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The ATP-driven Na^+-pump in the plasma membrane of the marine unicellular alga, *Platymonas viridis*

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Abstract

The ATP-supported $^{22}NA^+$ uptake by plasma membrane vesicles from the marine microalga, *Platymonas viridis*, was studied. At pH 7 in the medium, Na^+ uptake did not occur in the presence of ATP although ΔpH across the plasma membrane was generated. The ATP-dependent Na^+ uptake was induced by adding the protonophore, ClCCP. At pH 8, Na^+ uptake took place when ATP was added even without ClCCP. The ΔpH generated across the plasma membrane was negligible under these conditions. The Na^+ uptake at pH 8 was not affected by ClCCP and amiloride, an inhibitor of the Na^+/H^+ antiporter. It is concluded that the ATP-supported Na^+ uptake by *Pl. viridis* vesicles is catalyzed by Na^+-ATPase.

Key words: Na^+-pump; Plasmalemma; Marine alga; *Platymonas viridis*

1. Introduction

It is widely accepted that the Na^+/H^+ antiporter in the plasma membrane (PM) extrudes cellular Na^+ in exchange for H^+ influx and is energized by $\Delta\bar{\mu}H$ generated by the H^+-ATPase. Most experiments demonstrating the H^+-pump and Na^+/H^+ exchange in PM were performed on aminals, glycophytes, fungi, characean algae, and bacteria [1–5].

The question arose as to whether the PM H^+-ATPase and the Na^+/H^+ antiporter exert effective control over cytoplasmic Na^+ content in the cells of halotolerant microalgae, or whether some specialized Na^+-transferring systems exists in PM of these organisms. Our previous studies with highly purified PM vesicles from *Platymonas viridis* revealed the H^+-pump and the secondary Na^+/H^+ antiporter in these membranes [6]. Earlier, the Na^+/H^+ antiporter had been found in PM from the extreme halotolerant microalga, *Dunaliella salina* [7].

Indications suggesting a primary Na^+-pump functioning in the PM of halotolerant algae are also available. Namely, Na^+ transport in intact cells of *Dunaliella maritima* [8] and *Pl. viridis* [9] did not quite conform to the mechanism of the secondary Na^+/H^+ antiport. Na^+ extrusion demonstrated a low sensitivity to ClCCP and occurred at alkaline pH of the medium, i.e. under conditions unfavorable for Na^+ extrusion by the Na^+/H^+ antiporter. Studies on isolated PM vesicles from *Dunaliella* [10–12] and *Platymonas* [13] showed that the properties of ATP hydrolysis did not fully correspond to those of conventional plant H^+-ATPase of the p-type. The discrepancies might be connected with the functioning of two ATPases in the PM of halotolerant microalgae.

The idea of a second ATPase in the PM of halotolerant algae has been strongly supported by data from Wada et al. [14] obtained with PMs from the unicellular marine alga, *Heterosigma akashivo*. By using acidic polyacrylamide gel electrophoresis, the authors showed that PM 95 and 150 kDa polypeptides form acyl-phosphate bonds in the presence of ATP. Phosphorylation of the 150 kDa polypeptide required Na^+. According to [14], the 95 kDa polypeptide is similar to the PM H^+/K^+-ATPase from higher plants, and the 150 kDa polypeptide is the Na^+-activated ATPase similar to the Na^+,K^+-ATPase from animals.

In this paper, we have found ATP-dependent $^{22}Na^+$ accumulation by PM vesicles from *Pl. viridis* in the presence of protonophore and in the absence of ΔpH, i.e. in a proton motive force-independent fashion.

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Abbreviations: $\Delta\bar{\mu}H$, electrochemical H^+ potential difference; AO, Acridine orange; BTP, 1,3-bis-tris(hydroxymethyl)methylaminopropane; ClCCP, *m*-chlorocarbonylcyanide phenylhydrazone; DTT, dithiothreitol; EGTA, ethylenglycol-bis(*β*-aminoethyl ether)*N,N,N',N'* tetraacetic acid; HEPES, *N*-2-hydroxyethylpiperasine-*N*-2-ethanesulphonic acid; MES, 2-(*N*-morpholino)ethanesulphonic acid; PM, plasma membrane; PMSF, phenylmethylsulphonylfluoride; TI, trypsin inhibitor, chicken egg white purified ovomucoid; Tris, tris(hydroxymethyl)aminomethane.

