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Nature of the Light-Induced H^+ Efflux and Na^+ Uptake in Cyanobacteria[1]

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ABSTRACT

We investigated the nature of the light-induced, sodium-dependent acidification of the medium and the uptake of sodium by *Synechococcus*. The rate of acidification (net H^+ efflux) was strongly and specifically stimulated by sodium. The rates of acidification and sodium uptake were strongly affected by the pH of the medium; the optimal pH for both processes being in the alkaline pH range. Net proton efflux was severely inhibited by inhibitors of adenosine triphosphatase activity, energy transfer, and photosynthetic electron transport, but was not affected by the presence of inorganic carbon (C_i). Light and C_i stimulated the uptake of sodium, but the stimulation by C_i was observed only when C_i was present at the time sodium was provided. Amiloride, a potent inhibitor of Na^+/H^+ antiport and Na^+ channels, stimulated the rate of acidification but inhibited the rate of sodium uptake. It is suggested that acidification might stem from the activity of a light dependent proton excreting adenosine triphosphatase, while sodium transport seems to be mediated by both Na^+/H^+ antiport and Na^+ uniport.

Cyanobacteria possess a mechanism for concentrating CO_2 at the carboxylating site (1–3, 7, 8, 13, 27). The interconversion between different inorganic carbon (C_i[2]) species during uptake and accumulation of C_i (26, 28), and the subsequent utilization of CO_2 in photosynthesis may have a significant effect on intracellular pH. A considerable flux of H^+ across the plasma membrane (PM) may be involved in the maintenance and regulation of the intracellular pH.

Scherer *et al.* (22–25) demonstrated a fast phase of Na^+ dependent acidification of the medium, when dark adapted cells of *Anabaena* were illuminated. They attributed this phase to the interconversion between CO_2 and HCO_3^- during uptake of C_i (25). Molitor *et al.* (16), on the other hand, postulated that the acidification was due to the activity of a Na^+/H^+ antiporter. A third alternative that should perhaps be considered is a PM located proton pump (11) the activity of which is light and sodium dependent.

Sodium ions play a major role in cyanobacterial photosynthesis, particularly in the uptake of C_i (6, 9, 14, 21). Three different models were postulated (9, 21) to explain the role of Na^+ in C_i uptake:

1. A Na^+/H^+ antiporter (4) operates to regulate and maintain the intracellular pH during C_i uptake.
2. Na^+/HCO_3^- symport secondary to a primary Na^+ pump operates to accumulate C_i at the expense of the $\Delta\mu_{Na+}$.
3. Na^+ binds to the C_i carrier and alters its kinetic parameters.

The third alternative might be distinguished from 1 and 2 since Na^+ transport is not envisaged as a compulsory requirement, as in the case of models 1 and 2. It is difficult, however, to distinguish between models 1 and 2 since both would require large, C_i-dependent Na^+ fluxes into a small compartment. This flux of Na^+ should have the same magnitude regardless of which model is correct. Obviously, one does not expect to measure a sustained *net* Na^+ uptake on the supply of C_i (14), particularly in the case of model 2 since this would dissipate the $\Delta\mu_{Na+}$, the driving force for the accumulation of C_i in the case of model 2.

In the experiments reported here we determined the uptake of $^{22}Na^+$ and H^+ efflux by the mutant E_1, isolated from *Synechococcus* PCC 7942 (12). This mutant is capable of accumulating C_i like the wild type but is defective in its ability to utilize the intracellular C_i pool for photosynthesis and hence it requires high CO_2 for growth. Thus, possible complications due to the photosynthetic formation of OH^- ions were avoided.

MATERIALS AND METHODS

The mutant E_1, isolated from *Synechococcus* PCC 7942 (12), was grown on BG_{11} medium supplemented with 10 mM Hepes and 10 mM $NaHCO_3$ (pH 7.8). The cultures were aerated with 1.5% CO_2 in air at 30°C and continuous light (Osram white fluorescence lamps) at 12 $mW \cdot m^{-2}$ (400–700 nm). Cells of *Anabaena* were grown and spheroplasts isolated from them as described previously (29).

