Plant Physiol. (1980) 65, 397–402
0032-0889/80/65/0397/06/$00.50/0

# Evidence for $HCO_3^-$ Transport by the Blue-Green Alga (Cyanobacterium) *Coccochloris peniocystis*[1]

Received for publication May 17, 1979 and in revised form September 20, 1979

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## ABSTRACT

**The possibility of $HCO_3^-$ transport in the blue-green alga (cyanobacterium) *Coccochloris peniocystis* has been investigated. *Coccochloris* photosynthesized most rapidly in the pH range 8 to 10, where most of the inorganic C exists as $HCO_3^-$. If photosynthesis used only $CO_2$ from the external solution the rate of photosynthesis would be limited by the rate of $HCO_3^-$ dehydration to $CO_2$. Observed rates of photosynthesis at alkaline pH were as much as 48-fold higher than could be supported by spontaneous dehydration of $HCO_3^-$ in the external solution. Assays for extracellular carbonic anhydrase were negative. The evidence strongly suggests that $HCO_3^-$ was a direct C source for photosynthesis.**

**Weakly buffered solutions became alkaline during photosynthesis with a one-to-one stoichiometry between $OH^-$ appearance in the medium and $HCO_3^-$ initially added. Alkalization occurred only during photosynthesis and was blocked by 3-(3,4-dichlorophenyl)-1,1-dimethylurea, diuron. It is suggested that $HCO_3^-$ was transported into cells of *Coccochloris* in exchange for $OH^-$ produced as a result of $HCO_3^-$ fixation in photosynthesis.**

**The inorganic C concentration required to support a rate of photosynthesis of half the maximum rate ($K_m$) was 6 micromolar at pH 8.0 or, in terms of available $CO_2$, a $K_m$ of 0.16 micromolar. This value is two orders of magnitude lower than reported $K_m$ values for the D-ribulose-1,5-bisphosphate carboxylase for blue-green algae. It is suggested that the putative $HCO_3^-$ transport by *Coccochloris* serves to raise the $CO_2$ concentration around the carboxylase to levels high enough for effective fixation.**

It is generally accepted that various algal species are capable of transporting the bicarbonate ion across the cell membrane for use in photosynthesis (25), but substantive evidence is lacking in most cases. Raven (25) suggested that if the rate of photosynthesis by an alga is markedly higher at an alkaline pH, for a given $CO_2$ concentration, than at a more acid pH it can be concluded that the bicarbonate ion crosses the cell membrane and contributes C for photosynthesis. By this criterion, Raven (24) and Raven and Glidewell (26) have attributed $HCO_3^-$ transport ability to the green alga *Hydrodictyon africanum*. The same conclusion has been reached, on similar grounds, for other algal species (*e.g.* 11). However, as Raven (25) himself admits, an enhanced photosynthetic rate for a given $CO_2$ concentration at higher pH values may have causes other than an ability to transfer and use exogenous $HCO_3^-$.

The best evidence to date for $HCO_3^-$ transport by an alga is that of Lucas (16, 18) for the giant-celled alga *Chara corallina*. Unlike other reports, the evidence for $HCO_3^-$ transport in this case does not rest solely upon a comparison of photosynthetic rates at acid and alkaline pH values. Lucas (16) has shown that *C. corallina* at pH 9.0 has a rate of photosynthesis higher than can be supported solely by the fixation of $CO_2$ derived from the spontaneous dehydration of $HCO_3^-$ in the external medium. It is probable that these cells take up $HCO_3^-$ in exchange for $OH^-$ (generated by photosynthetic fixation of the transported $HCO_3^-$) on distinct ion carriers in the cell membrane (17). Talling (30) has shown that the algae *Microcystis aeruginosa* and *Ceratium hirudinella* both have photosynthetic rates at pH values of 10 or greater that are in excess of the rates that could be supported by the spontaneous dehydration of $HCO_3^-$ in the medium. Transport of $HCO_3^-$ across cell membranes is thus indicated.

In an interesting series of experiments Badger *et al.* (2) have shown that both *Chlamydomonas reinhardtii* and *Anabaena variabilis*, when grown at low ambient $CO_2$ concentrations, can substantially accumulate inorganic C. This accumulation is not the result of a more alkaline intracellular pH relative to the external medium (2) and a "$CO_2$-concentrating mechanism" is indicated, presumably across the cell membrane.

We have been studying the photosynthetic carbon metabolism of the blue-green alga *Coccochloris peniocystis* (7, 14). This alga has been placed in the genus *Synechococcus* by Stanier *et al.* (29), along with other blue-green algae with cylindrical cells that undergo repeated binary fission in a single plane, which frequently results (as with *C. peniocystis*) in the formation of short chains of cells. Recently, Birmingham and Colman (4) have shown that *C. peniocystis*, at pH 7.9 and a low inorganic C concentration, has a rate of photosynthesis 5-fold that is supportable by $CO_2$ production from the spontaneous dehydration of $HCO_3^-$ in the medium. In this paper we report further investigations of $HCO_3^-$ transport in *C. peniocystis*.

