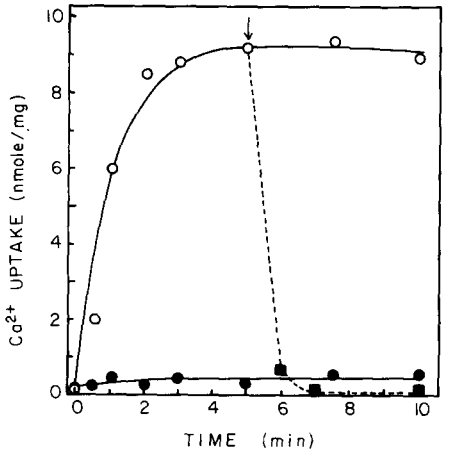

Fig. 5. Ca^{2+} uptake by *Tetrahymena* microsomes. Ca^{2+} uptake was assayed as described in Materials and Methods in the presence of 10 μM free Ca^{2+}. The following conditions are shown: ○——○, optimal medium; ●——●, ATP omitted; ■——■, 5 μM ionophore A23187 added at the arrow. The ionophore A23187 was dissolved in ethanol, which had no effect on measured uptake.

illustrated in Fig. 5. In the absence of ATP, approx. 0.5 nmol Ca^{2+} per mg protein became associated with the microsomes within 30 s of incubation. This most likely represents the Ca^{2+} bound to microsomes, since further incubation up to 10 min did not cause any changes in $^{45}Ca^{2+}$ radioactivity associated with microsomes. However, in the presence of Mg^{2+}, the addition of ATP initiated a considerable stimulation of Ca^{2+} uptake, leading to a maximal level of 9.2 nmol/mg protein in 5 min. No ATP-dependent uptake occurred when magnesium was omitted from the assay mixture. The accumulated Ca^{2+} was released rapidly from microsomal vesicles by a calcium ionophore A23187. Thus, in the presence of ATP, Ca^{2+} was indeed sequestered inside the microsomal vesicles and not bound to the membrane surface.

In order to attempt to link ATPase activity with calcium transport, effects of vanadate on (Ca^{2+} + Mg^{2+})-ATPase activity and Ca^{2+} uptake were examined. As shown in Fig. 6, the initial rate of Ca^{2+} accumulation was inhibited by vanadate to the same extent as for ATPase activity.

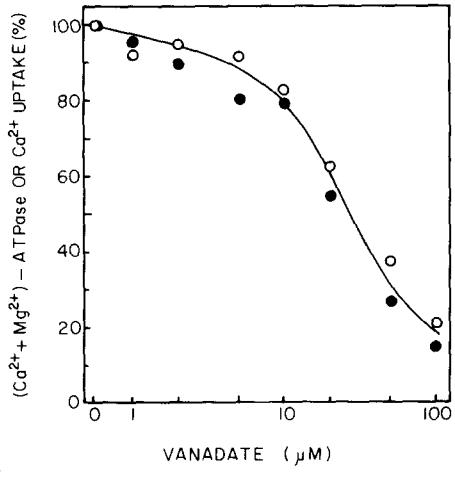

Fig. 6. Effects of vanadate on (Ca^{2+} + Mg^{2+})-ATPase activity and Ca^{2+} uptake. Microsomes were preincubated for 15 min at 37°C with vanadata at various concentrations, and then the ATPase activity or Ca^{2+} uptake was measured as described in Materials and Methods. In both cases, the reaction was started by the addition of ATP and the free Ca^{2+} concentration was maintained at 4 μM. Ordinate represents the percentage of initial velocity for the (Ca^{2+} + Mg^{2+})-ATPase (○——○) or the Ca^{2+} uptake (●——●).

Discussion

The ATPase activity of *Tetrahymena* microsomes appears to contain at least two components; Mg^{2+}-stimulated ATPase and an additional Ca^{2+}-stimulated activity ((Ca^{2+} + Mg^{2+})-ATPase). Although both activities exhibit similar profile of dependence on Mg^{2+} concentration, they may reflect the presence of two different enzymes, since the pH profile of the two activities is different and the Ca^{2+}-stimulated activity is potently inhibited by vanadate and trifluoperazine, whereas the Ca^{2+}-independent activity is not. Moreover, the (Ca^{2+} + Mg^{2+})-ATPase activity differs from the Mg^{2+}-ATPase in terms of K_m for ATP and substrate specificity. (Ca^{2+} + Mg^{2+})-ATPase is considered to be principally derived from endoplasmic reticulum, since the activity of a marker enzyme, adenylate cyclase of plasma membrane was not detected in the microsomal preparation (Materials and Methods) and the inhibitors for mitochondrial ATPase were without any effect on the (Ca^{2+} + Mg^{2+})-ATPase in microsomes (Table II). Furthermore, IC_{50} value for vanadate is rather similar to that for Ca^{2+}-transport ATPase in sarcoplasmic reticulum. In addition, the (Ca^{2+} + Mg^{2+})-ATPase activity reported here is different from Ca^{2+}-activated ATPase previously purified from the cytosolic fraction of *Tetrahymena* [25], which exhibits broad substrate specificity and low affinity for Ca^{2+} and ATP, compared with the (Ca^{2+} + Mg^{2+})-ATPase described here.

