Distinction between HCO_3^-- and CO_2-dependent photosynthesis in the cyanobacterium *Synechococcus leopoliensis* based on the selective response of HCO_3^- transport to Na^+

A.G. Miller and D.T. Canvin

Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada

Received 3 May 1985

At low concentrations of dissolved inorganic carbon (DIC) at pH 8.0 the rates of DIC transport and fixation by *Synechococcus leopoliensis* were markedly stimulated by the addition of Na^+. Carbonic anhydrase (CA) addition gave similar results. The Na^+-stimulated photosynthesis was inhibited by Li^+ whereas the CA-stimulated photosynthesis was not. The addition of a high DIC concentration (1 mM) overcame the need for either Na^+ or CA. We suggest that Na^+ stimulated HCO_3^- transport whereas CA stimulated CO_2 transport (by increasing the supply rate of CO_2 to the cells).

Synechococcus *Inorganic carbon uptake* *Photosynthesis* *Na^+-dependent HCO_3^- transport* *CO_2 transport*

1. INTRODUCTION

The photosynthesis and growth of cyanobacteria at low concentrations of dissolved inorganic carbon (DIC) at alkaline pH is usually based on the active transport of HCO_3^-, rather than CO_2, into the cells [1–7]. There is increasing evidence, however, that cyanobacteria may also be capable of active CO_2 transport [1,8]. We present in this report a method that allows distinction between HCO_3^-- and CO_2-dependent transport and photosynthesis in the cyanobacterium *Synechococcus leopoliensis*, based upon the recent finding that Na^+ is required for cyanobacterial photosynthesis at alkaline pH [9–11].

2. MATERIALS AND METHODS

Cells of *S. leopoliensis* (UTEX 625) were grown in unbuffered Allen's medium [12], to a chlorophyll density of 6–8 μg/ml, with air bubbling at 30°C. Cells were washed 3 times (by centrifugation for 2 min at 10000 × *g*) with 50 mM 1,3-bistris(hydroxymethyl)methylaminopropane adjusted to pH 8.0 with HCl and containing only low concentrations (12–20 μM) of DIC [5]. Photosynthetic O_2 evolution was measured with an O_2 electrode [5]. The transport, accumulation and photosynthetic fixation of ^{14}C-labelled DIC were measured by centrifugal filtration of the cells through silicone fluid as described [4], except that KOH rather than NaOH was used in the terminating solution.

Carbonic anhydrase (CA) from bovine erythrocytes (Sigma) was dialysed against 10 mM K-phosphate (pH 7.0) before use. These stock CA solutions (10 mg CA · ml^{-1}) contained no more than 170 μM Na^+ (determined by flame emission spectrometry) and would therefore have increased the Na^+ concentration in the algal suspensions by no more than 1.7 μM upon addition to give 100 μg CA · ml^{-1}.

3. RESULTS

We have shown that cells of *S. leopoliensis* harvested from HCO_3^--limited chemostats required

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

Na^+ in the extracellular medium in order to photosynthesize at normal rates [9]. Batch-grown cells also required the addition of Na^+ (as NaCl or Na_2SO_4) for optimal photosynthesis at a low concentration of DIC (25 μM) at pH 8.0 (fig.1A). A concentration of 3–5 mM NaCl was required for half-maximal stimulation (not shown). Li^+ inhibited Na^+-stimulated photosynthesis (fig.1B).

There is no evidence for the production of extracellular CA by cyanobacteria [1,3,7] and its absence would limit the rate of photosynthesis at low DIC concentrations at alkaline pH, if the cells were unable to transport HCO_3^- [3]. The addition of CA to cells in the presence of Na^+ stimulated photosynthesis slightly (fig.1B). The addition of CA to cells in the absence of Na^+, however, stimulated photosynthesis greatly and resulted in a restoration of the photosynthetic rate to that observed in the presence of Na^+ (fig.1B). Boiled CA gave no stimulation (fig.1B). Li^+ did not inhibit CA-stimulated photosynthesis (fig.1B).

