Presence of a Na^+-stimulated P-type ATPase in the plasma membrane of the alkaliphilic halotolerant cyanobacterium *Aphanothece halophytica*

Kanjana Wiangnon[1], Wuttinun Raksajit[1] & Aran Incharoensakdi[1,2]

[1]Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand; and [2]Center for Environmental Stress Tolerance in Plants, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

Correspondence: Aran Incharoensakdi, Department of Biochemistry and Center for Environmental Stress Tolerance in Plants, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand. Tel.: +662 2185419; fax: +662 2185418; e-mail: aran.i@chula.ac.th

Received 13 October 2006; revised 11 January 2007; accepted 11 January 2007.
First published online 15 February 2007.

DOI:10.1111/j.1574-6968.2007.00667.x

Editor: Karl Forchhammer

Keywords
Na^+-stimulated ATPase; halotolerant cyanobacterium; salt stress; Na^+ extrusion.

Abstract

Aphanothece cells could take up Na^+ and this uptake was strongly inhibited by the protonophore, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). Cells preloaded with Na^+ exhibited Na^+ extrusion ability upon energizing with glucose. Na^+ was also taken up by the plasma membranes supplied with ATP and the uptake was abolished by gramicidin D, monensin or Na^+-ionophore. Orthovanadate and CCCP strongly inhibited Na^+ uptake, whereas *N*, *N′*-dicyclohexylcarbodiimide (DCCD) slightly inhibited the uptake. Plasma membranes could hydrolyse ATP in the presence of Na^+ but not with K^+, Ca^{2+} and Li^+. The K_m values for ATP and Na^+ were 1.66 ± 0.12 and 25.0 ± 1.8 mM, respectively, whereas the V_{max} value was $0.66 \pm 0.05\ \mu mol\ min^{-1}\ mg^{-1}$. Mg^{2+} was required for ATPase activity whose optimal pH was 7.5. The ATPase was insensitive to *N*-ethylmaleimide, nitrate, thiocyanate, azide and ouabain, but was substantially inhibited by orthovanadate and DCCD. Amiloride, a Na^+/H^+ antiporter inhibitor, and CCCP showed little or no effect. Gramicidin D and monensin stimulated ATPase activity. All these results suggest the existence of a P-type Na^+-stimulated ATPase in *Aphanothece halophytica*. Plasma membranes from cells grown under salt stress condition showed higher ATPase activity than those from cells grown under nonstress condition.

Introduction

Salinity is one of the most important problems encountered by soil microorganisms and plants leading to the reduction in crop productivity worldwide. Specific mechanisms are needed to adjust the internal osmotic status for organisms thriving in hypersaline environments. One such mechanism involves the ability of the cells to accumulate compatible low molecular weight organic solutes such as glycine betaine (Incharoensakdi & Wutipraditkul, 1999; Sakamoto & Murata, 2002). Another mechanism for adaptation to high salinity is the removal of Na^+ ions from the cytoplasm out of the cells via plasma membrane or into the vacuoles via tonoplast membrane (Apse & Blumwald, 2002). *Aphanothece halophytica* is a halotolerant cyanobacterium, which is able to grow in a wide range of salinity from 0.25 to 3.0 M NaCl and in external alkaline conditions up to an external pH of 11.0 (Takabe *et al*., 1988; Waditee *et al*., 2003). Previous studies showed that Na^+/H^+ antiporters of alkaliphilic *A. halophytica* play a crucial role in Na^+ efflux with enhanced salt tolerance (Waditee *et al*., 2001; Wutipraditkul *et al*., 2005). Moreover, the overexpression of the NhaP antiporter from *A. halophytica* could allow the freshwater cyanobacterium *Synechococcus* to grow in sea water (Waditee *et al*., 2002). The efflux of Na^+ mediated by Na^+/H^+ is categorized as a secondary Na^+ transport because it utilizes proton motive force provided by a primary proton pump, H^+-ATPase, as a driving force.

In most plant cells, the extreme of Na^+ was mediated primarily through the Na^+/H^+ antiport system. However, there have been recent reports on the involvement of a primary Na^+ pump, Na^+-ATPase, in the efflux of Na^+ in bacteria and higher plants. V-type Na^+-ATPases were found in *Enterococcus hirae* and M-12, *Amphibacillus* sp. (Kaieda *et al*., 1998; Murata *et al*., 2001) whereas F-type ATPases were found in *Ilyobacter tartaricus* and *Acetobacterium woodii* (Neumann *et al*., 1998; Muller *et al*., 2001). An alkaliphilic *Exiguobacterium aurantiacum* contains a P-type Na^+-ATPase (Ueno *et al*., 2000). P-type Na^+-ATPases were also found in the plasma membranes of marine algae, *Heterosigma akashiwo* and *Tetraselmis viridis* (Shono *et al*., 1996; Popova *et al*.,

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Downloaded from https://academic.oup.com/femsle/article/270/1/139/599887 by guest on 23 April 2024