## MATERIALS AND METHODS

**Organism and Growth Conditions.** *C. peniocystis* Kutz (1548) was obtained as an axenic culture from the algal collection at Indiana University, Bloomington, and was cultured as previously described (21). The cell density at harvest was such that the Chl content was 6–11 μg/ml. Cells were harvested by centrifugation at 6,000*g* at 25 C and were then washed with the solution to be used in the subsequent experiment (usually 20 mM $K_2HPO_4$ phosphate adjusted to pH 8.0 with 1 N NaOH).

**$O_2$ Evolution.** Photosynthetic rates were based upon the rate of $O_2$ evolution, using a Clark-type electrode (Hansatech Ltd., Kings Lynn, Norfolk, U.K.), as described by Delieu and Walker (8). A cell density equivalent to 2–3 μg Chl/ml was used when a maximum rate of $O_2$ evolution per cell was required, or at 6–11 μg Chl/ml when a higher rate of $O_2$ evolution per unit volume of solution was required. Chl content was determined after extraction of cell pellets with ice-cold methanol (19). The light intensity at the surface of the $O_2$ electrode chamber was $2.6 \times 10^4$ μw/cm$^2$, or

[1] This work was supported by grants from the National Research Council of Canada.

about 31 klux, and was obtained using a 30-w reflector lamp (General Electric Co.).

The cell suspensions, before addition to the $O_2$ electrode chamber, were incubated in test tubes in a water bath at 25 C and bubbled with air that had been passed through a tube of closely packed NaOH pellets, a 50% (w/v) solution of NaOH, and finally, a volume of lightly acidified distilled $H_2O$. During this preincubation period (15–20 min) the $CO_2$ compensation point was reached, and upon addition of a sample of the cell suspension to the $O_2$ electrode chamber only a short burst of $O_2$ evolution was seen, followed by a steady trace indicative of the compensation point. Renewed $O_2$ evolution was then initiated by the addition of $\mu$l amounts of a freshly prepared $NaHCO_3$ solution. In some experiments the length of time needed to reach the $CO_2$ compensation point at about 0.5 $\mu$l/l $CO_2$ (4) was shortened by the resuspension of cells in solutions of low $CO_2$ content prepared by the addition of buffer salts ($K_2HPO_4$ for pH 8.0; the Tris base for pH 9.5) in amounts calculated to yield the desired pH, to $CO_2$-free distilled $H_2O$. In such cases the $CO_2$ compensation point was reached within 2–3 min.

**$^{14}C$ Photoassimilation.** After initiation of photosynthesis in the $O_2$ electrode chamber by addition of $NaH^{14}CO_3$, 30-$\mu$l samples of cell suspension were withdrawn through the capillary port and were quickly injected into 1 ml of the ethanol/formic acid extractant of Atkins and Canvin (1). The incorporation of radioactivity into acid-stable products was measured.

**Measurement of pH in External Solution.** Cells were usually incubated in solutions of 50 mM $K_2HPO_4$ (pH 8.0) and no significant pH changes in the external solution were evident for up to 3 h incubation in the light. To determine the ability of the cells to cause pH changes in the external solution, the K-phosphate concentration was reduced to 0.3 mM. Cell suspensions (10 ml) were placed in a temperature-controlled (15 C) and illuminated ($2.6 \times 10^4$ $\mu w/cm^2$) glass vial. With the pH electrode and a magnetic flea in place, a 2-cm layer of degassed paraffin oil was placed on top of the cell suspension to prevent $CO_2$ exchange with the atmosphere (Birmingham, personal communication). A combination electrode and a Fisher Accumet pH meter (model 320) with an expanded scale mode, coupled to a Sargent model SLGR stripchart recorder were used to monitor the pH changes. At maximum sensitivity, 1 pH unit corresponded to 27 cm on the strip chart. Increases in the external pH were equated with increasing $OH^-$ concentration by back titration of the cell suspension with aliquots of 0.1 N HCl (volumetric solutions, BDH Chemicals, Toronto).

**Carbonic Anhydrase Assay.** The assay was based upon the acidification that results from the conversion of $CO_2$ to $HCO_3^-$ at pH 8.4. Cells were suspended (up to 20 $\mu$g Chl/ml) in 25 mM $K_2HPO_4$ adjusted with 1 N NaOH to pH 8.36. The algal suspensions were then placed in the pH measuring chamber and chilled to 4–5 C. The rate of acidification following the addition of 0.5 ml of $CO_2$-saturated water was recorded. The rate was compared to the uncatalyzed rate obtained in the absence of cells.

