

# The ATP-driven Na<sup>+</sup>-pump in the plasma membrane of the marine unicellular alga, *Platymonas viridis*

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## Abstract

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

**Key words:** Na<sup>+</sup>-pump; Plasmalemma; Marine alga; *Platymonas viridis*

## 1. Introduction

It is widely accepted that the Na<sup>+</sup>/H<sup>+</sup> antiporter in the plasma membrane (PM) extrudes cellular Na<sup>+</sup> in exchange for H<sup>+</sup> influx and is energized by ΔpH generated by the H<sup>+</sup>-ATPase. Most experiments demonstrating the H<sup>+</sup>-pump and Na<sup>+</sup>/H<sup>+</sup> exchange in PM were performed on animals, glycophytes, fungi, characean algae, and bacteria [1–5].

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

Indications suggesting a primary Na<sup>+</sup>-pump functioning in the PM of halotolerant algae are also available. Namely, Na<sup>+</sup> transport in intact cells of *Dunaliella maritima* [8] and *Pl. viridis* [9] did not quite conform to the mechanism of the secondary Na<sup>+</sup>/H<sup>+</sup> antiporter. Na<sup>+</sup> extrusion demonstrated a low sensitivity to CCCP and occurred at alkaline pH of the medium, i.e. under conditions unfavorable for Na<sup>+</sup> extrusion by the Na<sup>+</sup>/H<sup>+</sup> 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<sup>+</sup>-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<sup>+</sup>. According to [14], the 95 kDa polypeptide is similar to the PM H<sup>+</sup>/K<sup>+</sup>-ATPase from higher plants, and the 150 kDa polypeptide is the Na<sup>+</sup>-activated ATPase similar to the Na<sup>+</sup>,K<sup>+</sup>-ATPase from animals.

In this paper, we have found ATP-dependent <sup>22</sup>Na<sup>+</sup> 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:** ΔpH, electrochemical H<sup>+</sup> potential difference; AO, Acridine orange; BTP, 1,3-bis-tris(hydroxymethyl)methylaminopropane; CCCP, *m*-chlorocarbonylcyanide phenylhydrazone; DTT, dithiothreitol; EGTA, ethyleneglycol-bis(β-aminoethyl ether)*N,N,N',N'*-tetraacetic acid; HEPES, *N*-2-hydroxyethylpiperazine-*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.

## 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  $K_2S_2O_8$ , 1 mM DTT, 0.5 mM ATP, 2 mM  $MgCl_2$ , 0.4 mM EGTA, 0.2 mg/ml TI, and 10  $\mu$ g/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  $\mu$ g/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  $\mu$ M AO, and the vesicles (20–30  $\mu$ g protein).

The  $^{22}Na^+$  uptake by vesicles was measured at room temperature in 260  $\mu$ l of the assay mixture containing 0.5 M mannitol, 25 mM BTP- $NO_3^-$ , 20 mM  $MgCl_2$ , and vesicles (180–250  $\mu$ g 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 equilibrate across the vesicle membrane, then ATP was added to initiate the  $Na^+$  uptake. After defined time intervals, aliquots of the suspension (60  $\mu$ l) were taken, and vesicles were separated from the medium by filtering through Synpore nitrocellulose filters with a pore size of 0.6  $\mu$ m. 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].

## 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. CCCP completely abolished the decrease in absorbance. The result indicates the generation of an ATP-dependent  $\Delta pH$  across the PM under these conditions. Fig. 1B presents the generated  $\Delta pH$  as a function of external pH. Maximal  $\Delta pH$  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  $\Delta pH/pH$  function drops abruptly at alkaline pH, being negligible at pH 8. The inability of the *Platymonas* PM vesicles to form an ATP-supported  $\Delta pH$  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  $\Delta pH$  generation by the  $H^+$ -ATPase and, consequently, for operation of the  $Na^+/H^+$  antiporter. Surpris-