Although the functions of (Ca^{2+} + Mg^{2+})-ATPase in *Tetrahymena* microsomes remain unestablished, it seems to be involved in Ca^{2+} pumping across the microsomal membrane. Indeed, in this study, we have demonstrated the presence of ATP-dependent Ca^{2+} uptake into the microsomes. The Ca^{2+} uptake and (Ca^{2+} + Mg^{2+})-ATPase activities were similar in their Mg^{2+}-requirement and inhibitory behavior by vanadate. Furthermore, the affinity of (Ca^{2+} + Mg^{2+})-ATPase for Ca^{2+} was high enough to respond effectively to changes in cytoplasmic calcium concentration. These findings indicate, in analogy with purified Ca^{2+}-transporting ATPases from sarcoplasmic reticulum [26] and erythrocyte plasma membrane [23], that the (Ca^{2+} + Mg^{2+})-ATPase activity in *Tetrahymena* microsomes represents the enzymatic

basis for the calcium pump. However, the ratio of Ca^{2+} transported per ATP hydrolyzed calculated from the initial velocity of Ca^{2+} uptake and (Ca^{2+} + Mg^{2+})-ATPase activity yields a low ratio (approx. 0.1), compared with those of the previously described Ca^{2+} pumps [19]. Such a low ratio has also been observed with the Ca^{2+} translocation in plasma membrane of sperm [27] or endoplasmic reticulum of islet cells [28]. Further characterization of Ca^{2+} uptake will be required to elucidate the interrelationship between ATP hydrolysis and Ca^{2+} translocation.

There is increasing recognition that ciliated protozoa offer an interesting and a typical example of Ca^{2+}-mediated control of cell functions [29]. Calcium has been shown to be a principal cation responsible for the depolarization-induced reversal of the ciliary beat [2], and calcium is also known to initiate the secretion of epinephrine by *Tetrahymena*, leading to an elevated cyclic AMP level [30]. A number of other physiological processes in ciliates, ranging from food vacuole formation [31] to sexual conjugation [32], appear to be controlled, at least in part, by calcium. The microsomal (Ca^{2+} + Mg^{2+})-ATPase demonstrated here may be essential to calcium homeostasis in *Tetrahymena* cells, when intracellular Ca^{2+} concentration is altered under changes of physiological conditions. Additionally, the microsome may serve as an important site of intracellular Ca^{2+} pools as suggested by Kusamran et al. [33]. On the other hand, there are several reports on Ca^{2+}-stimulated ATPases of ciliary membrane [34–36] or surface membrane (pellicle) [37,38] of *Paramecium*, and a possible implication of these enzymes in Ca^{2+} transport has been proposed by Nelson and colleagues [39,40]. These surface membranes also would be operative for the regulation of intracellular Ca^{2+} concentration in *Tetrahymena*. At present, although the relative contribution of microsomal (Ca^{2+} + Mg^{2+})-ATPase in calcium homeostasis in *Tetrahymena* cells remains to be clear, this system presumably plays some important role for maintaining the intracellular calcium concentration within an optimal range in this organism.

Acknowledgment

This work was supported in part by a research grant from the Ministry of Education, Culture and Science, Japan.