1998). Studies on ATPase in cyanobacteria were scarce. P-type ATPase gene was cloned from *Synechococcus* 7942 whereas its function was found to be involved in Cu^{2+} transport (Phung *et al.*, 1994). P-type ATPases were also reported in more freshwater cyanobacteria such as *Synechococcus* PCC 6301, *Synechocystis* PCC 6803 and *Anabaena* PCC 7120 (Neisser *et al.*, 1994). A P-type ATPase from *Synechococcus* PCC 7942 with a proposed role in K^+ influx under hyperosmotic condition has been reported (Kanamura *et al.*, 1993; Neisser *et al.*, 1994). ATPase with enhanced activity under hypersaline condition was reported in a marine cyanobacterium *Spirulina subsalsa* (Gabbay-Azaria *et al.*, 1994). Hitherto, ATPase involved in Na^+ transport has not been reported for cyanobacteria. This study demonstrates the presence of a P-type ATPase, specifically stimulated by Na^+. The transport of Na^+ by this cyanobacterium appeared to utilize the proton motive force generated by H^+-ATPase.

Materials and methods

Growth of organism

Axenic cells of *A. halophytica* were grown photoautotrophically in BG-11 medium supplemented with 18 mM $NaNO_3$ and Turk Island salt solution described previously (Incharoensakdi & Waditee, 2000). Cells were grown in cotton-plugged 250 mL conical flasks containing 100 mL medium on a rotary shaker at 30 °C under continuous illumination by cool white fluorescence tubes of 60 μmol photon m^{-2} s^{-1} without aeration.

Preparation of plasma membrane vesicles

Cells at exponential growth phase were harvested and suspended in 20 mM Tris-HCl pH 7.6 containing 1.0 M sucrose. The crude membrane vesicles were prepared by treatment of the cell suspension with 0.2% lysozyme and the resulting spheroplasts were subject to a French pressure cell at 900 psi. This crude membrane preparation was centrifuged twice (4000 ***g***, 20 min) to remove unbroken cells and cell debris. The resulting dark blue green supernatant is referred to as a mixture of crude vesicles. The plasma membrane vesicles were isolated by aqueous polymer two-phase partitioning using 5.6% (w/w) Dextran T-500 and 5.6% (w/w) polyethylene glycol as described by Norling *et al.* (1994). The final plasma membrane vesicles were suspended in 20 mM Tris-HCl pH 7.6 buffer containing 4 mM benzamidine and kept at − 80 °C until used.

Determination of Na^+ uptake by plasma membrane vesicles

The membrane vesicles were suspended in 1 mL of 20 mM Tris-HCl pH 7.6 (1 mg protein mL^{-1}) containing 20 mM $^{22}NaCl$ (0.45 μCi μmol^{-1}) with an illumination of 30 μmol photon m^{-2} s^{-1} provided by cool-white fluorescent lamps. After equilibration for 30 min, the mixture was added with 4 mM ATP to initiate the uptake of $^{22}Na^+$. Na^+-ionophore (ETH 2120, *N*,*N*,*N*′,*N*′,-tetracyclohexyl-1,2-phenylenedioxydiacetamide) and other ionophores including inhibitors were added at 10 min before the addition of ATP. At intervals, 50 μL of the reaction mixture was filtered through a 0.2 μm nitrocellulose membrane. The radioactivity trapped on the membrane filter was measured with a gamma counter.

ATPase activity

The activity of ATPase was assayed by measuring the release of inorganic phosphate resulting from the hydrolysis of ATP (Koyama *et al.*, 1980). The reaction mixture (1 mL) contained 20 mM Tris-HCl pH 7.6, 5 mM $MgCl_2$, 4 mM ATP, 100 mM NaCl and enzyme. The reaction was started by the addition of ATP.

Measurement of ATP and other methods

To determine ATP content in *A. halophytica*, cell suspensions were centrifuged at room temperature (2500 ***g***, 10 min) and the pellet was washed twice before suspending with 0.22 M phosphate buffer pH 6.8. This was followed by sonication to completely break the cells. Debris was removed and supernatant was determined for ATP by HPLC. The isocratic reverse phase HPLC with a water Resolve C18 (5-μM, spherical) column (Millipore, Milford, MA) was used and mobile phase was 0.22 M phosphate buffer pH 6.8. The eluate was monitored by absorption at 259 nm with a flow rate of 1 mL min^{-1}. ATP content was determined based on the standard curve constructed with the use of known concentrations of ATP injected vs. peak areas (Patil *et al.*, 1997). Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as standard.

In general, three independent experiments were performed and the mean values ± SEM are given in the figures.

Results

Na^+ uptake by intact cells and plasma membrane

To test whether *A. halophytica* could take up Na^+, the effect of NaCl at different concentrations on the uptake of Na^+ was investigated. *Aphanothece* cells showed an increased uptake of $^{22}Na^+$ with increasing concentration of $^{22}NaCl$ (Fig. 1a). Initial uptake rates at 2, 5, 10 and 20 mM NaCl were estimated to be 13.4 ± 0.5, 38.6 ± 1.6, 75.0 ± 3.1 and 130.3 ± 4.8 nmol min^{-1} mg^{-1}, respectively. The uptake of $^{22}Na^+$ was inhibited by the protonophore carbonyl cyanide

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Downloaded from https://academic.oup.com/femsle/article/270/1/139/599887 by guest on 23 April 2024

m-chlorophenylhydrazone (CCCP) (Fig. 1a, inset). Slight uptake of $^{22}Na^+$ in the CCCP-treated *Aphanothece* cells was observed at 10 and 20 mM NaCl, which might reflect diffusion of Na^+ by sodium symport systems.