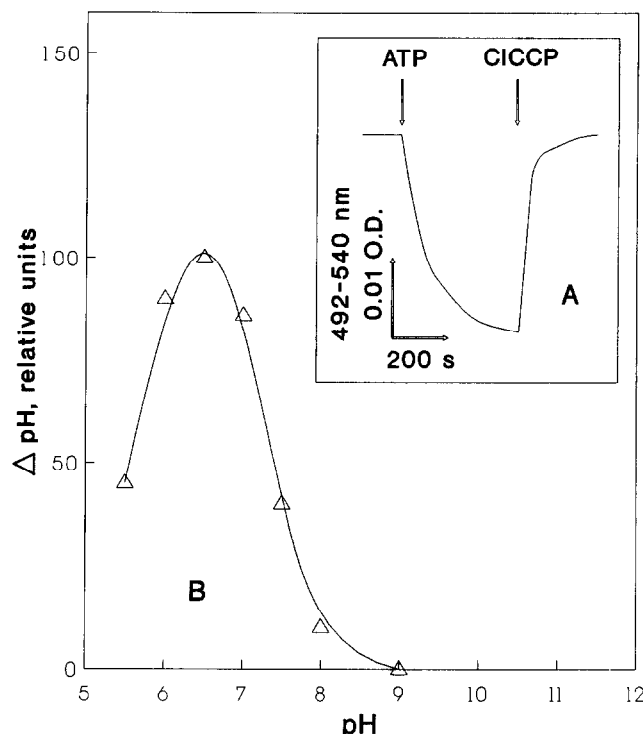


Fig. 1. ATP-dependent  $\Delta pH$  generation across PM vesicles from *Pl. viridis*. (A) ATP-dependent intravesicular acidification monitoring by changes in absorbance of Acridine orange at pH 7. Additions: ATP, 2 mM; CCCP, 12  $\mu$ M. (B) The  $\Delta pH$  generated across the PM in the presence of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

ingly, 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  $\Delta pH$  across *Platymonas* PM by ATP [6].

ATP-supported  $Na^+$  accumulation by vesicles at pH 7 was observed when the vesicles were pre-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  $\Delta pH$  decay by  $Na^+$ , the ATP-dependent  $\Delta pH$  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  $\Delta pH$  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

CICCP (Fig. 2). The presence of CICCP in the medium excluded  $\Delta pH$  generation (see Fig. 1A) and, hence, involvement of the  $\text{Na}^+/\text{H}^+$  antiporter in the ATP-supported  $\text{Na}^+$  accumulation.

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

Thus, the results of this work indicate that *Pl. viridis* possesses an ATP-supported  $\text{Na}^+$ -translocating system that is not mediated by  $\Delta pH$ . This system is apparently a primary electrogenic  $\text{Na}^+$ -pump ( $\text{Na}^+$ -ATPase). Electrogenicity of the pump is indicated by the fact that (i) CICCP is required for the ATP-linked  $\text{Na}^+$  uptake at neutral pH when the endogenous  $\text{H}^+$  conductance is low, and (ii) CICCP is not necessary at high pH when  $\text{H}^+$  conductance is high. Apparently, under neutral pH conditions, the CICCP-mediated  $\text{H}^+$  efflux from the vesicles discharges the electrical potential difference generated by the  $\text{Na}^+$ -ATPase-mediated  $\text{Na}^+$  influx. This allows for large-scale  $\text{Na}^+$  uptake to occur. Under alkaline conditions, the  $\text{H}^+$  efflux takes place even without CICCP (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  $\text{Na}^+$  export from the cell, i.e. (i) the conventional  $\text{Na}^+/\text{H}^+$  antiporter energized by the proton motive force, and (ii)  $\text{Na}^+$ -ATPase.