The stimulatory effect of CA was overcome by the addition of the CA inhibitor ethoxyzolamide (EA) (fig.2). Several other features of these experiments are shown in this figure. Upon the addition of CA the photosynthetic rate increased as the contaminant DIC in the buffer was used (fig.2A). Photosynthesis was rapidly resumed upon addition of 30 μM DIC (fig.2B) but after the addition of 30 μM EA (fig.2C) photosynthesis did not resume upon the addition of a further 30 μM DIC (fig.2D). The addition of 10 mM NaCl (fig.2E) allowed photosynthesis to proceed at a rate similar to that previously seen in the presence of active CA (fig.2B,F). The Na^+ effect was overcome by the addition of 20 mM LiCl (fig.2G,H) but photosyn-

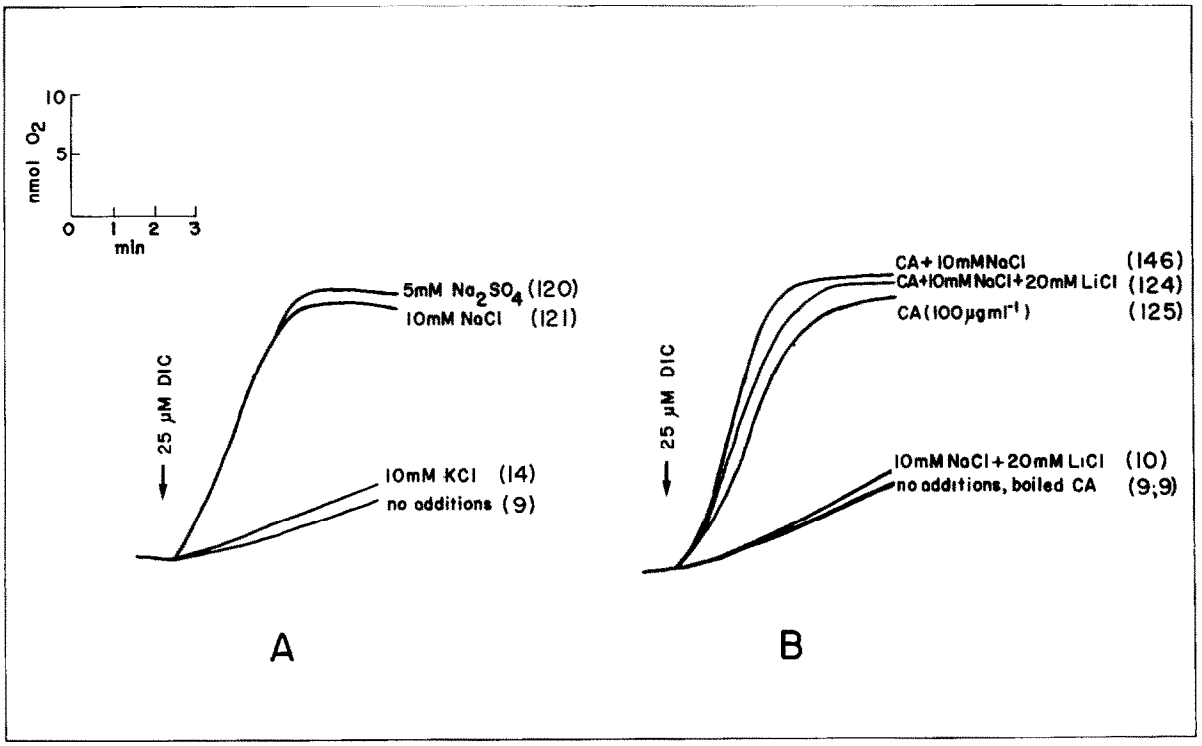

Fig.1. Effects of various salts (A) and CA (B) upon photosynthetic O_2 evolution at 25 μM DIC at pH 8.0. The salts (as 1 M stocks) and CA were added 3 min prior to the addition of sufficient 10 mM $KHCO_3$ to yield a final [DIC] of 25 μM. The numbers in brackets are the maximum rates of photosynthesis (in $\mu mol \cdot mg^{-1}$ Chl $\cdot h^{-1}$) determined from the respective traces. The highest rate of photosynthesis of these cells (251 $\mu mol \cdot mg^{-1}$ Chl $\cdot h^{-1}$) was observed in the presence of 20 mM NaCl and 1 mM DIC. A suboptimal Na^+ concentration (10 mM) was used in these experiments to clearly demonstrate the inhibitory effect of Li^+ (which is largely overcome by higher [Na^+]).

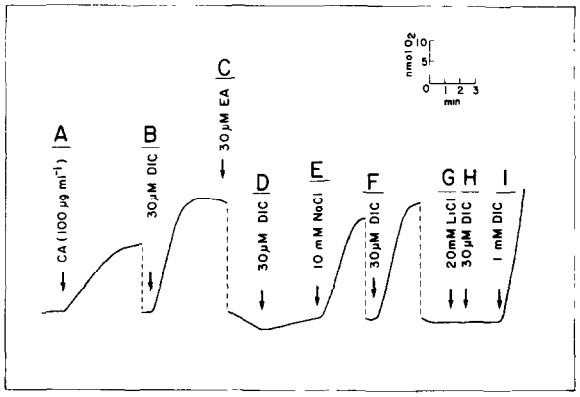

Fig.2. Further distinctions between CA- and Na^+-stimulated photosynthetic O_2 evolution at pH 8.0. Dotted lines indicate adjustment of recorder pen.

thesis proceeded rapidly upon the addition of a high concentration (1 mM) of DIC (fig.2I).

The transport and accumulation of DIC was stimulated by Na^+ (fig.3A) as was the rate of photosynthetic carbon fixation (fig.3B). The addition of CA gave results similar to those seen with Na^+ addition (fig.3).

4. DISCUSSION

When extracellular CA is absent, the DIC concentration is low and the pH is alkaline, the rate of cyanobacterial photosynthesis being limited by the rate of active HCO_3^- transport [1,3,7]. Under these conditions Na^+ was required for normal photosynthetic rates to occur (fig.1A). Upon the addition of CA (to accelerate $HCO_3^- \longrightarrow CO_2$ conversion) Na^+ was no longer required (fig.1B). When the rate of CO_2 supply to the cells was increased by the addition of a high DIC concentration (fig.2I) or by lowering the pH to 7.0 (not shown) there was also no need for Na^+. The CA-stimulated photosynthesis was not inhibited by Li^+ whereas the Na^+-stimulated photosynthesis was (fig.1B,2G). The stimulation of photosynthesis by both Na^+ and CA was the result of a stimulation of DIC transport (fig.3). We suggest that Na^+ stimulated

