# Evidence for a saturable transport component in the inorganic carbon uptake of *Chlamydomonas reinhardii*

Martin H. Spalding* and William L. Ogren

*Department of Agronomy, University of Illinois, Urbana, IL 61801* and *US Department of Agriculture, Agricultural Research Service, Urbana, IL 61801, USA*

Received 15 February 1983

Abstract not received

*Bicarbonate transport* Chlamydomonas *Transport kinetics* *Photosynthesis* *Algal transport* *Carbon dioxide*

## 1. INTRODUCTION

*Chlamydomonas reinhardii* and other unicellular green algae and cyanobacteria exhibit a high apparent affinity for $CO_2$ in photosynthesis when grown photoautotrophically at air levels of $CO_2$ [1–7]. This is believed to result from an inducible $CO_2$-concentrating system [2,4–10], which appears to involve active transport of inorganic carbon into the algal cells. A kinetic analysis of the inorganic carbon influx of the cyanobacterium *Anabaena variabilis* indicated that a saturable transport system was involved [5]. No direct evidence for the involvement of a saturable transport process has been reported in the green algae, however. This is an important point since it has been suggested that the high affinity for $CO_2$ observed in the green algae might be explained simply on the basis of high internal carbonic anhydrase activity [11,12]. In this paper we have demonstrated the presence of a saturable component (transport) in the uptake of inorganic carbon by cells of the green alga *C. reinhardii* and have obtained estimates of the kinetic parameters of this component.

* Present address: MSU/DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA

Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the University of Illinois or the US Department of Agriculture, and does not imply approval of the product to the exclusion of other products or vendors that may also be suitable

*Abbreviations:* MOPS, 3-(*N*-morpholino)propanesulfonic acid

## 2. MATERIALS AND METHODS

### 2.1. *Strain and culture conditions*

*Chlamydomonas reinhardii* strain 2137 ($mt^+$) [13] was grown photoautotrophically in liquid minimal medium at air levels of $CO_2$ as in [10].

### 2.2. *Total carbon accumulation*

Accumulation of total (inorganic plus fixed) carbon from $NaH^{14}CO_3$ was estimated using silicone oil-filtering centrifugation [8,14]. Algal suspensions free from inorganic carbon (200 $\mu$l, ~20 $\mu$g Chl/ml) were layered over 100 $\mu$l of silicone oil (Wäcker AR 20:AR 200, 4:1) in 400 $\mu$l centrifuge tubes in the light. Known concentrations of $NaH^{14}CO_3$ were then added to the suspensions with a spatula that simultaneously mixed the suspension. The algal cells were centrifuged at various times after $NaH^{14}CO_3$ addition (3–10 s)

*Published by Elsevier Science Publishers*
00145793/83/0000–0000/$3.00 

through the silicone oil into 20 $\mu$l of 5 N KOH. Total uptake of inorganic carbon into the cells (internal inorganic plus fixed $^{14}C$) was estimated after correcting for extracellular $NaH^{14}CO_3$ carried through the silicone oil using the non-permeable solute [$^{14}C$]sorbitol [14].

### 2.3. *Initial inorganic carbon influx rates*

Initial rates of inorganic carbon influx were estimated by a modification of the silicone oil filtering centrifugation technique. The 400 $\mu$l centrifuge tubes contained (bottom to top) 20 $\mu$l of 5 N KOH, 100 $\mu$l silicone oil (AR 200), 100 $\mu$l $CO_2$-free 50 mM MOPS–$KO^3H$ (pH 7.0) plus 1% dextran and excess carbonic anhydrase, and 100 $\mu$l silicone oil (AR 20:AR 200, 4:1). Just prior to centrifugation, known amounts of $NaH^{14}CO_3$ were injected into the buffer layer sandwiched between silicone oil, then 50 $\mu$l of a relatively concentrated (~ 100 $\mu$g Chl/ml) $CO_2$-free cell suspension was applied over the upper layer of silicone oil in the light. The tubes were then centrifuged (Beckman Microfuge B) in the light, thereby exposing the algal cells to $NaH^{14}CO_3$ for only a very short time as they passed through the $^{14}C$-containing buffer layer. The algal suspension and the buffer layer contained identical concentrations of $^3H_2O$, and by substituting [$^{14}C$]sorbitol for $NaH^{14}CO_3$ in the buffer layer it was possible to correct for extracellular $NaH^{14}CO_3$ carried through the lower silicone oil layer with the cells. In all experiments 12 tubes (2 replicates of 6 $NaHCO_3$ concentrations) were centrifuged at one time to minimize differences due to variations of the time of exposure of the cells to the $NaH^{14}CO_3$-containing buffer layer.

### 2.4. *Chlorophyll determination*

Chlorophyll was measured spectrophotometrically following extraction into 96% ethanol [15].

## 3. RESULTS AND DISCUSSION

Time courses of total carbon uptake upon short-term exposure of *C. reinhardii* cells to several concentrations of $NaH^{14}CO_3$ are illustrated in fig.1. Although the amount of carbon uptake at 3 s increased with increasing $NaHCO_3$ concentration, this probably does not represent an initial rate of inorganic carbon influx, since net uptake between 3 and 10 s did not extrapolate to zero. In these experiments more than half the $^{14}C$ in the cells was fixed carbon.