Measurements of net proton exchange were carried out as described previously (25). Cells were harvested and washed twice with 0.5 mM Hepes, 0.5 mM Mes, and 25 mM choline-Cl corrected with KOH to the required pH. The concentration of K^+ present in the experiments carried out at different pH values was kept constant by the addition of an appropriate amount of KCl. The cells were allowed to adjust to the medium in the dark, at 24°C, followed by illumination provided by two quartz-halogen lamps through a red filter (RG

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[2] Abbreviations: C_i, inorganic carbon; PM, plasma membrane.

610 cut off, Scott, Mainz, FRG). NaCl was supplied 1 min before illumination.

Uptake of $^{22}Na^+$ was determined following its supply for 3 to 7 s (in different experiments). The cells were suspended in 0.5 mL containing 10 mM Hepes, 10 mM Mes, 25 mM choline-Cl, 5 mM $CaCl_2$, KOH to the desired pH, and KCl to maintain a constant K^+ (20 mM) in all the experiments. In pH-jump experiments the cells were washed and resuspended in 1/10 the buffer capacity (pH 7.0) in the dark. $^{22}Na^+$ was provided for 7 s, together with HCl, buffer or KOH to give pH values 5.8, 7.0, and 8.5, respectively. KCl was also supplied in order to maintain the same concentration of K^+ at all pH values. These treatments were given to cells kept in the dark (D-D), and cells transferred to the light for 2 s (D-L) or 20 s (L-L) before the addition of $^{22}Na^+$.

The $^{22}Na^+$ (Amersham Buchler, Frankfurt, FRG) was diluted with 5 M NaCl to a final concentration of 11 mM (0.5 μCi per experiment). In some experiments the cells were allowed to equilibrate with 10 mM NaCl, in the buffer outlined above, in the light or dark, followed by a short period with $^{22}Na^+$ as a tracer in the presence or absence of C_i. In the latter case the buffer was bubbled for at least 1 h with CO_2-free air.

The exposure to $^{22}Na^+$ was terminated by washing the cells (four times, 5 mL each) with the same buffer on glass-fiber filters (Schleicher & Schuell No. 6), connected to a vacuum pump. The washing procedure took 3 to 4 s and was kept as short as possible to minimize the efflux of Na^+ from the cells. These experiments were carried out in the presence of 5 mM $CaCl_2$ and a relatively high ionic strength in an attempt to reduce the nonspecific binding of Na^+ to the cells. However, we could not accurately determine this parameter. The amount of label present on the filter in experiments where cells were omitted was very low and its reproducibility was very good (600 ± 30 cpm).

The rate of photosynthetic O_2 evolution as a function of the concentration of C_i was determined with the aid of an O_2 electrode (8). Uptake of C_i by spheroplasts of *Anabaena* was measured as previously described (29). Attempts to produce spheroplasts of *Synechococcus* capable of achieving a significant rate of C_i uptake were not successful. Amiloride (0.1 mM) treatments were given for 1 h in the corresponding buffers for the H^+ or Na^+ flux experiments.

RESULTS AND DISCUSSION

Measurements of the Acidification of the Medium

Figure 1 presents a typical response of the rate of acidification of the medium, to Na^+, when dark adapted cells of *Synechococcus* E_1 were illuminated. The rate of acidification was strongly and specifically affected by Na^+. We could not replace Na^+ by K^+, Ca^{2+} and Mg^{2+}, NaCl, and Na_2SO_4 gave the same response as was also the case in *Anabaena* (25). The maximal rate of acidification varied between different batches of cells, for an unknown reason (see also Refs. 17, 22, and 23). In some experiments it reached values as high as 900 μmol H^+ mg^{-1} Chl h^{-1} in the presence of Na^+. The extent of stimulation by Na^+ (Fig. 1), however, was fairly constant.

There appears to be a large species variability among cyanobacteria with respect to the concentration of Na^+ required,

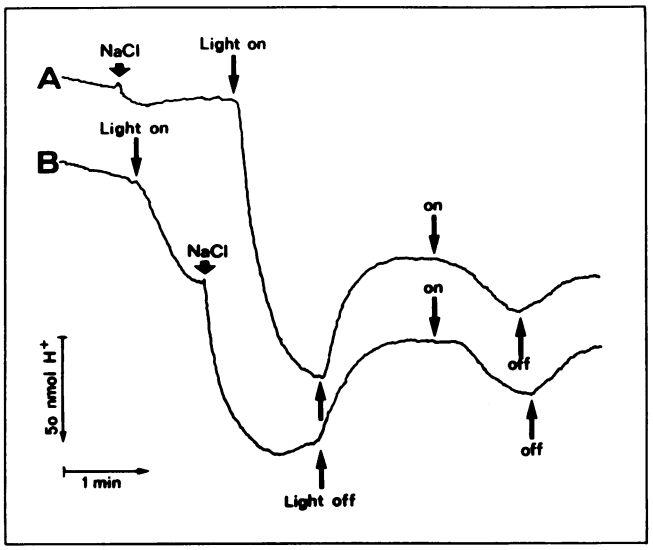

Figure 1. Typical response of the rate of light-dependent acidification of the medium to Na^+. Sodium (5 mM) was provided in the dark (A) or light (B). *Synechococcus* E_1 cells corresponding to 55 μg Chl were suspended in 2.8 mL of buffer (0.5 mM Hepes, 0.5 mM Mes, and 25 mM choline-Cl), pH 7.0, 24°C, 1500 μmol photons m^{-2} s^{-1}.

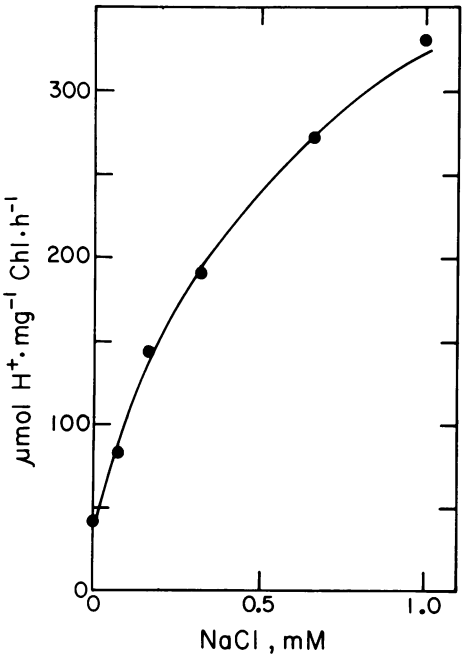

Figure 2. Dependence of the rate of light-induced acidification of the medium on the concentration of Na^+. Cells corresponding to 15 μg Chl per mL were used. Other conditions were as in Figure 1.

the dependence on extracellular pH and on C_i. In *Anabaena*, the concentration of Na^+ required to reach its half maximal effect on the rates of HCO_3^- uptake (9, 21) and of acidification (25) was 3 to 10 mM. On the other hand, data presented in Figure 2 demonstrate that in *Synechococcus*, the concentration of Na^+ required was considerably lower, in agreement with the relatively low concentration of Na^+ required to give maximal stimulation of the rate of photosynthesis (6, 14).

The light-induced, Na^+ dependent acidification in both *Anabaena* (25) and *Synechococcus* (Fig. 3) was strongly affected by the pH of the medium. However, while the optimum pH for *Anabaena* was 6.5, it was higher than 8.0 for *Synechococcus* (Fig. 3). C_i stimulated the rate of acidification in *Anabaena* (25) but using the same experimental conditions and the same measuring system, it hardly effected the rate of acidification in *Synechococcus* (Fig. 3). Ogawa and Kaplan (17), on the other hand, observed some stimulation of the rate of acidification by C_i and a 1:1 stoichiometry between the rates of CO_2 uptake and H^+ released in *Synechococcus*. The reasons for the apparent discrepancy between the results obtained here and those of Ogawa and Kaplan (17) are not yet understood but they might be due to the different measuring systems used in the two cases (the closed chamber containing 2.8 mL of cell suspension used here, compared with the open system containing 25 mL of cells, bubbled [1 L/min] with CO_2-free or 400 μL/L CO_2 in 2% O_2 and 98% N_2, used by Ogawa and Kaplan). Respiratory formation of CO_2, during the period of dark adaptation, might have led to the presence of a larger C_i concentration in the closed system used here, large enough to saturate the response of acidification to C_i in *Synechococcus*, unlike *Anabaena*.

The reasons for these differences between the two cyanobacteria are not yet understood, but they might be related to the nature of the C_i species actively taken up by the two organisms. Under the experimental conditions used here, CO_2 plays the major role in C_i uptake by *Synechococcus* (6, 26) while HCO_3^- appears to be the major C_i species taken up by *Anabaena* (28). Uptake of HCO_3^- from the medium and subsequent CO_2 fixation may impose a larger "load" on the pH regulating system than the uptake of CO_2. Thus, the differences between the two cyanobacteria could stem from the relative role played by different mechanisms which influence the regulation of the intracellular pH.

In an attempt to resolve the nature of the Na^+ dependent acidification we examined its response to several metabolic inhibitors (Table I). The uncoupler carbonylcyanide-*m*-chlorophenylhydrazone, the ATPase inhibitor *N,N*-dichlorohexylcarbodiimide, the energy transfer inhibitors nitrofen (23) and venturicidin, and the inhibitor of photosynthetic electron transport, DCMU, each severely inhibited the acidification. This might indicate that in *Synechococcus* the acidification depends on the activity of an ATPase (22, 23), or the pool size of ATP. Since the observed Na^+-dependent acidification could also stem from the activity of an Na^+/H^+ antiporter (4, 5, 9, 10, 16, 18, 20), we examined the effect of amiloride on the rate of acidification. Amiloride has been used as an inhibitor of the Na^+/H^+ antiport and/or Na^+ channel blocker

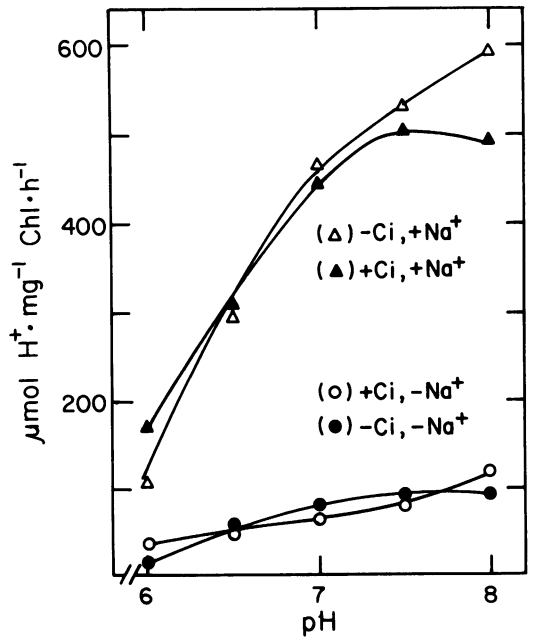

Figure 3. Rate of acidification of the medium as a function of the pH in the presence or absence of inorganic carbon (0.1 mM) and 10 mM NaCl. *Synechococcus* E_1 cells were suspended in 0.5 mM Hepes, 0.5 mM Mes, and 25 mM choline-Cl described in "Materials and Methods."

Table I. *Effect of Metabolic Inhibitors on the Light-Induced, Na^+-Dependent Acidification of the Medium by Synechococcus*

Cells (corresponding to 50 to 60 μg Chl/experiment, in different experiments) were suspended in 0.5 mM Hepes, 0.5 mM Mes, 5 mM NaCl, 25 mM choline-Cl, titrated to pH 7.0 with KOH at 24°C, 1500 μmol photons m^{-2} s^{-1}. Amiloride treatment for 1 h in the dark. Other inhibitors were provided for 2 min in the dark before the onset of light. The 100% corresponded to 310 to 520 μmol H^+ mg^{-1} Chl h^{-1} in different experiments but the effect of the various inhibitors was within a few percent of the average provided here.

Inhibitor[a]	Concentration	Percent of Control
	M	
None		100
CCCP	10^{-5}	0
DCCD	$5\star10^{-5}$	3
Venturicidin	$5\star10^{-5}$	2
Nitrofen	10^{-5}	48
DCMU	10^{-5}	54
Amiloride	10^{-4}	133

[a] CCCP, carboxylcyanide-*m*-chlorophenylhydrazone; DCCD, *N,N*-dichlorohexylcarbodiimide.

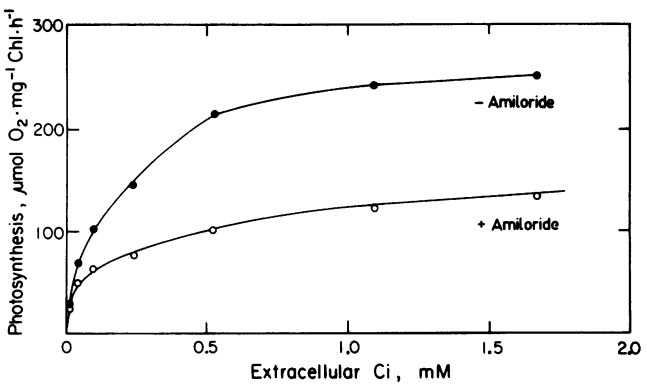

Figure 4. Rate of photosynthetic O_2 evolution by *Synechococcus* PCC7942 as a function of the concentration of C_i, in the presence or absence of amiloride. Cells were treated with amiloride (0.1 mM) for 1 h in the dark before the experiment. The cells were resuspended in 20 mM Hepes (pH 7.5), 4 mM NaCl, 30°C.

Table II. *Effect of Na^+ and Amiloride on C_i Uptake by Spheroplasts Isolated from Anabaena*

Spheroplasts were provided with 0.1 mM $H^{14}CO_3^-$ for 5 s (pH 8.0) in the presence or absence of 10 mM Na^+.

Treatment	C_i Accumulated
	nmol C_i/μg Chl
$-Na^+$, −amiloride	0.123 ± 0.021
$-Na^+$, +amiloride	0.165 ± 0.035
$+Na^+$, −amiloride	0.662 ± 0.064
$+Na^+$, +amiloride	0.122 ± 0.030

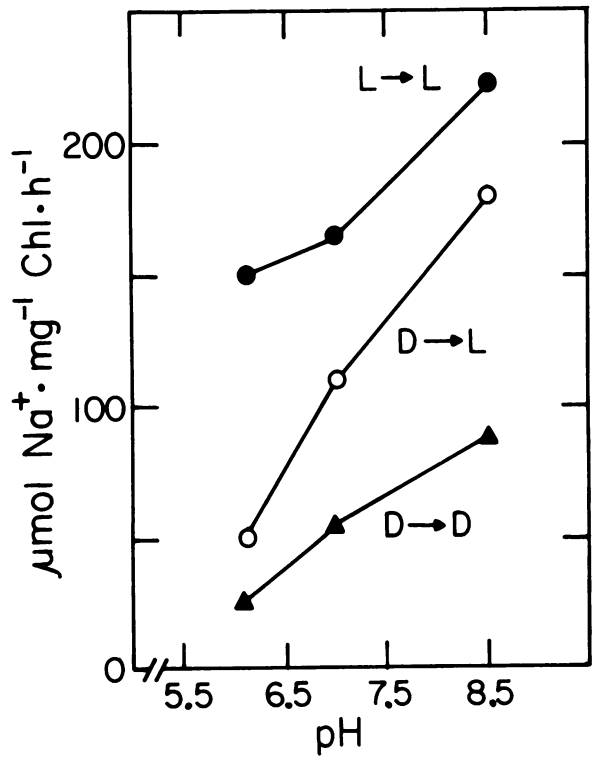

Figure 5. Uptake of $^{22}Na^+$ by *Synechococcus* (E_1) as a function of the pH in a pH-pump experiment. Cells (13 μg Chl/experiment) were suspended in 1 mM Hepes, 1 mM Mes, 25 mM choline-Cl, 5 mM $CaCl_2$, 0.1 mM Ci (pH 7.0) and kept in the dark (D-D), or exposed to 2 s (D-L) or 20 s (L-L), before the addition of $^{22}Na^+$. The latter was provided together with HCl or buffer or KOH (to give pH values of 5.8, 7.0, and 8.5, respectively). See "Materials and Methods" for experimental details.

(at different concentrations), in a wide variety of eukaryotic systems (10). Amiloride treatment of *Synechococcus* cells resulted in an enhanced rate of acidification (Table I). Since the observed acidification reflects the net result of all the processes involved in proton translocation, the stimulation by amiloride would tend to indicate that the net proton efflux (acidification) was inhibited by the Na^+/H^+ exchange rather than resulted from it. In prokaryotes, unlike eukaryotes, however, there are relatively few reports where amiloride treatments have been applied to either intact of cell-free systems (15). Hence it is not known whether the concentration of amiloride used here inhibited the Na^+/H^+ antiporter or the Na^+ channel or both. Studies with isolated intact vesicles of

Table III. *Uptake of $^{22}Na^+$ by Synechococcus as Affected by the Light Treatment and the Presence or Absence of C_i and Amiloride*

Cells were suspended in C_i-free buffer (pH 8.0) or provided with 0.1 mM C_i, in the presence or absence of amiloride (0.1 mM for 1 h in the dark). Light treatments as in Figure 5. Data are presented as the percentage of the rate observed in the dark (D-D) treatment. Value of 100% ranged between 90 and 210 nmol Na^+ mg^{-1} Chl h^{-1} in different batches of cells. The reproducibility of the effect of the different treatments, however, was very good, *i.e.* ±8%.

Treatment	Light Conditions		
	D-D	D-L	L-L
+Ci, −amiloride	100	130	195
+Ci, +amiloride	54	82	87
−Ci, −amiloride	63	57	66

cyanobacterial plasma membranes should provide this information. However, in most cases, PM enriched vesicles from *Synechococcus* and *Anabaena* were too leaky to allow for accurate measurements of H^+ fluxes as a function of the amiloride concentration (not shown).

The extent of stimulation of the acidification by amiloride was not affected by the pH (between 6.0 and 8.0, not shown) even though the rate of acidification itself was pH dependent (Fig. 3). Raising the external pH from 6.0 to 8.0 leads to a considerable decrease of the $\Delta\mu_H^+$ (19, 30) since the hyperpolarization of the PM, on alkalinization of the medium, is too small to compensate for the change in ΔpH between the cytoplasm and the medium. Thus, while the driving force for the different processes involved in net proton transport should have been affected by the pH, it is possible that the lack of a pH effect, on the stimulation of acidification by amiloride, was due to a kinetic effect.

The apparent photosynthetic affinity for extracellular C_i is believed to reflect the rate of C_i accumulation within the cells (7, 8). Amiloride depressed the maximal rate of photosynthesis in *Synechococcus* with only a small effect on the apparent photosynthetic affinity for extracellular C_i (Fig. 4). These data may suggest that amiloride had only a small effect on C_i uptake by *Synechococcus*. On the other hand, in spheroplasts isolated from *Anabaena*, where the uptake of C_i was markedly stimulated by Na^+ (Table II; see also Refs. 9 and 21), amiloride inhibited Ci uptake in the presence, but not in the absence of Na^+. These data provide another indication for the difference between the two cyanobacteria and the possible role played by the Na^+/H^+ antiporter in the uptake of C_i in cyanobacteria.

Uptake of $^{22}Na^+$ by *Synechococcus*

Sodium uptake was strongly stimulated by exposing the cells to a short period of light (Fig. 5). Changing the pH of the medium from 7.0 to 5.8 or 8.5, in the pH-jump experiments, led to a marked decrease or increase, respectively, in the net amount of $^{22}Na^+$ taken up (Fig. 5). These data are in agreement with the expected alterations of the electrochemical potential across the PM following illumination and the pH-jump. The fast, Na^+ dependent, acidification of the medium upon illumination (Fig. 1), might be involved in the alkalinization of the cytoplasm and the hyperpolarization of the PM

that have been observed when dark adapted cells of cyanobacteria were illuminated (30). Raising the pH of the medium, over the range used here, resulted in hyperpolarization of the PM (19, 30) and a fall in the $\Delta\mu_H^+$ (since the hyperpolarization was not large enough to compensate for the lower ΔpH). The resulting alterations in the pH and membrane potential should have a considerable effect on the driving force for the unidirectional fluxes of Na^+ and H^+, regardless of whether the transport of the two ions is coupled to one another.

Amiloride treatment inhibited the accumulation of $^{22}Na^+$ (Table III) as would have been expected if Na^+ uptake is mediated by the Na^+/H^+ antiporter or an amiloride-sensitive sodium channel. The stimulation of Na^+ uptake by light was smaller in the presence of amiloride for yet an unknown reason. Sodium uptake was stimulated when C_i was present in the medium (Table III). This result could stem from the activity of a Na^+-HCO_3^- symport system such as the one proposed to explain the role of Na^+ in HCO_3^- uptake by *Anabaena* (21). However, the effect of C_i on Na^+ uptake by *Synechococcus* (Table III) was mainly observed when the cells were exposed to C_i several seconds before the supply of $^{22}Na^+$. When C_i and Na^+ were simultaneously supplied, for 3 s, the effect of C_i was rather small (not shown). Hyperpolarization of the PM has been observed when C_i was provided to C_i-depleted cells of *Anabaena* (7). This aspect of bioenergetics has not yet been studied in *Synechococcus* but the effect of C_i on Na^+ uptake by *Synechococcus* could stem from such hyperpolarization. The latter might also explain the dependence of the stimulation of Na^+ uptake by light on the presence of C_i (Table III).

The interpretation of the data in Figure 5 and Table III is complicated by the following considerations: (a) although the cells were exposed to $^{22}Na^+$ for only 7 s, it is not clear whether unidirectional Na^+ influx was observed; (b) the kinetic parameters as well as the driving force for H^+ and Na^+ transport might also be affected by the alteration of the electrochemical potential of Na^+ and H^+ upon illumination; and (c) the relative role played by the Na^+/H^+ antiporter and the other means for Na^+ transport such as Na^+ uniport might be affected by the hyperpolarization of the PM upon illumination; supply of C_i or the alteration of extracellular pH (19, 30). A significant rate of Na^+ influx via a uniport system might explain the large effect of light on Na^+ uptake (Fig. 5) and the smaller effect of pH on Na^+ uptake by cells exposed to L-L treatment (Fig. 5). At present we can not rule out or examine these possibilities but some of these aspects might be studied with the aid of isolated vesicles of PM. However, while isolated PM-enriched fractions from *Synechococcus* clearly possess a Na^+/H^+ exchange activity (M Lerner, A Kaplan, unpublished data), these vesicles are, for yet an unknown reason, relatively leaky (compared with similar preparations from *Escherichia coli* (10, 20) and hence they can not serve as a useful tool to study these possibilities.

CONCLUSIONS

It is difficult, at present, to draw a firm conclusion as to the nature of the light-induced, Na^+ dependent acidification of the medium of cyanobacteria, particularly in view of the different response of the filamentous *Anabaena*, compared with the unicellular *Synechococcus*, to various treatments. Of the various explanations considered, however, the most likely is the involvement of a H^+-ATPase which depends on light and sodium for its energization and activation.

The parameters of the $\Delta\mu_H^+$, light and C_i each strongly affected Na^+ uptake, but the relative role of a Na^+/H^+ vs other possible means of sodium transport has yet to be established. Nevertheless, data presented here strongly suggest that the membrane potential may have a significant role in sodium transport. The possibility that, in addition to the role of the membrane potential as a driving force, voltage dependent sodium channels may be involved in cyanobacteria should be explored.

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LITERATURE CITED

1. **Abe T, Tsuzuki M, Miyachi S** (1987) Transport and fixation of inorganic carbon during photosynthesis of *Anabaena* grown under ordinary air. I. Active species of inorganic carbon utilized for photosynthesis. Plant Cell Physiol **28:** 273–281
2. **Badger MR, Andrews TJ** (1982) Photosynthesis and inorganic carbon usage by the marine cyanobacteria *Synechococcus* sp. Plant Physiol **70:** 517–523
3. **Badger MR, Basset M, Comins HN** (1985) A model for HCO_3^- accumulation and photosynthesis in the cyanobacterium *Synechococcus* sp. Plant Physiol **77:** 465–471
4. **Blumwald E, Wolosyn JM, Packer L** (1984) Na^+/H^+ exchange in the cyanobacterium *Synechococcus* 6311. Biochem Biophys Res Commun **122:** 452–459
5. **Erber WWA, Nitschmann WH, Muchl R, Peschek GA** (1986) Endogenous energy supply to the plasma membrane of dark aerobic cyanobacterium *Anacystis nidulans*: ATPase-independent efflux of H^+ and Na^+ from respiring cells. Arch Biochem Biophys **247:** 28–39
6. **Espie GS, Canvin DT** (1987) Evidence for Na^+-independent HCO_3^- uptake by the cyanobacterium *Synechococcus leopoliensis*. Plant Physiol **84:** 125–130
7. **Kaplan A** (1985) Adaptation to CO_2 levels: Induction and mechanism for inorganic carbon uptake. *In* WJ Lucas, JA Berry, eds, Inorganic Carbon Uptake by Aquatic Organisms. American Society of Plant Physiologists, Rockville, MD, pp 325–338
8. **Kaplan A, Badger MR, Berry JA** (1980) Photosynthesis and intracellular inorganic carbon pool in the blue-green algae *Anabaena variabilis*: response to external CO_2 concentration. Planta **149:** 219–226
9. **Kaplan A, Volokita M, Zenvirth D, Reinhold L** (1984) An essential role for sodium in the bicarbonate transporting system of the cyanobacterium *Anabaena variabilis*. FEBS Lett **176:** 166–168
10. **Krulwich TA** (1983) Na^+/H^+ antiporters. Biochim Biophys Acta **726:** 245–264
11. **Lockau W, Pfeffer S** (1982) A cyanobacterial ATPase distinct from the coupling factor of photophosphorylation. Z Naturforsch **37C:** 568–664
12. **Marcus Y, Schwarz R, Friedberg D, Kaplan A** (1986) High CO_2 requiring mutant of *Anacystis nidulans* R_2. Plant Physiol **82:** 610–612
13. **Miller AG, Colman B** (1980) Active transport and accumulation of bicarbonate by a unicellular cyanobacterium. J Bacteriol **143:** 1253–1259
14. **Miller AG, Canvin DT** (1987) Na^+-stimulation of photosynthesis