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2. Materials and methods

Pl. viridis cells were cultured in artificial sea water containing 0.46 M NaCl as described earlier [13].

The highly purified PM vesicles were prepared according to the method of [13] based on the partial proteolysis of the glycoprotein cell wall by trypsin followed by hypo-osmotic shock and subsequent membrane partitioning by differential centrifugation and centrifugation in a discontinuous sucrose gradient. Here, we employed this method with slight modifications. Fresh distilled glycerol was used at all stages of PM preparation. Cells were shocked hypo-osmotically in a solution of 0.5 M glycerol, 20 mM Tris-MES (pH 7.2), 2.5 mM $K_2S_2O_5$, 1 mM DTT, 0.5 mM ATP, 2 mM $MgCl_2$, 0.4 mM EGTA, 0.2 mg/ml TI, and 10 μg/ml PMSF. PM-enriched fractions from the sucrose gradient were collected by centrifugation, and the final membrane pellet was suspended in a medium of 0.5 M mannitol, 5 mM BTP-HEPES, 1 mM DTT, 0.2 mM EGTA, 2 mM $MgCl_2$, and 10 μg/ml PMSF. The pH of this medium was adjusted to 7 or 8 as required for the $^{22}Na^+$ transport experiments.

The ATP-dependent formation of the interior-acid pH gradient across the PM was assayed by monitoring the changes in absorbance of Acridine orange (AO) as described previously [6]. The assay was performed in 2 ml of reaction mixture containing 0.5 M mannitol, 25 mM BTP-NO_3^-, 20 mM $MgCl_2$, 8 μM AO, and the vesicles (20–30 μg protein).

The $^{22}Na^+$ uptake by vesicles was measured at room temperature in 260 μl of the assay mixture containing 0.5 M mannitol, 25 mM BTP-NO_3^-, 20 mM $MgCl_2$, and vesicles (180–250 μg protein). The labelled Na^+ was added to the assay mixture as $^{22}NaCl$ up to 0.5 MBq. Samples were pre-incubated for 30 min to allow the added Na^+ to equillibrate across the vesicle membrane, then ATP was added to initiate the Na^+ uptake. After defined time intervals, aliquots of the suspension (60 μl) were taken, and vesicles were separated from the medium by filtering through Synpore nitrocellulose filters with a pore size of 0.6 μm. Filters were washed three times with 1 ml assay solution free of label and ATP, and radioactivity was counted in a scintillation counter.

The protein content was assayed after Simpson and Somme [15].

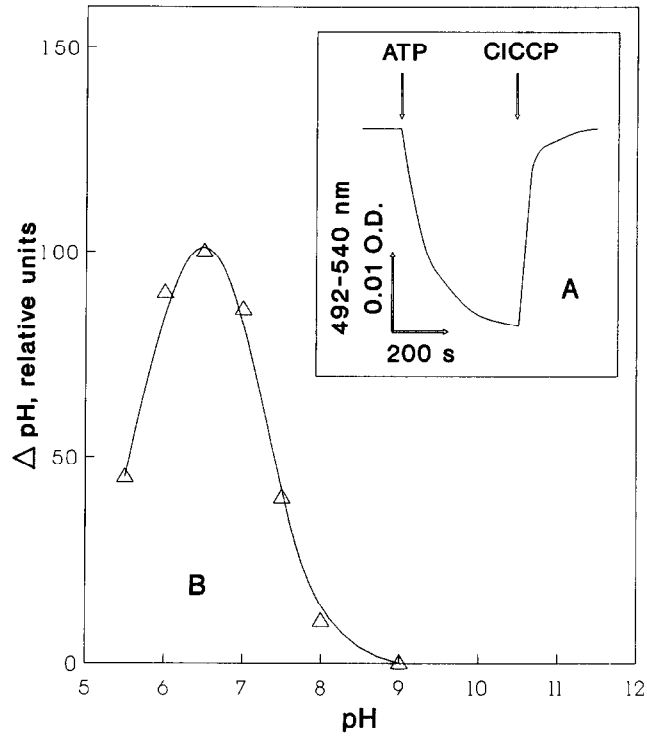

Fig. 1. ATP-dependent ΔpH generation across PM vesicles from *Pl. viridis*. (A) ATP-dependent intravesicular acidification monitoring by changes in absorbance of Acridine orange at pH 7. Additions: ATP, 2 mM; ClCCP, 12 μM. (B) The ΔpH generated across the PM in the presence of ATP (2 mM) as a function of assay medium pH.