## RESULTS

**Measurement of Inorganic C Assimilation.** Photosynthetic $O_2$ evolution was routinely used as a measure of inorganic C assimilation. The approach is justified because of the close correspondence between the time course of $O_2$ evolution and inorganic C assimilation (as measured with $^{14}C$) and a stoichiometry of about 0.96 between $O_2$ evolved and C fixed (Fig. 1).

The maximal rates of photosynthesis by *Coccochloris* were obtained in the pH range 8–10 (Fig. 2) and much reduced rates were obtained below pH 6.0. The ability to maintain higher photosynthetic rates at alkaline pH was consistent with the observation that growth of *Coccochloris* was almost as rapid in the pH range 9.5–10.0 as it was in the pH range 8.0–8.5 (data not shown) while growth below pH 7.0 was slow. The drop in photosynthetic rate at pH 10.5 could be due to $CO_3^{2-}$ inhibition of $HCO_3^-$ transport (16), but since no inhibition was observed at pH 10.0, where $CO_3^{2-}$ accounts for about 35% of the total DIC[2], this inhibition appears to require a high concentration of $CO_3^{2-}$.

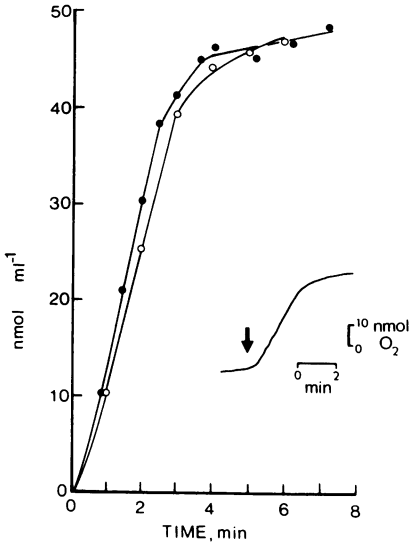


FIG. 1. Comparison of photosynthetic $^{14}C$ incorporation and $O_2$ evolution. Cells were suspended in phosphate buffer (pH 8.0). Photosynthesis was initiated by the addition (↓) of $NaH^{14}CO_3$ (0.5 $\mu$Ci) to yield an inorganic C concentration of 50 $\mu$M. Inset figure shows the recorder trace of $O_2$ evolution. Main figure shows the course of $O_2$ evolution (○) (derived from the recorder trace) from the time of $NaHCO_3$ addition, and the course of $^{14}C$ incorporation (●) into acid-stable products.

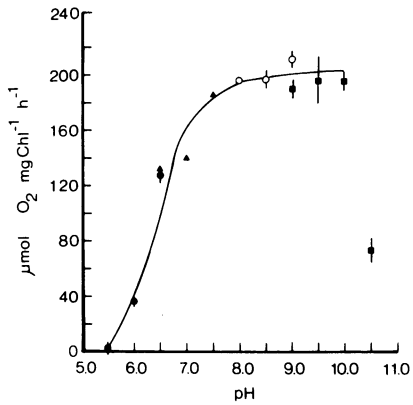


FIG. 2. Rate of photosynthetic $O_2$ evolution with respect to pH. Cells were suspended in one of the following buffers: 50 mM 2-(*N*-morpholino)ethanesulfonic acid (●) adjusted to pH 5.5, 6.0, or 6.5 with 1 N KOH; 50 mM potassium dihydrogen Pi (▲) adjusted to pH 6.5, 7.0, or 7.5 with 1 N KOH; 50 mM Tris (hydroxymethyl)methylaminopropanesulfonic acid (○) adjusted to pH 8.0, 8.5, or 9.0 with 1 N KOH; and 50 mM glycine (■) adjusted to pH 9.5, 10.0, or 10.5 with 1 N KOH. The inorganic C concentration was 5 mM. Triplicate determinations were made in those cases where a stable rate of photosynthesis was maintained (results presented as mean ± SE). For cells suspended in K-phosphate or pH 8.0 solutions the photosynthetic rate was at a maximum soon after preparation of the suspension and replicate determinations on the same suspension could not be made.

**Rate of Photosynthesis at Alkaline pH.** At high levels of inorganic C the photosynthetic rate was maximal at alkaline pH (Fig. 2). A rapid rate was maintained in the pH range 8–10 even at a low inorganic C concentration (Fig. 3). In these experiments, the rate of $O_2$ evolution was greater than the rate that could have been supported solely by the assimilation of $CO_2$ resulting from the

[2] Abbreviation: DIC: dissolved inorganic carbon.

