An essential role for sodium in the bicarbonate transporting system of the cyanobacterium *Anabaena variabilis*

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The apparent photosynthetic affinity of *Anabaena variabilis* for extracellular inorganic carbon (C_i) was strikingly increased by Na^+. The effect was highly specific for Na^+ and was maximal at 40 mM Na^+. Na^+ supply decreased the apparent K_m (C_i) of the C_i transporting system and to a lesser extent increased V_{max}. It did not affect photosynthetic rate expressed as a function of intracellular C_i. We infer an effect of Na^+ on the C_i transporting system rather than on the photosynthetic machinery itself. We propose several possible models, including Na^+–H^+ antiport for maintenance of intracellular pH during HCO_3 uptake, and Na^+–HCO_3^- symport.

Anabaena *Inorganic carbon uptake* *Photosynthesis* *Sodium-proton antiport* *Sodium-bicarbonate symport*

1. INTRODUCTION

Active transport and accumulation of inorganic carbon (C_i) in cyanobacteria involves a primary electrogenic pump [1], but the molecular mechanism of transport has not yet been elucidated. Bicarbonate appears to be the C_i species arriving at the inner side of the plasmalemma [2]. Since CO_2 is the species utilized by the carboxylating enzyme [3], hydroxyl ions must be released to the medium in order to maintain intracellular pH. Alkalization of the medium following the supply of HCO_3^- has in fact been observed in *Anabaena* [4]. The mechanism involved in OH^- efflux is not understood, but the efflux (or H^+ influx) appears to occur along its electrochemical potential gradient [5]. It has recently been suggested that an Na^+–H^+ antiport system is involved in the maintenance of intracellular pH in bacteria [6–8]. We have examined the possibility that a similar system may operate in cyanobacteria. Here, we demonstrate that Na^+ plays a mojor role in the mechanism for HCO_3^- uptake in these organisms.

2. MATERIALS AND METHODS

Cells of *Anabaena vaiabilis* were grown as in [1,9], at a CO_2 level equal to that in air. Cells were harvested by centrifugation (500 × *g*, 5 min) and resuspended in 40 mM 1,3-bis(tris-hydroxymethylmethylamino)propane (BIP) brought to pH 9.0 with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes).

Photosynthetic O_2 evolution was measured in an O_2 electrode as in [1,9]. Accumulation of acid-stable and -labile ^{14}C and intracellular C_i concentration were determined by a filtering centrifugation technique after the supply of $NaH^{14}CO_3$ [1,9].

3. RESULTS AND DISCUSSION

Fig.1. gives the photosynthetic rate as a function of external C_i concentration in the presence and absence of Na^+ in the medium. It shows that the apparent photosynthetic affinity for C_i was strongly affected by Na^+. Maximum photosynthetic rate at saturating C_i level was also affected but to a considerably lesser extent (fig.1). It has been observed that apparent photosynthetic affinity for C_i in the

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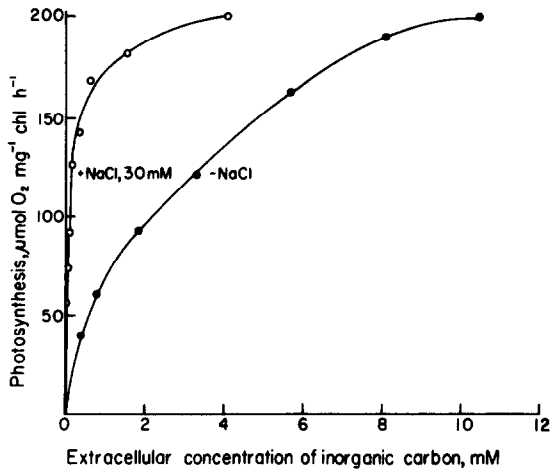

Fig.1. Rate of photosynthetic O_2 evolution as a function of external C_i concentration in the presence and absence of Na^+. Light intensity was 7 mW·cm^{-2} (400–700 nm), 30°C, ±NaCl (40 mM), pH 9.0.

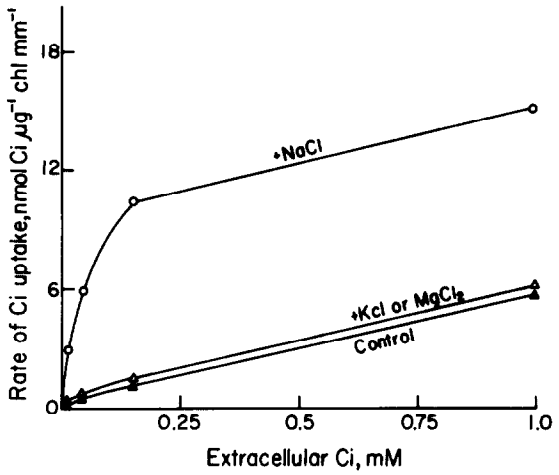

Fig.2. The effect of NaCl, KCl and $MgCl_2$ on the curve relating rate of C_i uptake to external C_i concentration. Cells were exposed for 5 s to the desired $^{14}C_i$ concentration in the presence or absence of the various salts (30 mM each). Other conditions as in fig.1.

medium depends on HCO_3^- transport capacity [10]. Moreover, the oblique curve obtained in the absence of Na^+ resembles that earlier observed for cells adapted to high ambient CO_2 conditions. The lesser apparent photosynthetic affinity of such cells for C_i in the medium, as compared with cells adapted to low ambient CO_2, is attributed to their lower capacity for HCO_3^- transport [10]. These findings therefore suggested that the capacity for active HCO_3^- uptake by *A. variabilis* may depend on the presence of Na^+ in the medium.

Data presented in fig.2,3 support this suggestion. Direct measurement of C_i uptake from the medium (estimated over a time interval so brief that 90% of the C_i absorbed was still in inorganic form) was strongly promoted in the presence of 30 mM Na^+. This effect appears to be highly specific for Na^+, as KCl, $MgCl_2$ and $CaCl_2$ (not shown) could not replace NaCl (fig.2). LiCl countered the NaCl stimulation, while Na_2SO_4 had an effect equivalent to that of NaCl (not shown).

Photosynthesis itself was not directly affected by the presence or absence of Na^+ at the concentrations used here, as can be seen when the photosynthetic rate (accumulation of acid-stable ^{14}C) is plotted against the intracellular C_i pool (fig.3). The points for control and Na^+-treated cells lie on the same line. It may thus be concluded that the lower apparent photosynthetic capacity for extracellular C_i (fig.1) resulted from the reduced capacity for C_i transport in the absence of Na^+ (fig.2).

Fig.4 gives the dependence of HCO_3^- uptake on Na^+ concentration. The highest rate of HCO_3^- uptake from a medium containing 0.15 mM HCO_3^-, was obtained in the presence of 40 mM Na^+.

One possible explanation for the alteration in the K_m and V_{max} of the C_i transporting system by

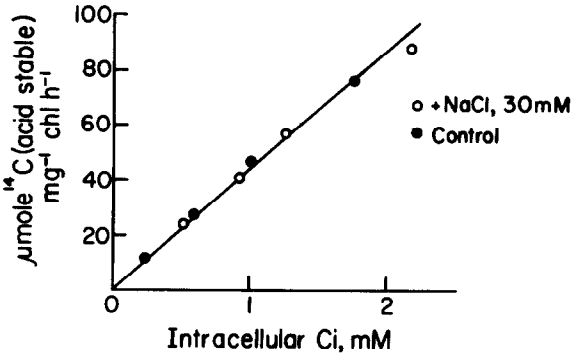

Fig.3. Rate of accumulation of photosynthetic products as a function of the intracellular C_i concentration in the presence or absence of NaCl. Data calculated from the rate of accumulation of ^{14}C acid-stable products and the corresponding intracellular C_i concentration in experiments such as the one presented in fig.2.

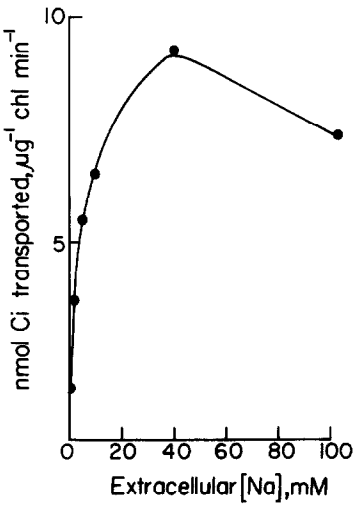

Fig.4. Dependence of HCO_3^- uptake on Na^+ concentration. Cells supplied with 0.15 mM $NaH^{14}CO_3$ for 5 s. Other conditions as in fig.1.

Na^+ (fig.2, and in preparation) could be a specific effect of Na^+ on the HCO_3^- porter, leading to altered HCO_3^- binding parameters. However, bearing in mind the very large influx of HCO_3^-, which yields OH^- within the cell, and the major role recently suggested for Na^+–H^+ exchange mechanisms in the maintenance of intracellular pH in bacteria [6–8] it seems likely that Na^+ might be required for the regulation of intracellular pH during HCO_3^- uptake. This model would predict that addition of HCO_3^- in the absence of Na^+ would lead to alkalization of the cytoplasm. There are various ways in which this local alkalization could alter the kinetic parameters for HCO_3^- uptake, including a change in the rate of dissociation of the carrier-HCO_3^- complex at the inner side of the membrane. This model would also predict that the unidirectional fluxes of Na^+ would show dependence on the presence of HCO_3^-; and the magnitude of these fluxes would be required to be as large as that of the HCO_3^- flux.

Confirmation that the latter requirement is fulfilled could also support an alternative model. It might be postulated that the HCO_3^- uptake process is in fact Na^+–HCO_3^- symport. In this case the various criteria used to establish the connection between 'driven' and 'driver' substrates would have to be met [11,12].

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REFERENCES

[1] Kaplan, A., Zenvirth, D., Reinhold, L. and Berry, J.A. (1982) Plant Physiol. 69, 978–982.
[2] Volokita, M., Zenvirth, D., Kaplan, A. and Reinhold, L. (1984) Plant Physiol., in press.
[3] Badger, M.R. (1980) Arch. Biochem. Biophys. 201, 247–254.
[4] Kaplan, A. (1981) Plant Physiol. 67, 201–204.
[5] Zenvirth, D., Volokita, M. and Kaplan, A. (1984) J. Membr. Biol., in press.
[6] Padan, E., Zilberstein, D. and Schuldiner, S. (1981) Biochim. Biophys. Acta 650, 151–166.
[7] Garcia, M.L., Guffanti, A.A. and Krulwich, T.A. (1983) J. Bacteriol. 156, 1151–1157.
[8] Ken-Dror, S., Shnaiderman, R. and Avi-Dor, Y. (1984) Arch. Biochem. Biophys. 229, 640–649.
[9] Marcus, Y., Zenvirth, D., Harel, E. and Kaplan, A. (1982) Plant Physiol. 69, 1008–1012.
[10] Kaplan, A., Badger, M.R. and Berry, J.A. (1980) Planta 149, 219–226.
[11] Reinhold, L. and Kaplan, A. (1984) Annu. Rev. Plant Physiol., in press.
[12] Sanders, D., Hansen, U.P., Gradmann, D. and Slayman, C.L. (1984) J. Membr. Biol. 77, 123–152.