in the cyanobacterium *Synechococcus* UTEX 625 grown on high levels of inorganic carbon. Plant Physiol **84:** 118–124

15. **Mochizuki-Oda N, Oosawa F** (1985) Amiloride-sensitive Na^+-H^+ antiporter in *Escherichia coli*. J Bacteriol **163:** 395–397
16. **Molitor V, Erber W, Peschek GA** (1986) Increased levels of cytochrome oxydase and sodium-proton antiporter in the plasma membrane of *Anacystis nidulans* after growth in sodium-enriched media. FEBS Lett **204:** 251–256
17. **Ogawa T, Kaplan A** (1987) The stoichiometry between CO_2 and H^+ fluxes involved in the transport of inorganic carbon in cyanobacteria. Plant Physiol **83:** 888–891
18. **Paschinger H** (1977) DCCD induced sodium uptake by *Anacystis nidulans*. Arch Microbiol **113:** 285–291
19. **Peschek GA, Czerny T, Schmetterer G, Nitschmann WH** (1985) Transmembrane proton electrochemical gradients in dark aerobic and anaerobic cells of the cyanobacterium (blue-green alga) *Anacystis nidulans*. Plant Physiol **79:** 278–284
20. **Radan E, Zilberstein D, Schuldiner S** (1981) pH homeostasis in bacteria. Biochim Biophys Acta **650:** 151–166
21. **Reinhold L, Volokita M, Zenvirth D, Kaplan A** (1984) Is HCO_3^- transport in *Anabaena* a Na^+-symport ? Plant Physiol **76:** 190–192
22. **Scherer S, Boger P** (1984) Vanadate-sensitive proton efflux by filamentous cyanobacteria. FEMS Microbiol Lett **22:** 215–218
23. **Scherer S, Hinrichs I, Boger P** (1986) Effect of monochromatic light on proton efflux of the blue-green alga *Anabaena variabilis*. Plant Physiol **81:** 939–941
24. **Scherer S, Riege H, Boger P** (1989) Light-induced proton efflux of the cyanobacterium *Anabaena variabilis*. *In* J Gallon, LJ Rogers, eds, Biochemistry of Algae and Cyanobacteria, Oxford University Press (in press)
25. **Scherer S, Riege H, Boger P** (1988) Light-induced proton release by the cyanobacterium *Anabaena variabilis* dependence on CO_2 and Na^+. Plant Physiol **86:** 769–772
26. **Schwarz R, Friedberg D, Kaplan A** (1988) Is there a role for the 42 kilodalton polypeptide in inorganic carbon uptake by cyanobacteria ? Plant Physiol **88:** 284–288
27. **Tu CK, Spiller H, Wynns GC, Silverman DN** (1987) Carbonic anhydrase and the uptake of inorganic carbon by *Synechococcus* sp. (UTEX 2380). Plant Physiol **85:** 72–77
28. **Volokita M, Zenvirth D, Kaplan A, Reinhold L** (1984) Nature of the inorganic carbon species actively taken up by the cyanobacterium *Anabaena variabilis*. Plant Physiol **76:** 599–602
29. **Zenvirth D, Kaplan A** (1984) Photosynthesis and inorganic carbon uptake by spheroplasts isolated from the cyanobacterium *Anabaena variabilis*. J Gen Microbiol **130:** 1995–1997
30. **Zenvirth D, Volokita M, Kaplan A** (1984) Evidence against H^+-$HCO3^-$ symport as the mechanism for $HCO3^-$ transport in the cyanobacterium *Anabaena variabilis*. J Membr Biol **79:** 271–274