References

1 Rasmussen, H. (1982) in Hormone Receptors (Kohn, L.D., ed.), pp. 175–197, John Wiley & Sons, New York
2 Eckert, R. and Brehm, P. (1979) Annu. Rev. Biophys. Bioeng. 8, 353–383
3 Eckert, R. (1972) Science 176, 473–481
4 Onimaru, H., Ohki, K., Nozawa, Y. and Naitoh, Y. (1980) Proc. Jap. Acad. 56, 538–543
5 Naitoh, Y. and Kaneko, H. (1973) J. Exp. Biol. 58, 657–675
6 Yazawa, M., Yagi, K., Toda, H., Kondo, K., Narita, K., Yamazaki, R., Sobue, K., Kakiuchi, S., Nagao, S. and Nozawa, Y. (1981) Biochem. Biophys. Res. Commun. 99, 1051–1057
7 Shimizu, T., Hatano, M., Muto, Y. and Nozawa, Y. (1984) FEBS Lett. 166, 373–377
8 Kakiuchi, S., Sobue, K., Yamazaki, R., Nagao, S., Umeki, S., Nozawa, Y., Yazawa, M. and Yagi, K. (1981) J. Biol. Chem. 256, 19–22
9 Muto, Y., Kudo, S. and Nozawa, Y. (1983) Biochem. Pharm. 32, 3559–3563
10 Klumpp, S., Kleefeld, G. and Schultz, J.E. (1983) J. Biol. Chem. 258, 12455–12459
11 Schultz, J.E. and Jantzen, H.M. (1980) FEBS Lett. 116, 75–78
12 Carafoli, E. and Crompton, M. (1978) Curr. Top. Membrane Transp. 10, 151–216
13 Nozawa, Y. and Thompson, G.A. (1971) J. Cell Biol. 49, 712–721
14 Nozawa, Y. (1975) in Methods in Cell Biology, Vol. 10 (Prescott, D.M., ed.), pp. 105–133, Academic Press, New York
15 Fukushima, H., Watanabe, T., Sasaki, N., Takeda, T. and Nozawa, Y. (1983) FEBS Lett. 161, 261–264
16 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
17 Pershadsingh, H.A. and McDonald, J.M. (1980) J. Biol. Chem. 255, 4087–4093
18 Chen, P.S., Toribara, T.Y. and Warner, H. (1956) Anal. Chem. 28, 1756–1758
19 Schuurmans Stekhoven, F. and Bonting, S.L. (1981) Physiol. Rev. 61, 1–76
20 Jones, L.R., Besch, H.R. and Wanabe, A.M. (1977) J. Biol. Chem. 252, 3315–3323
21 Duggan, P.F. (1977) J. Biol. Chem. 252, 1620–1627
22 O'Neal, S.G., Rhoads, D.B. and Racker, E. (1979) Biochem. Biophys. Res. Commun. 89, 845–850
23 Niggli, V., Adunyah, E.S., Penniston, J.T. and Carafoli, E. (1981) J. Biol. Chem. 256, 395–401
24 Nagao, S., Kudo, S. and Nozawa, Y. (1983) Biochem. Pharm. 32, 2501–2504

25 Chua, B., Elson, C. and Shrago, E. (1977) J. Biol. Chem. 252, 7548–7554
26 Knowles, A.F. and Racker, E. (1975) J. Biol. Chem. 250, 3538–3544
27 Breitbart, H., Stern, B. and Rubinstein, S. (1983) Biochim. Biophys. Acta 728, 349–355
28 Colca, J.R., Kotagal, N., Lacy, P.E. and McDaniel, M.L. (1983) Biochim. Biophys. Acta 729, 176–184
29 Watanabe, Y. and Nozawa, Y. (1982) in Calcium and Cell Function, Vol. 2 (Cheung, W.Y., ed.), pp. 297–323, Academic Press, New York
30 Nandini-Kishore, S.G. and Thompson, G.A., Jr. (1979) Proc. Natl. Acad. Sci. USA 76, 2708–2711
31 Nilsson, J.R. (1972) C.R. Trav. Lab. Carlsberg 39, 83–100
32 Allewell, N.M., Oles, J. and Wolfe, J. (1976) Exp. Cell Res. 97, 394–405
33 Kusamran, K., Mattox, S.M. and Thompson, G.A., Jr. (1980) Biochim. Biophys. Acta 598, 16–26
34 Doughty, M.J. (1978) Comp. Biochem. Physiol. 60B, 339–345
35 Andrivon, C., Brugerolle, G. and Delachambre, D. (1983) Biol. Cell 47, 351–364
36 Doughty, M.J. and Kaneshiro, E.S. (1983) J. Protozool. 30, 565–573
37 Bilinski, M., Plattner, H. and Tiggemann, R. (1981) Eur. J. Cell Biol. 24, 108–115
38 Tiggemann, R. and Plattner, H. (1982) FEBS Lett. 148, 226–230
39 Browning, J.L. and Nelson, D.L. (1976) Biochim. Biophys. Acta 448, 338–351
40 Riddle, L.M., Rauh, J.J. and Nelson, D.L. (1982) Biochim. Biophys. Acta 688, 525–540