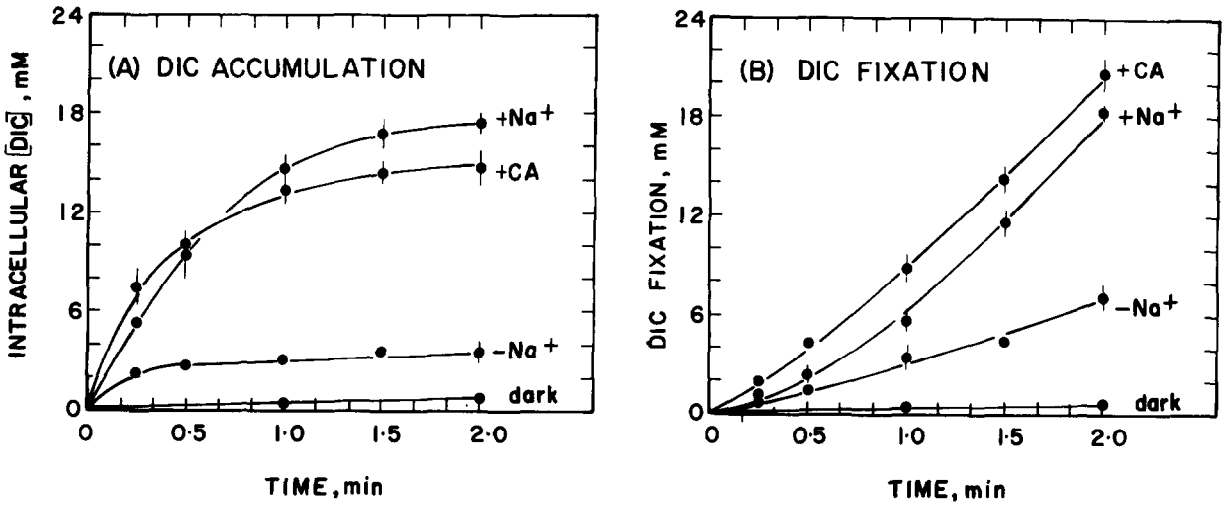

Fig.3. Effects of Na^+ and CA upon DIC transport, accumulation and fixation at pH 8.0. DIC transport was initiated by the addition of a pH 8.0 solution containing $KH^{14}CO_3$ (45 mCi/mmol) to illuminated cells. The [DIC] upon initiation of transport was 75 μM. (A) DIC accumulation refers to the accumulation of unmetabolized, acid-labile DIC within the cells and was measured as in [9]. Concentrations (mM) were calculated on the basis of the sorbitol impermeable space [9] measured in this experiment as 95 μl $H_2O \cdot mg^{-1}$ Chl. (B) DIC fixation represents the formation of photosynthetic, acid-stable products from transported DIC [9]. The total amount of DIC transported into the cells can be obtained by addition of corresponding values for 'DIC accumulation' and 'DIC fixation'. If the intracellular pH in illuminated cells is assumed to be 7.5 [13] then the intracellular [CO_2] can be estimated for each intracellular [DIC] (A). For example, cells incubated in CA accumulated CO_2 to a concentration of about 1100 μM after 2 min (A), compared to a concentration of only 0.86 μM remaining in the medium. The calculated accumulation ratios for CO_2 of 1279 will be an overestimate due to the formation of acid-labile carbamates, but does strongly suggest that CO_2 was accumulated by these cells against its concentration gradient.

HCO_3^- transport (inhibited by Li^+) whereas CA stimulated CO_2 transport (not inhibited by Li^+).

ACKNOWLEDGEMENT

This work was supported by a grant to D.T.C. from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

[1] Badger, M.R. and Andrews, T.J. (1982) Plant Physiol. 70, 517–523.
[2] Kaplan, A., Badger, M.R. and Berry, J.A. (1980) Planta 149, 219–226.
[3] Miller, A.G. and Colman, B. (1980) Plant Physiol. 65, 397–402.
[4] Miller, A.G. and Colman, B. (1980) J. Bacteriol. 143, 1253–1259.
[5] Miller, A.G., Turpin, D.H. and Canvin, D.T. (1984) Plant Physiol. 75, 1064–1070.
[6] Shelp, B.J. and Canvin, D.T. (1984) Can. J. Bot. 62, 1398–1403.
[7] Espie, G.S., Gehl, K.S., Owttrim, G.W. and Colman, B. (1984) in: Advances in Photosynthetic Research (Sybesma, C. ed.) vol.3, pp.457–460, Martinus Nijhoff/Junk, The Netherlands.
[8] Volokita, M., Zenvirth, D., Kaplan, A. and Reinhold, L. (1984) Plant Physiol. 76, 599–602.
[9] Miller, A.G., Turpin, D.H. and Canvin, D.T. (1984) J. Bacteriol.
[10] Kaplan, A., Volokita, M., Zenvirth, D. and Reinhold, L. (1984) FEBS Lett. 176, 166–168.
[11] Reinhold, L., Volokita, M., Zenvirth, D. and Kaplan, A. (1984) Plant Physiol. 76, 1090–1092.
[12] Allen, M.M. (1968) J. Phycol. 4, 1–4.
[13] Coleman, J.R. and Colman, B. (1981) Plant Physiol. 67, 917–921.