Aphanothece cells also possessed Na^+ extrusion capacity upon energizing with glucose (Fig. 1b). Cells showed an increased intracellular content of $^{22}Na^+$ with increasing time of incubation with 20 mM ^{22}NaCl. Addition of glucose to the cell suspension at 10 min resulted in a slight decrease of intracellular $^{22}Na^+$ at 15 min. An almost complete loss of intracellular $^{22}Na^+$ was evident at 20 min, suggesting $^{22}Na^+$ extrusion capacity of *Aphanothece* cells. To determine the involvement of ATP in the uptake and extrusion of Na^+, the levels of ATP in *Aphanothece* cells with and without glucose during the transport process were measured. In deenergized cells, ATP levels were decreased (Fig. 1b, inset) concomitant with the increase of Na^+ uptake (Fig. 1b). Similarly, the energized cells also showed the decreased ATP levels (Fig. 1b, inset) accompanying the increase of Na^+ extrusion capacity (Fig. 1b). $^{22}Na^+$ uptake was also demonstrated using plasma membrane vesicles upon addition of 4 mM ATP (Fig. 1c). An increase of $^{22}Na^+$ uptake into the vesicles was observed with saturation at around 6 min of incubation. The uptake of $^{22}Na^+$ was dependent on ATP. Na^+-ionophore as well as gramicidin D and monensin abolished the uptake of $^{22}Na^+$. The protonophore, CCCP and orthovanadate strongly inhibited $^{22}Na^+$ uptake. In contrast, *N*, *N′*-dicyclohexylcarbodiimide (DCCD) caused no inhibition of $^{22}Na^+$uptake in the first 4 min although slight inhibition was observed afterwards.