A better estimate of the initial rate of inorganic carbon uptake could be obtained by modifying the silicone oil centrifugation technique so that the cells were simply centrifuged through a layer containing $NaH^{14}CO_3$. Data using this modified system are illustrated in fig.2. The uptake rates are

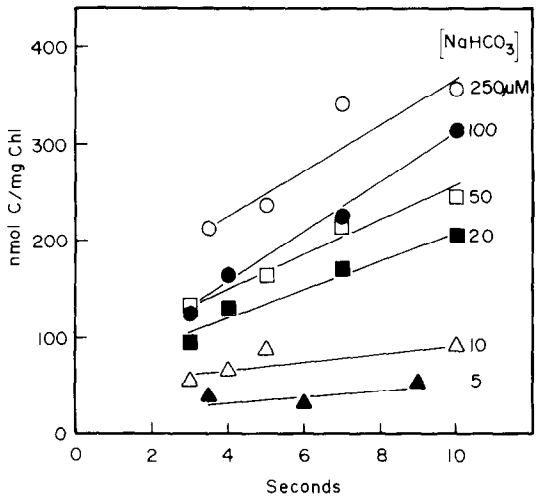


Fig.1. Time courses for the uptake of inorganic carbon into *C. reinhardii* cells at various external concentrations of $NaHCO_3$. Each point represents the mean value of 4 replicates.

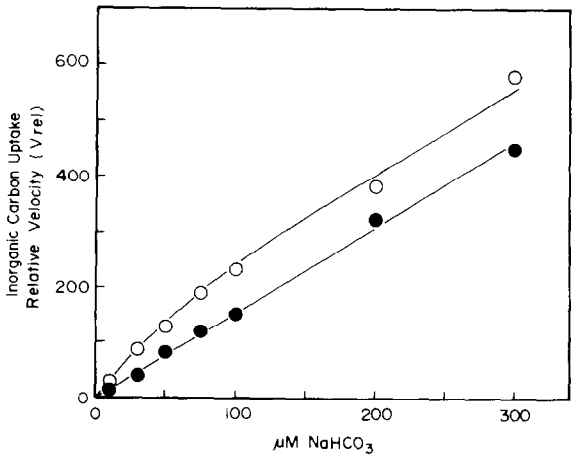


Fig.2. Relative velocities of the initial rate of inorganic carbon uptake in *C. reinhardii* as a function of external $NaHCO_3$ concentration (pH 7.0) in the absence (○) or presence (●) of 5 $\mu$M FCCP. Each point represents the mean value of 4 replicates.

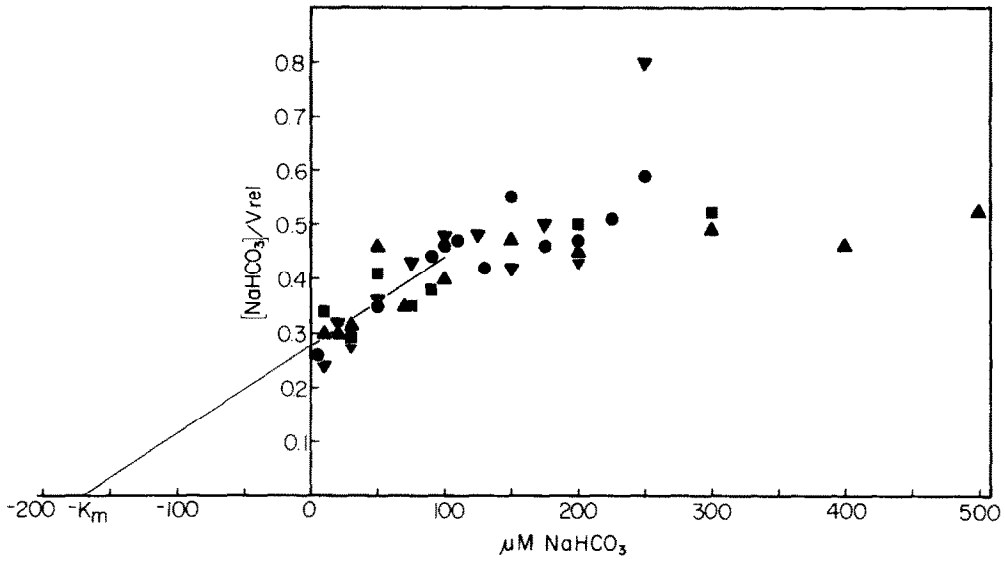


Fig.3. Plot of $[S]/V_{rel}$ *vs* $[S]$ for initial inorganic carbon uptake velocity in *C. reinhardii*. Each point represents the mean value of 4 replicates, and each symbol represents a separate experiment.

expressed in relative units, since it was not possible to obtain an accurate estimate of the time of exposure of the cells to the $NaH^{14}CO_3$-containing layer. The response of the estimated initial uptake rate to increasing concentrations of $NaHCO_3$ is typical of a system with both a saturable component (transport) and a nonsaturable component (diffusion) [16]. Uptake of inorganic carbon in the presence of FCCP, which inhibits inorganic carbon accumulation [8], exhibited only the non-saturable component (fig.2).