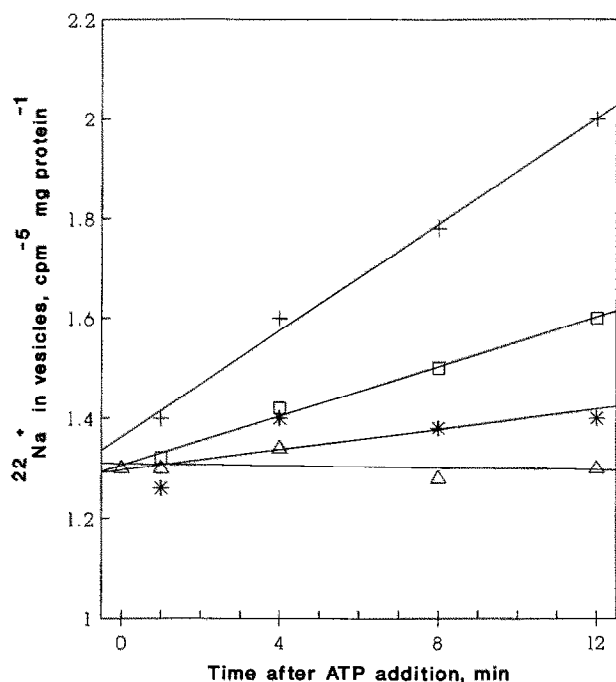


Fig. 2. ATP-dependent  $^{22}\text{Na}^+$  uptake by PM vesicles from *Pl. viridis* at pH 7 with 20 mM NaCl in the assay medium. Additions: (Δ) none; (∗) ATP, 2 mM; (◻) ATP plus CICCP, 12  $\mu\text{M}$ ; (+) ATP plus monensin, 12  $\mu\text{M}$ . ATP was added at 0 min, CICCP or monensin at  $-30$  min.

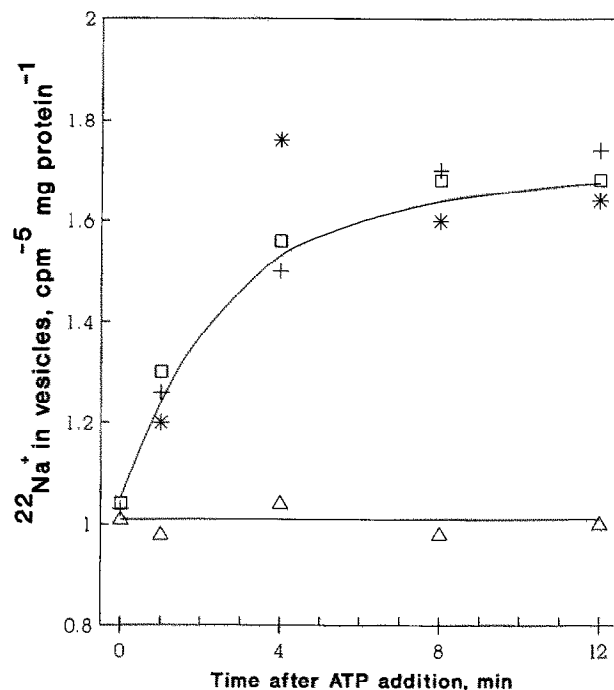


Fig. 3. ATP-dependent  $^{22}\text{Na}^+$  uptake by PM vesicles from *Pl. viridis* at pH 8 and 50 mM NaCl in the assay medium. Additions: (Δ) none; (∗) ATP, 2 mM; (◻) ATP plus CICCP, 12  $\mu\text{M}$ ; (+) ATP plus amiloride, 50  $\mu\text{M}$ . ATP was added at 0 min, CICCP or amiloride at  $-30$  min.

The functioning of two  $\text{Na}^+$ -transporting systems, the  $\text{Na}^+/\text{H}^+$  antiporter and the primary  $\text{Na}^+$ -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  $\text{Na}^+$ -pump in the PM is related to adaptation of the organisms to changes in the surrounding conditions. The PM  $\text{Na}^+$ -pump of *Pl. viridis* operates probably when the  $\Delta pH$  value is low or is oppositely directed (lower pH inside the cell), i.e. when  $\text{Na}^+$  extrusion by the  $\text{Na}^+/\text{H}^+$  antiporter is hardly possible.

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