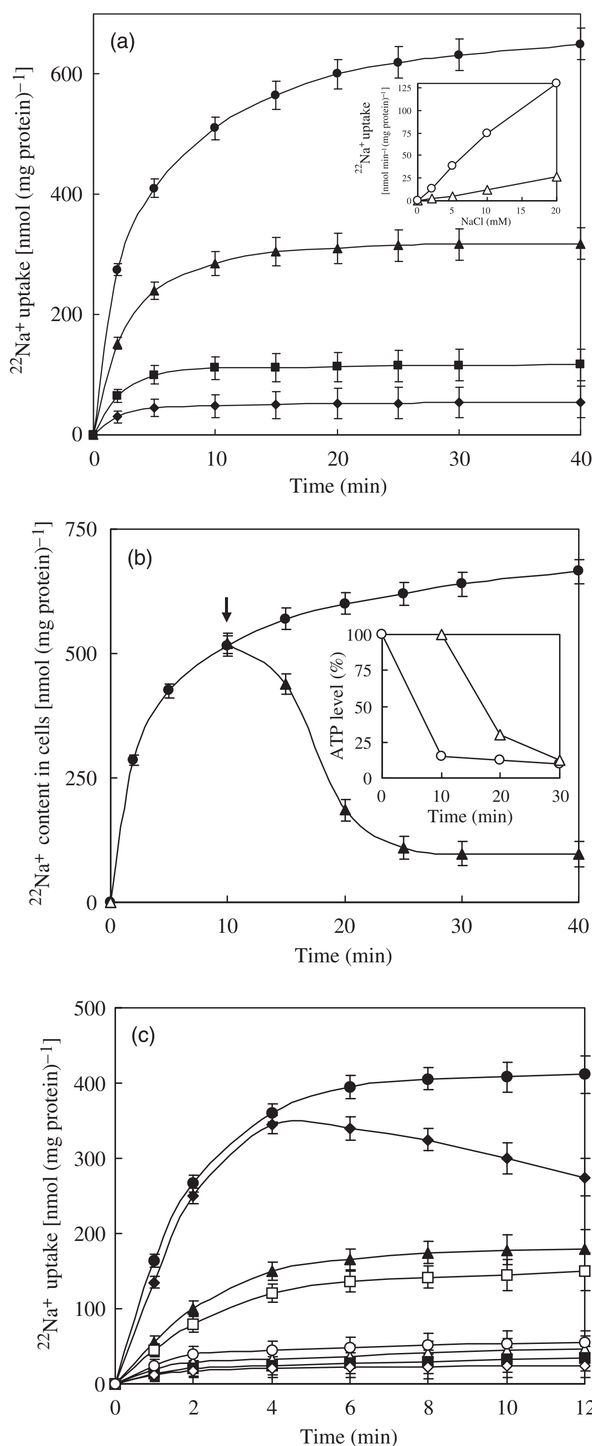

Fig. 1. Na^+ uptake (a), Na^+ extrusion (b) in *Aphanothece halophytica* and Na^+ uptake in plasma membrane vesicles (c). In (a), cells at the exponential growth phase (2 mg protein mL^{-1}) were incubated in 20 mM Tris-HCl buffer pH 7.6 containing 2 (♦), 5 (■), 10 (▲) and 20 mM (●) ^{22}NaCl (0.45 µCi µmol^{-1}). At indicated times, the radioactivity trapped in the cells after filtering through a 0.45 µm nitrocellulose membrane was counted in a gamma counter. Inset shows $^{22}Na^+$ uptake rates at different concentrations of ^{22}NaCl without treatment (○) or pretreated 10 min with 1 mM CCCP (△). In (b), the experiments were carried out as in (a) with 20 mM ^{22}NaCl (●). At 10 min, the reaction mixture was divided into two portions with the addition of 10 mM glucose (indicated by arrow) to one portion (▲). Inset shows ATP levels of deenergized (no glucose) cells (○) and energized (with 10 mM glucose) cells (△) at indicated times of Na^+ uptake and Na^+ extrusion. In (c), the membrane vesicles (1 mg protein mL^{-1}) were incubated in 20 mM Tris-HCl buffer pH 7.6 containing 20 mM ^{22}NaCl (0.45 µCi µmol^{-1}) with 4 mM ATP (●), without ATP (◇), with 4 mM ATP plus 1 mM DCCD (♦), with 4 mM ATP plus 1 mM CCCP (▲), with 4 mM ATP plus 10 µM orthovanadate (□), with 4 mM ATP plus 100 µM monensin (○), with 4 mM ATP plus 25 µM Na^+-ionophore (■) and with 4 mM ATP plus 10 µM gramicidin D (△).

Na^+-dependent ATPase

Before the characterization of ATPase, it was necessary to determine the ratio of the inside-out and right-side-out vesicles in the membrane preparations. This was accomplished by measuring ATPase activity of the prepared membrane vesicles treated with 3% toluene according to

Downloaded from https://academic.oup.com/femsle/article/270/1/139/599887 by guest on 23 April 2024

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

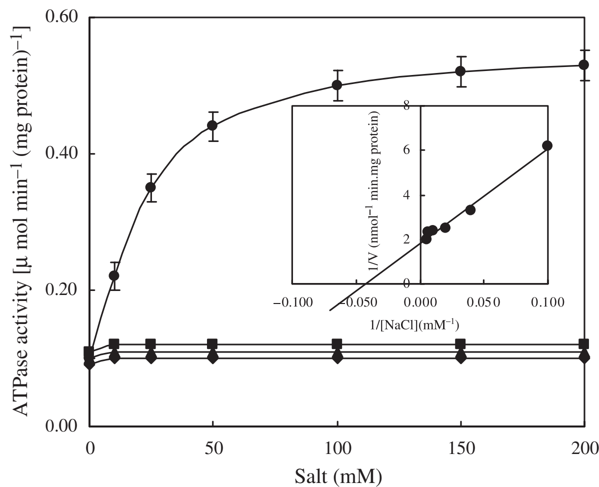

Fig. 2. Effect of cations on ATPase. Membrane vesicles (1 mg protein mL^{-1}) were incubated in 20 mM Tris-HCl buffer pH 7.6 containing 4 mM ATP, 5 mM $MgCl_2$ with NaCl (●), KCl (■), $CaCl_2$ (▲) and LiCl (◆) at indicated concentrations. Inset shows a double reciprocal plot of the activity against the NaCl concentration.

Heise *et al.* (1992). Toluene destroyed the membrane integrity resulting in increased ATPase activities if the membrane preparations contained right-side-out vesicles. The stimulation of ATPase activity by toluene treatment (data not shown) could not be observed, indicating that the membrane preparations consisted almost entirely of inside-out vesicles.

The ATPase activity was only marginal without the addition of NaCl (Fig. 2). Increasing NaCl concentration led to an increase of ATPase activity with saturation at about 100 mM. No stimulation of the activity was observed by the addition of monovalent cations such as K^+ or Li^+ or divalent cation such as Ca^{2+}, suggesting that ATPase was specific for Na^+. The K_m value for Na^+ was estimated from the Lineweaver–Burke plot (Fig. 2, inset) to be 25.0 ± 1.8 mM. The ATPase activity increased with the ATP concentration with saturation being reached at about 4 mM (Fig. 3a). The K_m value for ATP and V_{max} estimated by the Lineweaver–Burke plot were 1.66 ± 0.12 mM and 0.66 ± 0.05 µmol min^{-1} mg^{-1} protein, respectively (Fig. 3a, inset). The ATPase activity required the presence of $MgCl_2$ with optimal activity at 5 mM (Fig. 3b). Concentration of $MgCl_2$ higher than 5 mM led to a decline in ATPase activity. Optimal pH for the ATPase was around pH 7.5 (Fig. 3c). It is noted that considerable ATPase activity was retained at pH 9.

It was also checked whether salt stress could affect Na^+-stimulated ATPase activity of the plasma membrane. Na^+-stimulated ATPase was present in high amounts in cells grown at low salt and stimulated slightly in cells grown at high salt (Fig. 4).