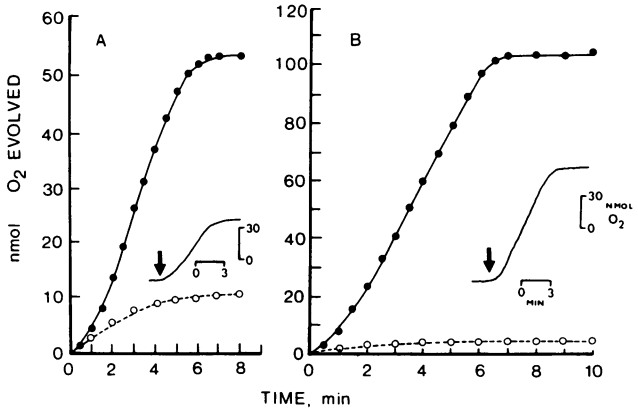


FIG. 3. Observed photosynthetic rate compared to the photosynthetic rate that could be supported by the theoretical $CO_2$ supply rate (given by equation 10). Cells were suspended in $CO_2$-free phosphate buffer at pH 8.16 (A) or in $CO_2$-free 50 mM Tris (hydroxymethyl)aminomethane solution at pH 9.58 (B). Photosynthesis was initiated by the addition (↓) of $NaHCO_3$ to give final inorganic C concentrations of 50 or 100 μM, respectively. Inset figures show the recorder traces of $O_2$ evolution. Main figures show the courses of $O_2$ evolution (●) (derived from the recorder traces), from the time of $NaHCO_3$ addition, and the calculated course of $O_2$ evolution (○) that could be supported solely by the use of $CO_2$.

spontaneous dehydration of $HCO_3^-$ in the external solution. At both pH 8.16 (Fig. 3A) and pH 9.58 (Fig. 3B) the final number of nmol $O_2$ evolved was within 5% of the number of nmol $NaHCO_3$ added to initiate photosynthesis. In the experiment carried out at pH 8.16, the inorganic C concentration after 4 min was about 16 μM. The $CO_2$ supply rate from the spontaneous dehydration of $HCO_3^-$ at this inorganic C concentration and pH would have been no more than 0.9 nmol $ml^{-1}$ $min^{-1}$ (calculated from equation 11 of Appendix). The observed photosynthetic rate was 11.4 nmol $ml^{-1}$ $min^{-1}$ and was thus about 13-fold the rate that could have been supported by use of $CO_2$ from the spontaneous dehydration of $HCO_3^-$. Furthermore, in the experiment carried out at pH 9.58, the inorganic C concentration remaining after 5 min was about 25 μM (Fig. 3B) and the maximal $CO_2$ supply rate would have been about 0.40 nmol $ml^{-1}$ $min^{-1}$. The observed photosynthetic rate was 19 nmol $ml^{-1}$ $min^{-1}$ and was thus about 48-fold the rate that could have been supported by the use of $CO_2$ from the spontaneous dehydration of $HCO_3^-$. Discrepancies between the rate of inorganic C assimilation and the rate of spontaneous dehydration of $HCO_3^-$ can be expected if cells are capable of using $HCO_3^-$ directly. Such a discrepancy would be most readily observable at low levels of inorganic C and at alkaline pH (both factors result in a low supply rate of $CO_2$ as a result of spontaneous $HCO_3^-$ dehydration, see equation 11). Both these factors apply to the experiments described in Figure 3.

**Alkalization of Medium.** Most experiments were performed with solutions buffered sufficiently to prevent significant changes in the pH. When the buffering capacity was drastically lowered (to 0.6% N) an alkalization concomitant with photosynthesis was readily detectable (Fig. 4). This alkalization was observed only when cells were illuminated in the presence of $NaHCO_3$ and it was completely abolished by 10 μM DCMU which also completely abolished photosynthesis (Fig. 4C). The pH change expressed as a molar increase in hydroxyl ions was equal, within experimental error, to the molar amount of $NaHCO_3$ initially added to the medium to support photosynthesis. Thus, in the 14 cases that were analyzed, the average increase in the amount of hydroxyl ion was 1,030 ± 33 nmol (± SE) compared to the 1,000 nmol $NaHCO_3$ initially added.

At compensation point levels of inorganic C in the light, the pH of the medium remained effectively constant (Fig. 4B). In the dark an acidification of the medium was observed (Fig. 4D). The rate of acidification was always substantially lower than the rate of alkalization observed in the light and appeared to be dependent upon the length of the preceding photosynthetic period. The acidification process has not yet been studied in any greater detail.

A consideration of reactions 1, 2, and 3 shows that alkalization will occur if $CO_2$, produced from the spontaneous dehydration of $HCO_3^-$, is removed from the medium by cells for use in photosynthesis. That is, the dehydration of $HCO_3^-$ to redress the balance caused by removal of $CO_2$ results in $OH^-$ production in the medium that will equal the rate of $HCO_3^-$ dehydration (or $CO_2$ supply rate), as shown in equation 11. There is a very close stoichiometry between alkalization and inorganic C consumption

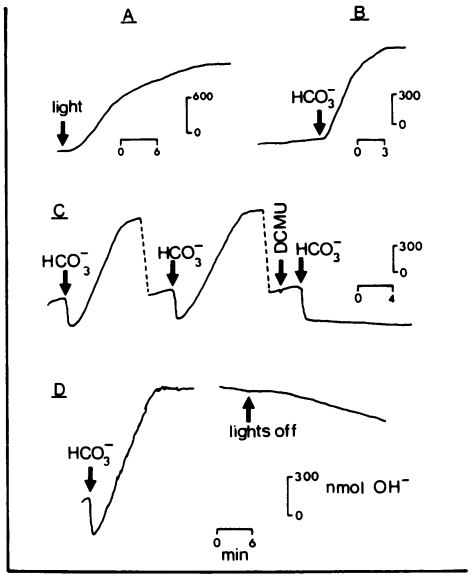


FIG. 4. Alkalization of the medium during photosynthesis. Cells were suspended in 0.3 mM phosphate buffer and the pH was adjusted to the desired value with 0.1 N NaOH. When $NaHCO_3$ was added it was to yield an initial inorganic C concentration of 100 μM. A: initial pH = 7.20; final pH = 9.52. B: initial pH = 8.17; final pH = 9.10. C: initial pH = 9.83; final pH = 10.02. The pH was readjusted (---) to the initial value by the addition of 0.1 N HCl. DCMU concentration was 10 μM. D: initial pH = 9.85; final pH = 9.98.

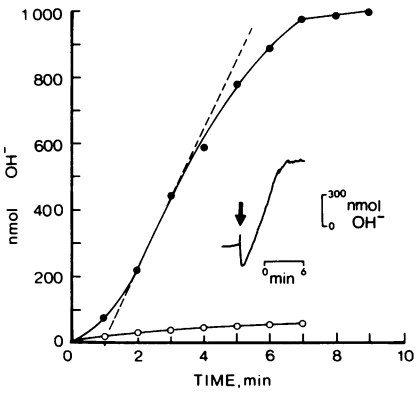


FIG. 5. Observed alkalization during photosynthesis compared to the course of alkalization expected if $CO_2$ were the only C source for photosynthesis. Initial pH was 9.64. Inorganic C concentration was raised to 100 μM by the addition (↓) of $NaHCO_3$. Inset figure shows the pH recorder trace. Main figure shows the course of $OH^-$ appearance (●), derived from the recorder trace, in the medium, and the calculated course of $OH^-$ appearance (○) that would occur if $CO_2$ were the sole C source for photosynthesis.

in photosynthesis (see above) and thus, the amount of inorganic C remaining at any given time can be estimated from the extent of alkalization that has occurred up to that time. If photosynthesis is initiated by the addition of a known amount of $NaHCO_3$ to a lightly buffered medium it is possible to measure the pH at any given time and to estimate the amount of inorganic C consumed. If the pH and inorganic C concentration are known it is possible, over any given interval, to calculate the maximum $CO_2$ supply rate due to dehydration of $HCO_3^-$ (equation 11). An experiment was performed to test the hypothesis that alkalization resulted solely from the result of spontaneous dehydration of $HCO_3^-$ (Fig. 5). At all times the rate of alkalization was considerably greater than could be accounted for by the dehydration of $HCO_3^-$ and uptake by the cells of the $CO_2$ produced (Fig. 5). Thus, after 2.5 min the inorganic C concentration was estimated to about 5 $\mu$M and the observed rate of alkalization was 210 nmol $OH^-$ $min^{-1}$. The calculated dehydration rate under these conditions was no more than 10 nmol $OH^-$ $min^{-1}$. The discrepancy was even greater as photosynthesis progressed and the inorganic C concentration decreased and the medium became more alkaline (Fig. 5).

**Search for External Carbonic Anhydrase.** Because the rates of photosynthesis and alkalization were faster than could be accounted for by the rate of spontaneous dehydration of $HCO_3^-$ in the medium, it was possible that *Coccochloris* possessed an extracellular carbonic anhydrase to catalyze the dehydration. Even at cell densities up to twice as great as those used in photosynthetic measurements, no evidence for carbonic anhydrase activity in the extracellular space was detectable (Fig. 6). Small levels of activity resulting from the addition of commercially obtained carbonic anhydrase were readily detected in the assay (Fig. 6).

**Photosynthesis and Inorganic C Concentration.** *Coccochloris* was evidently capable of maintaining rapid rates of photosynthesis at low inorganic C concentrations (*e.g.* Fig. 3). In a number of experiments a more detailed study of the relationship between the rate of photosynthesis and inorganic C concentration was carried out. Typical results are shown in Figure 7. An apparent $K_m$ with regard to the inorganic C concentration has been calculated as 6 $\mu$M (Fig. 7).

## DISCUSSION

*C. peniocystis* grows and photosynthesizes optimally at alkaline pH values and was thus a logical candidate for testing the ability of a photosynthetic cell to use exogenous $HCO_3^-$ as a C source, rather than $CO_2$. If a photosynthetic cell can use only $CO_2$ from the external medium then its rate of photosynthesis will be limited

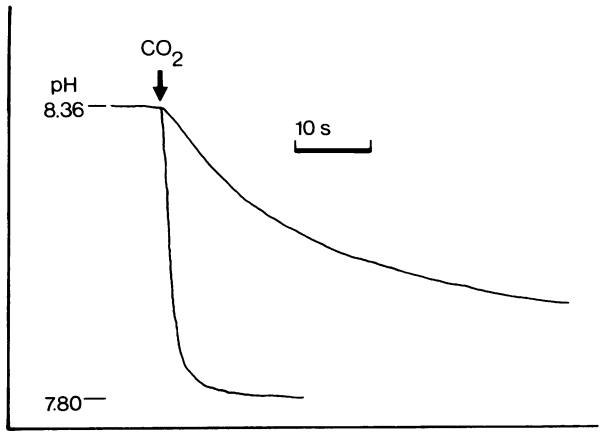


FIG. 6. Assay for extracellular carbonic anhydrase. Cells were suspended at a density of 18.0 $\mu$g Chl $ml^{-1}$ in phosphate buffer. Upper trace shows the pH change that took place in either the absence or presence of cells. Lower trace shows the pH change in the presence of 0.2 $\mu$g/ml carbonic anhydrase (Sigma).

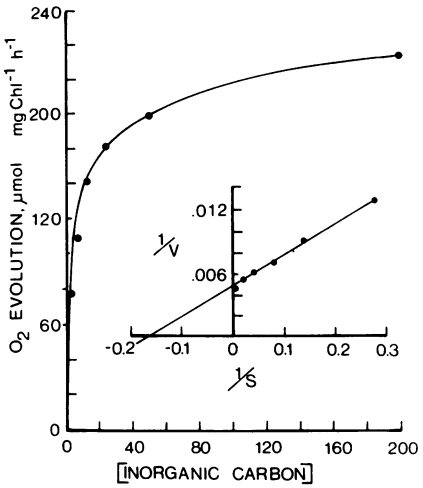


FIG. 7. Rate of photosynthetic $O_2$ evolution with respect to the total inorganic C concentration.

by the rate at which $CO_2$ can be formed in the medium from $HCO_3^-$. The theoretical basis for the calculation of maximum rate of supply of $CO_2$ from $HCO_3^-$ is given in the Appendix. In the absence of an extracellular carbonic anhydrase the dehydration of $HCO_3^-$ to form $CO_2$ and $OH^-$ will proceed no faster than described by equation 11. Thus, if photosynthetic rates are measured that exceed the maximum $CO_2$ supply rate, and if no evidence for an extracellular carbonic anhydrase can be found, it can reasonably be postulated that $HCO_3^-$ itself crosses the cell membrane as the source of inorganic C for photosynthesis. The same reasoning has been used to demonstrate $HCO_3^-$ transport in *C. corallina* (16). The photosynthetic rates of *Coccochloris* at alkaline pH were 13- to 48-fold greater than the rates that could have been realized if the cells had been able only to use $CO_2$ generated by the spontaneous breakdown of $HCO_3^-$ in the medium (Fig. 3). No evidence for an extracellular carbonic anhydrase at sufficient activity to enhance the dehydration of $HCO_3^-$ to account for the 13- to 48-fold discrepancy was found (Fig. 6). The carbonic anhydrase assay used could readily have detected a 13-fold enhancement of the $HCO_3^-$ dehydration rate. In any event, it is highly unlikely that an extracellular carbonic anhydrase (even if present in the periplasmic space) could be useful in the pH range 9.58–10.00 (Figs. 3B and 5) because in this range reaction 1 is very significant (15) and the back reaction constant (for the formation of $HCO_3^-$ from $CO_2$ and $OH^-$) is $4.25 \times 10^7$-fold the forward rate constant (for the formation of $CO_2$ by dehydration). Thus, the half-life of a $CO_2$ molecule produced from $HCO_3^-$ will be very short indeed and any $CO_2$-scavenging mechanism would have to be remarkably efficient. The high photosynthetic rates of *Coccochloris* at alkaline pH are most readily explained by the ability of the cells to use $HCO_3^-$ itself.

The uptake of inorganic C was concomitant with an alkalization of the medium (Fig. 4) and a stoichiometry of close to 1:1 existed between $OH^-$ appearance in the medium and $HCO_3^-$ initially added. The rate of alkalization was much greater than could have resulted from the uptake by the cells of $CO_2$ produced by the spontaneous dehydration of $HCO_3^-$ in the medium (Fig. 5). We suggest, instead, that the alkalization was the result of $OH^-$ efflux by photosynthesizing cells to balance the influx of $HCO_3^-$. Lucas (16) has shown the same exchange for *C. corallina*. The $OH^-$ ions would be produced as a result of $HCO_3^-$ assimilation in photosynthesis.

Electroneutrality could, of course, also be maintained by an equivalent uptake of $H^+$ with $HCO_3^-$ and the observed alkalization (Fig. 4) would still occur. However, the rate of photosynthesis was as rapid at pH 10 as at pH 8 (Fig. 2) and thus a $H^+$ co-

transporting system would have to possess a $K_m$ with respect to $H^+$ of considerably less than $10^{-10}$ M. The intracellular pH of *Coccochloris* has not been measured, but in other blue-green algae it is greater than pH 7 (2, 10, 20). The intracellular $OH^-$ will, therefore, be at least $10^{-7}$ M and $OH^-$ is a more likely substrate than $H^+$. Lucas (18) has reached the same conclusion in the case of *Chara*. Photosynthetic fixation of C supplied as $HCO_3^-$ will result in the production of 1 mol $OH^-$ for each mol of carbon fixed, independent of the fixation pathway.

Lucas (16) has measured a $V_{max}$ for $HCO_3^-$ transport by *C. corallina* of 71 pmol $cm^{-2}$ $s^{-1}$. From a knowledge of the cell dimensions of an average *Coccochloris* cell (cylinders 0.9 μm wide × 4.2 μm long) and the number of cells per unit suspension volume (by hemocytometer) we have been able to calculate the putative $HCO_3^-$ influxes in terms of pmol $cm^{-2}$ $s^{-1}$. Assuming a $V_{max}$ of 210 μmol mg $Chl^{-1}$ $h^{-1}$. (Fig. 7) a value of 5.5 pmol $cm^{-2}$ $s^{-1}$ is obtained.

Few studies on an ion transport by blue-green algae have been published. Dewar and Barber (9) measured what appeared to be mainly $Cl^-/Cl^-$ exchange across the cell membrane and found a $V_{max}$ of 0.2 pmol $cm^{-2}$ $s^{-1}$, considerably less than $V_{max}$ of $HCO_3^-$ transport into *Coccochloris* of 5.5 pmol $cm^{-2}$ $s^{-1}$. It should be noted that the $HCO_3^-$ fluxes estimated in the present study are probably minimum estimates reflecting final steady-state values. The initial influx rates obtained before saturation of the intracellular inorganic C pool could be much greater (*cf.* 2).

The larger $OH^-$ efflux, or less likely a $H^+$ influx, by *Coccochloris* upon illumination is worthy of note because it is often assumed that $H^+$ translocation is outwardly directed across the cell membrane of photosynthetic prokaryotes, as it is in heterotrophic ones (27). When cells of *A. variabilis* or *Plectonema boryanum* were illuminated, the external medium was acidified (20, 27). It is interesting that these cells were grown on elevated levels of $CO_2$ (5% v/v) rather than air. When *Coccochloris* is grown on elevated $CO_2$ levels an induction period is required before high rates of photosynthesis with bicarbonate are attained (14). It is possible that cells grown on high levels of $CO_2$ do not possess the efficient $HCO_3^-$-scavenging mechanism of those grown on air and would thus not carry out a $HCO_3^-/OH^-$ exchange process across the cell membrane. Berry *et al.* (3) have presented evidence suggesting that low $CO_2$ adapted *C. reinhardtii* possesses a $HCO_3^-$ influx pump whereas high $CO_2$ cells do not. Thus, we believe it unwise to make *a priori* assumptions regarding the direction of $H^+$ translocation in blue-green algae. Padan and Schuldiner (22), in the absence of any flux measurements, have postulated that glucose transport in the dark and the light by *P. boryanum* is driven by energy stored in a proton electrochemical gradient resulting from outward $H^+$ translocation. Their assumption for this polarity was based solely on the work of Scholes, Mitchell, and Moyle (27).

The significance of the large $OH^-$ efflux upon the membrane potential of *Coccochloris* will depend upon whether or not there is a tightly coupled obligate exchange of $OH^-$ for $HCO_3^-$ or whether there are electrogenic components. Also unknown is the nature of the intracellular pH during the $OH^-$ efflux.

The inorganic C concentration required to support a rate of photosynthesis of half the maximum rate was 6 μM (Fig. 7). At pH 8.0 only a small percentage of the inorganic C exists as $CO_2$ in a closed system and thus $K_m$ calculated on the basis of the free $CO_2$ available to the cells would be about 0.16 μM. For *Aphanocapsa* 6308, Codd and Stewart (6) have reported a $K_m$ for ribulose-1,5-bisphosphate carboxylase $CO_2$ concentration of 71 μM. The observed $K_m$, with respect to free $CO_2$, of whole cells of *Coccochloris* is more than two orders of magnitude lower than the expected $K_m$ for the final enzyme involved in $CO_2$ fixation. Thus, a $CO_2$-concentrating mechanism is required if the $CO_2$ concentration around the carboxylase is to be high enough for effective fixation. The postulated $HCO_3^-$ transport system would serve this function.

## APPENDIX

**Calculation of Maximum $CO_2$ Supply Rate.** The $HCO_3^-$ ion dehydrates to form $CO_2$ by two concurrent reactions (15). Dehydration of the ion can be direct

$$HCO_3^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} CO_2 + OH^- \quad (1)$$

This mechanism is especially significant at pH values greater than 8 (15). Published values for the rate constants at 25 C are $k_1 = 2 \times 10^{-4}$ $s^{-1}$ and $k_2 = 8.5 \times 10^3$ $M^{-1}$ $s^{-1}$ (23). The second reaction giving rise to $HCO_3^-$ dehydration is the indirect one involving $H_2CO_3$ as an intermediate (15). The ionic reaction

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \quad (2)$$

is almost instantaneous (28) and will not be a rate-limiting step under physiological conditions. The breakdown of $H_2CO_3$ is, however, quite a slow step

$$H_2CO_3 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} CO_2 + H_2O \quad (3)$$

Values for the rate constants at 25 C are $k_3 = 18$ $s^{-1}$ and $k_4 = 3.7 \times 10^{-2}$ $s^{-1}$ (23). Considering equations 1 and 3, an over-all rate equation for $CO_2$ formation from $HCO_3^-$ can be derived

$$\frac{d[CO_2]}{dt} = k_1[HCO_3^-] - k_2[CO_2][OH^-] + k_3[H_2CO_3] - k_4[CO_2] \quad (4)$$

Equation 4 can be simplified following the procedure of Lucas (16), by making the assumption that photosynthesizing cells scavenge all $CO_2$ molecules as rapidly as they are formed by the dehydration of $HCO_3^-$. Thus, the $CO_2$ tends to zero. Under this rather optimistic assumption a maximal rate of $CO_2$ formation is calculated

$$\frac{d[CO_2]}{dt} = k_1[HCO_3^-] + k_3[H_2CO_3] \quad (5)$$

Attainment of equilibrium in reaction 2 is almost instantaneous (28), so that the following relationship will hold at all times during $HCO_3^-$ dehydration

$$[H_2CO_3] = \frac{[HCO_3^-][H^+]}{K_1} \quad (6)$$

where $K_1$ is the equilibrium constant for reaction 2 and has a value at 25 C of $1.72 \times 10^{-4}$ (12, 31). At pH values greater than 8 the predominant inorganic C species (DIC) are $HCO_3^-$ and $CO_3^{2-}$ (4) and the equilibration between them is almost instantaneous (28). Thus, at all times during $HCO_3^-$ dehydration

$$[HCO_3^-] = \frac{[DIC]}{1 + \frac{[H^+]}{K} + \frac{K_2}{[H^+]}} \quad (7)$$

where $K_2$ is the equilibrium constant for the reaction

$$H^+ + CO_3^= \rightleftharpoons HCO_3^- \quad (8)$$

and at 25 C has a value of $4.69 \times 10^{-11}$ (13), and K has a value of $4.45 \times 10^{-7}$ (12) and is the product of the equilibrium constants for reactions 2 and 3. Substitution of equations 6 and 7 into equation 5 gives

$$\frac{d[CO_2]}{dt} = k_1\frac{[DIC]}{1 + \frac{[H^+]}{K} + \frac{K_2}{[H^+]}} + k_3\frac{[DIC]}{1 + \frac{[H^+]}{K} + \frac{K_2}{[H^+]}}[H^+]\cdot\frac{1}{K_1} \quad (9)$$

Substitution of values for the rate and equilibrium constants yields

$$\frac{d[CO_2]}{dt} = \frac{[DIC]}{5000A} + \frac{1.05 \times 10^5[DIC][H^+]}{A} \quad (10)$$

where

$$A = 1 + \frac{[H^+]}{4.45 \times 10^{-7}} + \frac{4.69 \times 10^{-11}}{[H^+]}$$

Equation 10 allows contributions of reaction 1, first term, and reaction 3, second term, to be individually calculated. For routine calculations equation 10 is expressed as

$$\frac{d[CO_2]}{dt} = \frac{[DIC]}{5000A} \times (1 + 5.25 \times 10^8[H^+]) \quad (11)$$

This equation applies to a closed system in which the sole extracellular source of $CO_2$ is from the spontaneous dehydration of $HCO_3^-$. $dCO_2/dt$ is subsequently referred to as the $CO_2$ supply rate. $CO_2$ supply rates calculated by means of equation 11 are about 2-fold higher at pH 9.0 than those calculated by Lucas (15), but the discrepancy does not invalidate the conclusion that *C. corallina* transports $HCO_3^-$ (15).

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