Estimates of the $K_m$ and $V_{max}$ of the transport system and the permeation coefficient of the diffusion component can be obtained by replotting data such as those in fig.2 as the ratio of substrate concentration to uptake velocity *vs* the substrate concentration ($[S]/V$ *vs* $[S]$, [16]). Fig.3 has been constructed with data from 4 experiments of this type. In this analysis, uptake which occurred by diffusion only would result in a horizontal line, since velocity would be a linear function of substrate concentration. A completely saturable system, such as an enzyme, would result in a straight line of slope $1/V_{max}$ intercepting the abscissa at $-K_m$. With a system containing both a saturable and a non-saturable component the result should be a curve which is asymptotic to a line representing only the saturable component at low substrate concentration and asymptotic to the non-saturable component at high substrate concentration. Therefore, by approximating the asymptotic line at low substrate concentration it should be possible to estimate the kinetic parameters of the transport system [16]. Since only relative inorganic carbon velocities could be obtained, it was not possible to estimate $V_{max}$ for the transport system. However, the estimation of the apparent $K_m$ for transport remains valid. The apparent $K_m$ for inorganic carbon transport was estimated to be about 160 $\mu$M by linear regression analysis of the data from fig.3 for $NaHCO_3$ concentrations up to 100 $\mu$M. Assuming that bicarbonate is the substrate for transport, this corresponds (at pH 7.0) to an apparent $K_m$ of 130 $\mu$M bicarbonate. From the $K_m$-value and the data in fig.1, it was calculated that the minimum required $V_{max}$ for transport was about 1400 $\mu$mol bicarbonate.(mg Chl)$^{-1}$.h$^{-1}$, or roughly 7-fold higher than the maximum rate of photosynthesis. This estimated $V_{max}$ corresponds to an exposure time for the cells of 0.5–1 s in the modified silicone oil centrifugation technique (fig.2,3).

The data presented here indicate that the initial rate of inorganic carbon uptake in *Chlamydomonas* can be separated into a saturable component (transport) and a non-saturable component (diffusion). This represents the first direct demonstration of the involvement of a saturable transport process in the inorganic carbon uptake in green

algae. Due to the scattered data (see fig.3) the quantitative value obtained for the $K_m$ of transport can only be considered to be a rough estimate but should prove useful in developing an understanding of the $CO_2$-concentrating system in this and other microalgae. The estimated $K_m$ (160 $\mu$M $NaHCO_3$) and the predicted minimum required $V_{max}$ (1400 $\mu$mol C.(mg Chl)$^{-1}$.h$^{-1}$) for inorganic carbon transport into *C. reinhardii* are similar to the kinetic parameters estimated for inorganic carbon transport in *Anabaena* [5]. This suggests that the transport systems of unicellular green algae and cyanobacteria may be similar.

## ACKNOWLEDGEMENT

The authors would like to thank A.R. Portis for helpful discussions and for critical reading of the manuscript.

## REFERENCES

[1] Berry, J., Boynton, J., Kaplan, A. and Badger, M. (1976) Carnegie Inst. Wash. Yearbook, 423–432.
[2] Findenegg, G.R. (1976) Z. Pflanzenphysiol. 79, 428–437.
[3] Hogetsu, D. and Miyachi, S. (1977) Plant Cell Physiol. 18, 347–352.
[4] Raven, J.A. and Glidewell, S.M. (1978) Plant Cell Environ. 1, 185–197.
[5] Kaplan, A., Badger, M.R. and Berry, J.A. (1980) Planta 149, 219–226.
[6] Coleman, J.R. and Colman, B. (1980) Plant Physiol. 65, 980–983.
[7] Zenvirth, D. and Kaplan, A. (1981) Planta 152, 8–12.
[8] Badger, M.R., Kaplan, A. and Berry, J.A. (1980) Plant Physiol. 66, 407–413.
[9] Beardall, J. and Raven, J. (1981) J. Phycol. 17, 134–141.
[10] Spalding, M.H. and Ogren, W.L. (1982) FEBS Lett. 145, 41–44.
[11] Hogetsu, D. and Miyachi, S. (1979) Plant Cell Physiol. 20, 747–756.
[12] Tsuzuki, M., Shiraiwa, Y. and Miyachi, S. (1980) Plant Cell Physiol. 21, 677–688.
[13] Spreitzer, R.J. and Mets, L. (1981) Plant Physiol. 67, 565–569.
[14] Heldt, H. (1980) Methods Enzymol. 69, 604–613.
[15] Wintermans, J.F.G.M. and De Mots, A. (1965) Biochim. Biophys. Acta 109, 448–453.
[16] Neame, K.D. and Richards, T.G. (1972) Elementary Kinetics of Membrane Carrier Transport, Wiley, New York.