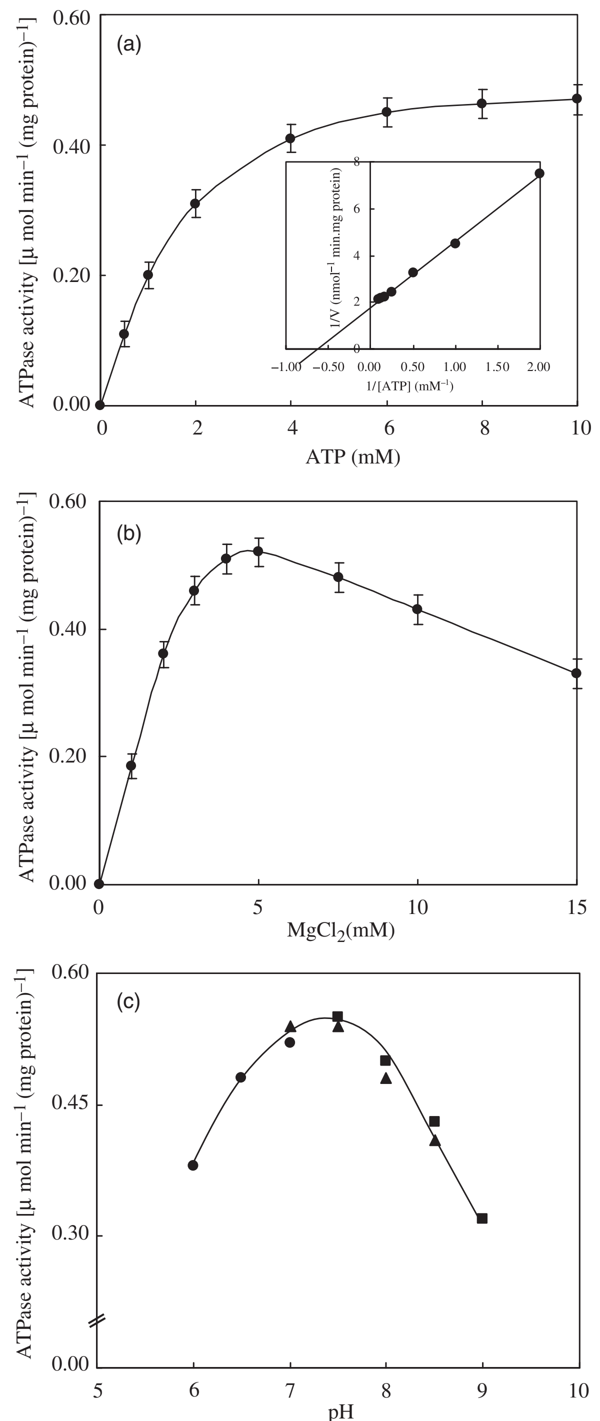

Fig. 3. Dependence of ATPase on ATP, Mg^{2+} and pH. Membrane vesicles (1 mg protein mL^{-1}) were incubated in 20 mM Tris-HCl buffer pH 7.6 containing 100 mM NaCl, 5 mM $MgCl_2$ and indicated concentrations of ATP (a), or containing 100 mM NaCl, 4 mM ATP and indicated concentrations of $MgCl_2$ (b), or containing 100 mM NaCl, 4 mM ATP, 5 mM $MgCl_2$, at indicated pH values (c) of 6.0–7.0 (●, 20 mM Mes-KOH buffer), 7.0–8.5 (▲, 20 mM Hepes-KOH) and 7.5–9.0 (■, 20 mM Tris-HCl). Inset in (a) shows a double-reciprocal plot of the activity against the ATP concentration.

Downloaded from https://academic.oup.com/femsle/article/270/1/139/599887 by guest on 23 April 2024

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

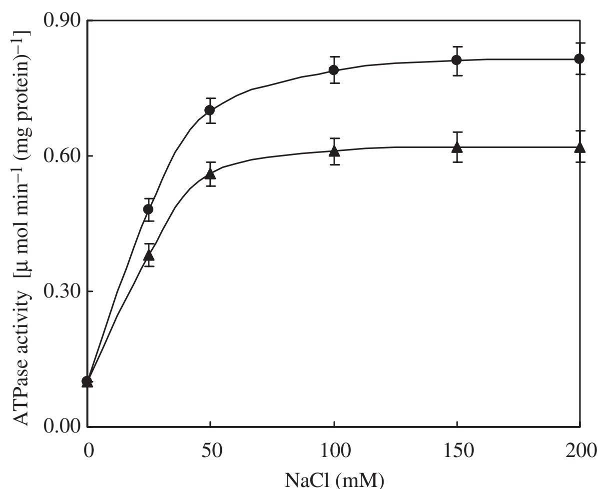

Fig. 4. Effect of salt stress during growth on Na^+-ATPase. Membrane vesicles were prepared from cells grown under nonstress condition (▲) with 0.5 M NaCl or under salt-stress condition (●) with 2.0 M NaCl. The ATPase assay was carried out as described in 'Materials and methods' at indicated concentrations of NaCl.