3. Results and discussion

The conventional Na^+/H^+ antiporter is energized by the proton motive force. In contrast, operation of a primary Na^+-pump does not require any proton motive force. To discriminate between these Na^+-translocating systems, ATP-dependent transport of H^+ and Na^+ was measured in everted PM vesicles of *Pl. viridis*.

Fig. 1A shows the H^+ uptake by the vesicles, assayed as a decrease in absorbance of AO, at pH 7 in the presence of ATP. ClCCP completely abolished the decrease in absorbance. The result indicates the generation of an ATP-dependent ΔpH across the PM under these conditions. Fig. 1B presents the generated ΔpH as a function of external pH. Maximal ΔpH values are observed at pH 6–7 coinciding with the maximum of the pH profile of the PM ATPase activity found in *Platymonas* PM (see [13] and Fig. 7 therein). However, unlike the pH profile of the ATPase activity, the ΔpH/pH function drops abruptly at alkaline pH, being negligible at pH 8. The inability of the *Platymonas* PM vesicles to form an ATP-supported ΔpH at alkaline pH is most probably a result of high H^+-conductivity of PMs at this pH [16].

Fig. 2 shows the $^{22}Na^+$ uptake at pH 7, which is favorable for ΔpH generation by the H^+-ATPase and, consequently, for operation of the Na^+/H^+ antiporter. Surprisingly, the addition of ATP did not result in sodium uptake by the vesicles. In other words, we failed to demonstrate ATP-supported sodium accumulation mediated by the Na^+/H^+ antiporter. This contradicts the results of previous experiments performed at pH 7 which showed a Na^+-dependent decay of the initially generated ΔpH across *Platymonas* PM by ATP [6].

ATP-supported Na^+ accumulation by vesicles at pH 7 was observed when the vesicles were pri-incubated with the Na^+/H^+ antiporter, monensin (Fig. 2). It still remains to be elucidated why the endogenous Na^+/H^+ antiporter is inactive under these experimental conditions. A similar phenomenon was observed on isolated PM vesicles from *Streptococcus faecalis* [17]. When an artificial pH gradient (interior acid) was imposed upon the membrane no sodium accumulation was seen except when the medium was supplemented with monensin. In our previous experiments on *Pl. viridis* vesicles, which demonstrated a ΔpH decay by Na^+, the ATP-dependent ΔpH had been formed before Na^+ was introduced into the medium [6]. In the Na^+ uptake experiments carried out in the present work, the ATP was added after Na^+ (Figs. 2 and 3). Perhaps pre-incubation for some time under high ΔpH is required to activate the native Na^+/H^+ antiporter of *Pl. viridis*.

Some ATP-supported $^{22}Na^+$ influx into *Platymonas* PM vesicles at pH 7 was also induced by the addition of

ClCCP (Fig. 2). The presence of ClCCP in the medium excluded ΔpH generation (see Fig. 1A) and, hence, involvement of the Na^+/H^+ antiporter in the ATP-supported Na^+ accumulation.

The ATP-supported $^{22}Na^+$ accumulation by *Platymonas* PM vesicles at pH 8 is presented in Fig. 3. Addition of ATP to the assay medium resulted in Na^+ uptake by the vesicles even in the absence of ClCCP or monensin. ClCCP and amiloride, an inhibitor of the *Pl. viridis* Na^+/H^+ antiporter [6], did not influence this process.

Thus, the results of this work indicate that *Pl. viridis* possesses an ATP-supported Na^+-translocating system that is not mediated by ΔpH. This system is apparently a primary electrogenic Na^+-pump (Na^+-ATPase). Electrogenicity of the pump is indicated by the fact that (i) ClCCP is required for the ATP-linked Na^+ uptake at neutral pH when the endogenous H^+ conductance is low, and (ii) ClCCP is not necessary at high pH when H^+ conductance is high. Apparently, under neutral pH conditions, the ClCCP-mediated H^+ efflux from the vesicles discharges the electrical potential difference generated by the Na^+-ATPase-mediated Na^+ influx. This allows for large-scale Na^+ uptake to occur. Under alkaline conditions, the H^+ efflux takes place even without ClCCP (for discussion, see [18]).

Taking into account the results of our previous paper [6], we can conclude that in *Platymonas* PM there are in fact two mechanisms of Na^+ export from the cell, i.e. (i) the conventional Na^+/H^+ antiporter energized by the proton motive force, and (ii) Na^+-ATPase.

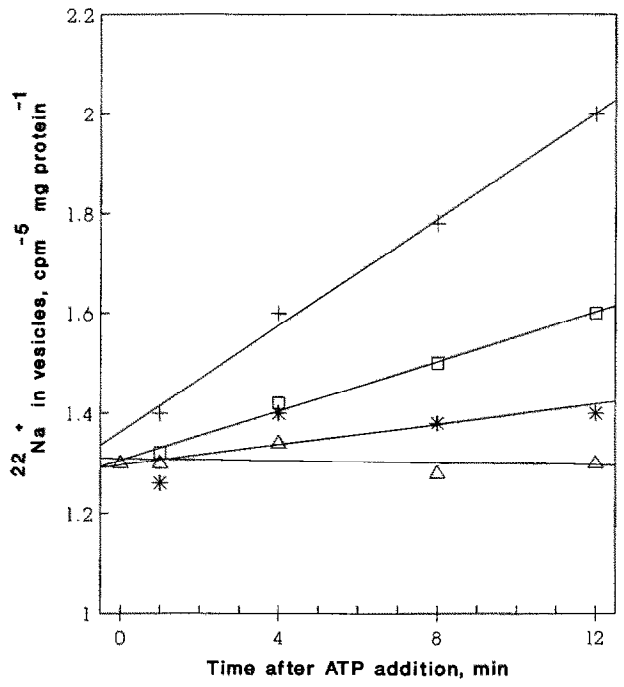

Fig. 2. ATP-dependent $^{22}Na^+$ uptake by PM vesicles from *Pl. viridis* at pH 7 with 20 mM NaCl in the assay medium. Additions: (△) none; (*) ATP, 2 mM; (□) ATP plus ClCCP, 12 μM; (+) ATP plus monensin, 12 μM. ATP was added at 0 min, ClCCP or monensin at −30 min.

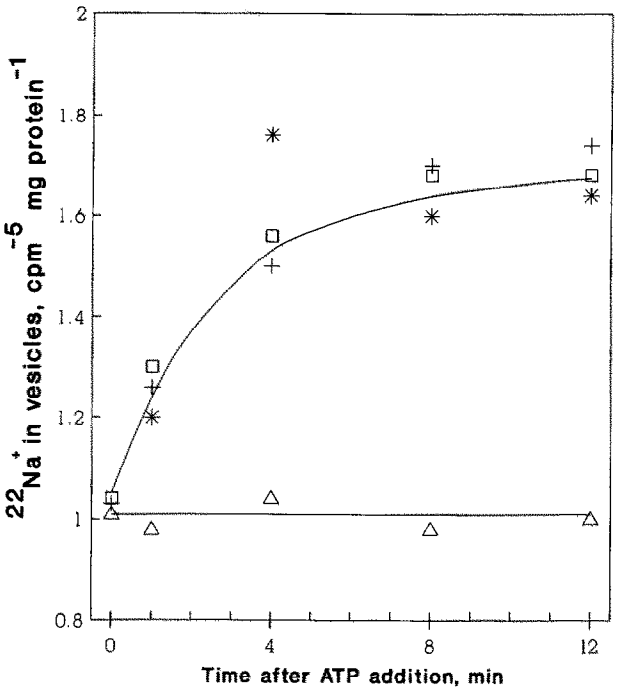

Fig. 3. ATP-dependent $^{22}Na^+$ uptake by PM vesicles from *Pl. viridis* at pH 8 and 50 mM NaCl in the assay medium. Additions: (△) none; (*) ATP, 2 mM; (□) ATP plus ClCCP, 12 μM; (+) ATP plus amiloride, 50 μM. ATP was added at 0 min, ClCCP or amiloride at −30 min.

The functioning of two Na^+-transporting systems, the Na^+/H^+ antiporter and the primary Na^+-pumps, in PMs is a feature inherent also in some prokaryotes living under high salinity. In this case, the former mechanism is operative at neutral pH and the latter at high pH [3,18]. Apparently, the presence of the Na^+-pump in the PM is related to adaptation of the organisms to changes in the surrounding conditions. The PM Na^+-pump of *Pl. viridis* operates probably when the ΔpH value is low or is oppositely directed (lower pH inside the cell), i.e. when Na^+ extrusion by the Na^+/H^+ antiporter is hardly possible.

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