Table 1. Effect of inhibitors on the Na^+-ATPase of plasma membrane vesicles*

Reagent	Concentration (mM)	Remaining activity (%)
None	–	100
NEM	0.5	98
KNO_3	50	98
KSCN	20	88
Azide	5	100
CCCP	1	95
Ouabain	1	96
Amiloride	0.2	85
Orthovanadate	0.01	52
DCCD	1	63
Gramicidin D	0.001	135
Monensin	0.001	120

*ATP hydrolytic activity was assayed as described in 'Materials and methods'. Each inhibitor was added to the reaction mixture 10 min before the start of the reaction. One hundred percent activity corresponded to 0.48 μmol min^{-1} mg^{-1}.

Effect of inhibitors on ATPase

N-Ethylmaleimide, KNO_3 and KSCN, which are inhibitors of V-type ATPase, had little or no effect on ATPase (Table 1). Similarly, no inhibition was caused by either azide, an inhibitor of F-type ATPase, or the protonophore CCCP. Ouabain, an animal Na^+/K^+-ATPase inhibitor, did not inhibit ATPase. Amiloride, a potent inhibitor of many Na^+-coupled transport systems including Na^+/H^+ antiporter, had no significant effect on ATPase. However, orthovanadate, an inhibitor of P-type ATPase, and DCCD could inhibit ATPase. Na^+-gradient dissipators, gramicidin D and monensin, caused some stimulation of ATPase. All the results suggested that *A. halophytica* contains a P-type ATPase.

Discussion

This study demonstrated that *A. halophytica* could take up Na^+ in a concentration-dependent fashion (Fig. 1a). The uptake of Na^+ by *Aphanothece* cells could be strongly inhibited by the protonophore CCCP, suggesting the involvement of electrochemical proton gradient in the uptake system (Fig. 1a, inset). Furthermore, *Aphanothece* cells also had Na^+ extrusion capacity when energized with glucose (Fig. 1b). The utilization of glucose for growth has been reported in some photoautotrophic cyanobacteria, i.e. *Anabaena variabilis* (Ohki & Katoh, 1975), *Aphanocapsa* 6714 (Pelroy *et al.*, 1972) and *Plectonema boryanum* (White & Shilo, 1975). Previously, the uptake of nitrate in the presence of glucose was shown to increase in the starved *Aphanothece* cells (Incharoensakdi & Laloknam, 2005), suggesting that glucose could be utilized as energy source by *A. halophytica*.

The Na^+ extrusion is likely due to the contribution by ATPase because glucose can yield ATP during its metabolism. The decreased ATP levels inside the cells during the course of Na^+ uptake (Fig. 1b, inset) also support the contention that ATP was hydrolyzed by ATPase to provide a driving force for Na^+ uptake and Na^+ extrusion. This was further substantiated by Na^+ uptake experiments using membrane vesicles (Fig. 1c). Inhibition of ATPase by orthovanadate resulted in strong inhibition of Na^+ uptake. Na^+-stimulated ATPase activity was observed in plasma membrane vesicles (Fig. 2). This ATPase was specific for Na^+ with no activity toward K^+, Li^+ and Ca^{2+}. The ATPase was inhibited by orthovanadate and DCCD, whereas no inhibition was observed by CCCP, amiloride, KNO_3, KSCN, *N*-ethylmaleimide, azide and ouabain (Table 1). On the other hand, both gramicidin D and monensin, which can dissipate electrochemical sodium ion gradient, were able to stimulate *Aphanothece* ATPase. This is to be expected because the rate of ATP hydrolysis is controlled by ion potential, i.e. the lower the potential, the higher the activity. Taken together, it is thus likely that the Na^+-stimulated ATPase in *A. halophytica* is a P-type ATPase.

The results in Fig. 1c provide strong evidence for the coupling of ATPase and Na^+ transport. An increased uptake of $^{22}Na^+$ occurred upon the addition of ATP. The hydrolysis of ATP by ATPase is the driving force for Na^+ transport. Furthermore, the abolition of Na^+ transport observed under conditions when ATP was added in the presence of either the Na^+ ionophore or gramicidin D or monensin suggested that Na^+ gradient might be involved in the transport. Strong inhibition of Na^+ uptake by CCCP (Fig. 1c) indicated that

Downloaded from https://academic.oup.com/femsle/article/270/1/139/599887 by guest on 23 April 2024

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

μH^+-dependent Na^+/H^+ antiporter might be involved in the ATP-dependent Na^+ transport. The participation of the P-type ATPase in Na^+ transport is also suggested by the strong suppression of Na^+ uptake by orthovanadate (Fig. 1c).

Membrane vesicles prepared from *Aphanothece* cells grown under stress condition (2.0 M NaCl) showed higher Na^+-ATPase activity than that from vesicles of nonstressed (0.5 M NaCl) cells (Fig. 4). Similar observation was reported in a marine cyanobacterium *Spirulina subsalsa* subject to prolonged adaptation to hypersaline conditions (Gabbay-Azaria *et al.*, 1994).

Previous studies on the response of *A. halophytica* to an increase in salinity showed the increased expression of Na^+/H^+ antiporters under salt stress conditions (Waditee *et al.*, 2001; Wutipraditkul *et al.*, 2005). At least three Na^+/H^+ antiporters, namely Nha P1, Nap A1-1 and Nap A1-2, have been identified and characterized. NhaP-type antiporter is more efficient than NapA1-type antiporter for the adaptation to salt stress. The former also retains high activity over a pH range of 6.0–9.0 whereas the latter shows a decline in activity toward the acidic pH. It is of interest to note that Na^+-stimulated ATPase in the present study also retained high activity over a pH range of 6.0–9.0 although a peak activity occurred at pH 7.5 (Fig. 3c).

Organisms thriving in external high salinity and alkaline pH where proton motive force is not large enough utilize primary Na^+ pumps for Na^+ extrusion (Gimmler, 2000). Whether this is the case for *A. halophytica*, which can thrive under high salinity and alkaline pH, remains to be further investigated. Monovalent cation/proton antiporters play dominant roles in alkaline pH homeostasis in cells that have several antiporters catalyzing similar reactions (Padan *et al.*, 2005). For alkaliphiles under alkaline pH conditions, uptake of H^+ by Na^+/H^+ antiporter is important to keep the cytoplasmic pH neutral. The Na^+/H^+ antiporter would then extrude Na^+ out of the cells. To maintain H^+ homeostasis at alkaline pH, a reentry route for Na^+ to be a substrate for the Na^+/H^+ antiporter is required (Padan *et al.*, 2005). The Na^+/solute symporter could be a reentry route. The Na^+/betaine symporter has recently been reported in *A. halophytica* (Laloknam *et al.*, 2006). This Na^+/betaine symporter is beneficial to *A. halophytica* by providing Na^+ to serve as a substrate for Na^+/H^+ antiporter, thus maintaining H^+ homeostatsis under alkaline pH. At the same time, the symporter can take up betaine to serve as an osmolyte essential for the adjustment of osmotic balance under external high salinity.

In conclusion, the evidence obtained in the present study indicated that H^+-pump ATPase specific for and stimulated by Na^+ is involved in Na^+ uptake possibly mediated by Na^+/H^+ antiporter in *A. halophytica*. To search for the existence of a primary Na^+ pump in *A. halophytica*, further study using certain defined mutants such as the Na^+/H^+ antiporter deficient mutant is needed.

Acknowledgements

This work was supported by the Thailand Research Fund (BRG 4880004) to A. I. and partly by the scholarship from the Faculty of Graduate Studies of Chulalongkorn University to K. W.

References

Apse MP & Blumwald E (2002) Engineering of salt tolerance in plants. *Curr Opin Biotechnol* **13**: 146–150.

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254.

Gabbay-Azaria R, Pick U, Ben-Hayyim G & Tel-Or E (1994) The involvement of a vanadate-sensitive ATPase in plasma membranes of a salt tolerant cyanobacterium. *Physiol Plant* **90**: 692–698.

Gimmler H (2000) Primary sodium plasma membrane ATPase in salt-tolerant algae: facts and fictions. *J Exp Bot* **51**: 1171–1178.

Heise R, Muller V & Gottschalk G (1992) Presence of a Na^+-translocating ATPase in membrane vesicles of the homoacetogenic bacterium *Acetobacterium woodii*. *Eur J Biochem* **206**: 553–557.

Incharoensakdi A & Laloknam S (2005) Nitrate uptake in the halotolerant cyanobacterium *Aphanothece halophytica* is energy-dependent driven by ΔpH. *J Biochem Mol Biol* **38**: 468–473.

Incharoensakdi A & Waditee R (2000) Degradation of glycine betaine by betaine homocysteine methyltransferase in *Aphanothece halophytica*: effect of salt downshock and starvation. *Curr Microbiol* **41**: 227–231.

Incharoensakdi A & Wutipraditkul N (1999) Accumulation of glycine betaine and its synthesis from radioactive precursors under salt-stress in the cyanobacterium *Aphanothece halophytica*. *J Appl Phycol* **11**: 515–523.

Kaieda N, Wakagi T & Koyama N (1998) Presence of Na^+-stimulated V-type ATPase in the membrane of a facultatively anaerobic and halophilic alkaliphile. *FEMS Microbiol Lett* **167**: 57–61.

Kanamura K, Kashiwagi S & Mizuno T (1993) The cyanobacterium, *Synechococcus* sp. PCC 7942, possesses two distinct genes encoding cation-translocating P-type ATPase. *FEBS Lett* **330**: 99–104.

Koyama N, Koshiya K & Nosoh Y (1980) Purification and properties of ATPase from an alkalophilic *Bacillus*. *Arch Biochem Biophys* **199**: 103–109.

Laloknam S, Tanaka K, Buaboocha T, Waditee R, Incharoensakdi A, Hibino T, Tanaka Y & Takabe T (2006) Halotolerant cyanobacterium *Aphanothece halophytica* contains a betaine transporter active at alkaline pH and high salinity. *Appl Environ Microbiol* **72**: 6018–6026.

Downloaded from https://academic.oup.com/femsle/article/270/1/139/599887 by guest on 23 April 2024

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Muller V, Aufurth S & Rahlfs S (2001) The Na^+ cycle in *Acetobacterium woodii*: identification and characterization of a Na^+-translocating F_1F_0-ATPase with a mixed oligomer of 8 and 16 kDa proteolipids. *Biochim Biophys Acta* **1505**: 108–120.
Murata T, Kawano M, Igarashi K, Yamato I & Kakinuma Y (2001) Catalytic properties of Na^+-translocating V-ATPase in *Enterococcus hirae*. *Biochim Biophys Acta* **1505**: 75–81.
Neisser A, Fromwald S, Schmatzberger A & Paschek GA (1994) Immunological and functional localization of both F-type and P-type ATPase in cyanobacterial plasma membranes. *Biochem Biophys Res Commun* **200**: 884–892.
Neumann S, Matthey U, Kaim G & Dimroth P (1998) Purification and properties of F_1F_0 ATPase of *Ilyobacter tartaricus*, a sodium ion pump. *J Bacteriol* **180**: 3312–3316.
Norling B, Mirzakhanian V, Nilsson F & Andersson B (1994) Subfractional analysis of cyanobacterial membranes and isolation of plasma membranes by aqueous polymer two-phase partitioning. *Anal Biochem* **218**: 103–111.
Ohki K & Katoh T (1975) Photoorganotrophic growth of a blue-green alga, *Anabaena variabilis*. *Plant Cell Physiol* **16**: 53–64.
Padan E, Bibi E, Ito M & Krulwich TA (2005) Alkaline pH homeostasis in bacteria: new insights. *Biochim Biophys Acta* **1717**: 67–88.
Patil S, Kaplan JE & Minnear FL (1997) Protein, not adenosine or adenine nucleotide, mediates platelet decrease in endothelial permeability. *Am Physiol Soc* **12**: 199–207.
Pelroy RA, Rippka R & Stanier RY (1972) Metabolism of glucose by unicellular blue-green algae. *Arch Microbiol* **87**: 303–322.
Phung LT, Ajlami G & Haselkorn R (1994) P-type ATPase from the cyanobacterium *Synechococcus* 7942 related to the human Menkes and Wilson disease gene products. *Proc Natl Acad Sci USA* **91**: 9651–9654.
Popova L, Balnokin Y, Dietz K-J & Gimmler H (1998) Na^+-ATPase from the plasma membrane of the marine alga *Tetraselmis* (*Platymonas*) *viridis* forms a phosphorylated intermediate. *FEBS Lett* **426**: 161–164.
Sakamoto A & Murata N (2002) The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant Cell Environ* **25**: 163–171.
Shono M, Hara Y, Wada M & Fujii T (1996) A sodium pump in the plasma membrane of the marine alga *Heterosigma akashiwo*. *Plant Cell Physiol* **37**: 385–388.
Takabe T, Incharoensakdi A, Arakawa K & Yokota S (1988) CO_2 fixation and RuBisCO content increase in a highly halotolerant cyanobacterium *Aphanothece halophytica*, grown in high salinity. *Plant Physiol* **88**: 1120–1124.
Ueno S, Kaieda N & Koyama N (2000) Characterization of a P-type Na^+-ATPase of a facultatively anaerobic alkaliphilic, *Exiguobacterium aurantiacum*. *J Biol Chem* **275**: 14537–14540.
Waditee R, Hibino T, Tanaka Y, Nakamura T, Incharoensakdi A & Takabe T (2001) Halotolerant cyanobacterium *Aphanothece halophytica* contains an Na^+/H^+ antiporter, homologous to eukaryotic ones, with novel ion specificity affected by C terminal tail. *J Biol Chem* **276**: 36931–36938.
Waditee R, Hibino T, Nakamura T, Incharoensakdi A & Takabe T (2002) Overexpression of a Na^+/H^+ antiporter confers salt tolerance on a freshwater cyanobacterium, making it capable of growth in sea water. *Proc Natl Acad Sci USA* **99**: 4109–4114.
Waditee R, Tanaka Y, Aoki K, Hibino T, Jikuya H, Takano J, Takabe T & Takabe T (2003) Isolation and functional characterization of N-methyltransferases that catalyze betaine synthesis from glycine in a halotolerant photosynthetic organism *Aphanothece halophytica*. *J Biol Chem* **278**: 4932–4942.
White AW & Shilo M (1975) Heterotrophic growth of filamentous blue-green alga *Plectonema boryanum*. *Arch Microbiol* **102**: 123–127.
Wutipraditkul N, Waditee R, Incharoensakdi A, Hibino T, Tanaka Y, Nakamura T, Shikata M, Takabe T & Takabe T (2005) Halotolerant cyanobacterium *Aphanothece halophytica* contains NapA-type Na^+/H^+ antiporters with novel ion specificity that are involved in salt tolerance at alkaline pH. *Appl Environ Microbiol* **71**: 4176–4184.

Downloaded from https://academic.oup.com/femsle/article/270/1/139/599887 by guest on 23 April 2024

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved