Characterization of the Na⁺-Requirement in Cyanobacterial Photosynthesis

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ABSTRACT
The Na⁺ requirement for photosynthesis and its relationship to dissolved inorganic carbon (DIC) concentration and Li⁺ concentration was examined in air-grown cells of the cyanobacterium Synechococcus leopoliensis UTEX 625 at pH 8. Analysis of the rate of photosynthesis (O2 evolution) as a function of Na⁺ concentration, at fixed DIC concentration, revealed two distinct regions to the response curve, for which half-saturation values for Na⁺ (Kd[Na⁺]) were calculated. The value of both the low and the high Kd(Na⁺) was dependent upon extracellular DIC concentration. The low Kd(Na⁺) decreased from 1000 micromolar at 5 micromolar DIC to 200 micromolar at 140 micromolar DIC whereas over the same DIC concentration range the high Kd(Na⁺) decreased from 10 millimolar to 1 millimolar. The most significant increases in photosynthesis occurred in the 1 to 20 millimolar range. A fraction of total photosynthesis, however, was independent of added Na⁺ and this fraction increased with increased DIC concentration. A number of factors were identified as contributing to the complexity of interaction between Na⁺ and DIC concentration in the photosynthesis of Synechococcus. First, as revealed by transport studies and mass spectrometry, both CO₂ and HCO₃⁻ transport contributed to the intracellular supply of DIC and hence to photosynthesis. Second, both the CO₂ and HCO₃⁻ transport systems required Na⁺, directly or indirectly, for full activity. However, micromolar levels of Na⁺ were required for CO₂ transport while millimolar levels were required for HCO₃⁻ transport. These levels corresponded to those found for the low and high Kd(Na⁺) for photosynthesis. Third, the contribution of each transport system to intracellular DIC was dependent on extracellular DIC concentration, where the contribution from CO₂ transport increased with increased DIC concentration relative to HCO₃⁻ transport. This change was reflected in a decrease in the Na⁺ concentration required for maximum photosynthesis, in accord with the lower Na⁺ requirement for CO₂ transport. Lithium competitively inhibited Na⁺-stimulated photosynthesis by blocking the cells’ ability to form an intracellular DIC pool through Na⁺-dependent HCO₃⁻ transport. Lithium had little effect on CO₂ transport and only a small effect on the size of the pool it generated. Thus, CO₂ transport did not require a functional HCO₃⁻ transport system for full activity. Based on these observations and the differential requirement for Na⁺ in the CO₂ and HCO₃⁻ transport system, it was proposed that CO₂ and HCO₃⁻ were transported across the membrane by different transport systems.

It has long been known that Na⁺ is essential for the growth of cyanobacteria at alkaline pH (3, 10, 16, 22). In addition to other possible roles (5, 6, 8, 10, 22, 24), the requirement for Na⁺ in growth is directly related to a requirement for Na⁺ in photosynthesis (2, 3, 6, 10, 11, 15, 18, 19, 22). Two sites of action for Na⁺ have been identified; the HCO₃⁻ transport system (11, 15, 18, 22) and the CO₂ transport system (1, 2, 19, 20). Together, these transport systems generate the large intracellular pool of DIC which serves as the immediate source of CO₂ for photosynthetic fixation (7, 9, 14, 18, 21). Whether Na⁺ acts directly on the transport carriers or on some other component of the system, indirectly influencing CO₂ or HCO₃⁻ transport, is not yet known. Sodium does not appear to be directly required for photosynthetic carbon metabolism (1, 11, 15, 18, 22) and there is a suggestion that Na⁺ does not directly influence the rate of the photosynthetic electron transport to methyl viologen (24).

Although a role for Na⁺ in photosynthesis has been clearly established, the Na⁺-requirement has only been partially characterized. In the present study we examine the effects of DIC and Li⁺ concentration on the Na⁺-requirement for photosynthesis and DIC transport in air-grown cells of the cyanobacterium Synechococcus leopoliensis UTEX 625. The results provide additional insight into the mechanism of inorganic carbon acquisition by cyanobacteria.

MATERIALS AND METHODS
Organism and Growth Conditions. The unicellular cyanobacterium Synechococcus leopoliensis UTEX 625, obtained from the University of Texas Culture Collection (Austin, TX), was grown with air-bubbling (0.05% v/v CO₂) in unbuffered Allen's medium (4) as described previously (11). Cultures were continuously illuminated during growth by fluorescent lamps (40–90 μE·m⁻²·s⁻¹ PAR) and maintained between 28 and 30°C. Cells were harvested for experiments when the [Chl] reached 6 to 9 μg·mL⁻¹. At this time the pH of the culture medium was between 9.5 and 10.2.

Experimental Conditions. Prior to experiments, the cells were washed three times by centrifugation (1.0 min at 10,000g, Beckman Microfuge B) and resuspended in 25.0 mM BTP/23.5 mM HCl buffer (pH 8.0). The buffer contained about 15 μM DIC and 5 μM Na⁺ (11, 22). Experiments were conducted at 30°C, and light was provided by a quartz-halogen projector lamp at 200 to 600 μE·m⁻²·s⁻¹, which was saturating for photosynthesis. The [Chl] of cell suspensions was determined spectrophotometrically at 665 nm after extraction in methanol (11).

Photosynthetic Kinetics. The rate of photosynthesis was measured as O₂ evolution using a thermostatted Clark-type electrode (Hansatech, Kings Lynn, Norfolk, UK). Washed cells (4–9 μg Chl·mL⁻¹) were placed in the O₂ electrode chamber, purged with N₂ and allowed to deplete the medium of residual DIC. The

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2 Abbreviations: DIC, dissolved inorganic carbon (CO₂ + HCO₃⁻ + CO₃²⁻); BTP, 1,3-bis [tris (hydroxymethyl)methylamino] propane; CA, carbonic anhydrase; Kd, substrate concentration required to give half-maximum response.
[Na+] was adjusted to the desired level by adding a known volume of a concentrated NaCl solution to the cell suspension. Photosynthesis was initiated by the addition of a defined concentration of KHCO3 and the maximum rate determined. Following consumption of the DIC, the [NaCl] was increased and photosynthesis was reinitiated at the same [DIC] by the addition of another volume of KHCO3. This procedure was repeated until Na+ addition elicited no further increase in the rate of photosynthesis. In some experiments, the [Na+] was increased prior to the cells consuming all the DIC. In these cases, photosynthesis was measured at the new [Na+] following the addition of KHCO3 calculated to yield the original [DIC]. Both methods gave similar results but the latter method provided a considerable saving in time, particularly at low Na+ concentrations. During the experiments, the cell suspension was periodically purged with N2 to maintain the [O2] below 100 μM during rate measurements.

The [Na+] required to give one-half the maximum rate of photosynthesis (K0.5[Na+]), at a defined [DIC], was determined from Eadie-Hofstee or Lineweaver-Burke plots by linear regression analysis.

The effect of Li+ on Na+-stimulated photosynthesis was assessed in experiments similar to those described above where the [LiCl] and [DIC] were held constant while the [NaCl] was varied.

**Intracellular DIC Pool.** Transport and intracellular accumulation of DIC was measured using a modified version of the silicone fluid filtering centrifugation technique (9, 14, 20). The 400 μL microcentrifuge tubes were prepared by first adding 100 μL of terminating solution (2 mM KOH in 10% v/v methanol) and 100 μL of degassed silicone fluid (Ar 20:Ar 200, 0.625:1.0 v/v; 1 mM KHCO3, 0.625:1.0 v/v; Wacker Chemie, Munich, FRG), followed by a brief (30 s) centrifugation at 12,000g (Beckman Microfuge B) to compact the layers. Subsequently, 75 μL of reaction buffer (50 mM BTP/46 mM HCl, pH 8.0), containing twice the desired concentration of 14C-DIC, (as KH14CO3) was added to each microcentrifuge tube and the head space flushed with N2. Washed cells (75 μL), in 25 mM BTP/46 mM HCl buffer (pH 8.0) containing twice the desired [Chl] were then introduced into each tube in such a way as to leave an N2-filled gap between the cell suspension and the reaction buffer. The tubes were flushed again with N2 and capped until used (5 min). The cells had previously been equilibrated in the O2 electrode chamber at 30°C and allowed to deplete the medium of DIC. The [Na+] and/or [Li+] in the cell suspension was adjusted to twice the final concentration before adding the cells to the microcentrifuge tubes. Four tubes were placed in the microcentrifuge and illuminated for 2 min by water-shielded Sylvania (300 W) flood-lamps at about 350 μE·m−2·s−1 PAR. The temperature was maintained between 27 and 30°C (20). Uptake and accumulation of DIC was initiated by mixing the cells with the reaction buffer. This was accomplished by quickly turning the microcentrifuge on then off. Test runs with colored solutions and cells indicated that the brief centrifugation uniformly mixed the cell suspension with the reaction buffer without contaminating the other layers. The major benefit to this procedure was that multiple reactions could be conducted at one time.

The uptake of DIC was subsequently stopped at timed intervals by centrifuging (60 s) the cells through the silicone fluid into the basic terminating solution. The tubes were then quickly frozen in liquid N2 and stored at −20°C until analyzed.

To determine the amount of acid stable and acid labile 14C associated with the cell pellets, the bottom of the tubes were cut off, and the acid stable fluid/terminating solution interphase and the cells plus terminating solution (100 μL) removed upon thawing. The excised tips were rinsed with 100 μL of 5 mM KOH and this was combined with the terminating solution. Total 14C activity of 50 μL samples was measured directly by liquid scintillation counting in 6 mL of ACS scintillation fluid (Amersham, Toronto). Acid stable radioactivity was determined by first acidifying 50 μL samples with 300 μL of 4 N acetic acid and then evaporating the sample to dryness at 90°C. The residue was resuspended in 200 μL of distilled H2O and counted as described above. After correcting for specific activity, cell recovery and extracellular contamination of 14C-DIC (9, 14, 20), the intracellular pool was determined as the difference between total C and acid stable C and expressed as a concentration on a cell volume basis. The intracellular volume and extracellular 14C contamination was determined by the 14C-sorbitol-H2O method as described previously (9, 14). Cell recovery, typically greater than 90%, was determined in parallel experiments by comparing the Chl recovered from the cell pellet to that of an equivalent volume of cells before centrifugation. For this measurement, the terminating solution was replaced by a 6% v/v sorbitol solution. In five separate experiments (not shown) the intracellular volume was determined just prior to DIC transport studies (e.g. Fig. 5) and found to be about 56 ± 5 μg Chl/μL. This value is about 1.7-fold lower than previous measurements (9, 18). This difference may be due to differences in growth conditions. In mass spectrometry experiments, where the intracellular volume was not measured, we used an average of previous and present determinations (75 ± 6 μL·mg−1 Chl) for calculating the intracellular DIC pool.

**CO2 Transport.** The light-dependent uptake of CO2 by Synechococcus was followed by measuring the disappearance of dissolved CO2 (m/z = 44) from the medium with a mass spectrometer (VG Gas Analysis, MM 14-80 SC, Middlewich, England) equipped with a membrane inlet system (12, 13, 20). The instrument was calibrated as previously described (12, 20). Corrections to the calibration were applied to compensate for the increase in ionic strength due to the addition of millimolar levels of NaCl or LiCl. The initial rate of CO2 uptake was determined from chart recorder tracings as the slope of the line. The steady state rate of CO2 uptake was calculated from the equation of Miller et al. (20). The intracellular pool of DIC, generated predominantly by CO2 uptake, was measured by MS in iodoacetamide (3.3 mM) poisoned cells as described previously (20).

**RESULTS**

**Interaction Between [Na+] and [DIC].** Figure 1 illustrates the response of photosynthetic O2 evolution to increasing [Na+] (as NaCl) at various [DIC] and pH 8.0. At all [DIC] shown, the rate of photosynthesis increased with increased [Na+] up to a saturation level, above which no further increase in photosynthesis occurred. The rate of this increase, however, was dependent upon the [DIC] in the medium (Fig. 1). Either in the absence or presence of Na+ the rate of photosynthesis also increased with increased [DIC] although the measured rates were lower for cells lacking extracellular Na+ (Fig. 1). At [DIC] above 1 mM (not shown), however, near maximum rates of photosynthesis occurred in the absence of Na+, with Na+ addition causing only a small enhancement of the rate (11). The occurrence of near-maximum rates of photosynthesis with high [DIC] indicated that Na+ was not directly required for photosynthetic carbon metabolism. These results and those of Figure 1 indicated that the Na+ requirement for photosynthesis was dynamically related to the extracellular [DIC].

The relationship between photosynthesis and [Na+] (Fig. 1) was further analyzed using Eadie-Hofstee plots of the data in Figure 1 and other similar data. An example of one such plot is shown in Figure 2. As is evident, there were two distinct phases to the Eadie-Hofstee plot, one with a low K0.5[Na+] (phase 1; 410 μM) while the other had a much higher K0.5[Na+] (phase 2; 4.2 mM). In 25 of 31 experiments similar to those shown in Figure 1, biphasic responses (Fig. 2) were observed. In those instances where a monophasic response (not shown) was observed, the [DIC] was usually above 70 μM and it was the low K0.5[Na+]
between the apparent exponential two component which was absent. At high [DIC], the resolution of the two phases was more difficult because the difference in value between the high and the low $K_s$(Na$^+$) was smaller (Fig. 3).

Figure 3, A and B, provides a summary of the $K_s$(Na$^+$) data obtained for a range of [DIC] up to 140 μM, which was saturating for photosynthesis. The value of the high $K_s$(Na$^+$) declined in an apparent exponential manner with increased [DIC], from about 10 mM at 5 μM DIC to about 1 mM at 140 μM DIC (Fig. 3B).

The low $K_s$(Na$^+$), however, declined in an apparent linear manner from about 1000 μM at 5 μM DIC to 200 μM at 140 μM DIC. Estimates of the value of the high $K_s$(Na$^+$) are inherently more accurate due to the large percentage increase in the rate of photosynthesis at Na$^+$ concentrations higher than about 1 mM (Fig. 1).

Interaction between Na$^+$ and Li$^+$. At 25 μM DIC, photosynthesis was stimulated 18-fold by 30 mM Na$^+$, This enhancement by Na$^+$ was progressively reduced as the Li$^+$ concentration was increased from 0 to 5 mM (Fig. 4; 11, 18). Lithium acted as a

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**FIG. 1.** Effect of [Na$^+$] on the rate of photosynthetic O$_2$ evolution measured at 6 DIC concentrations. The [DIC] (μM) is indicated at the right hand side of the figure. Photosynthesis was measured at 30°C (pH 8.05) and light was supplied at 250 μE·m$^{-2}$·s$^{-1}$ PAR. The Chl concentration range between 4.5 and 6.5 μg·ml$^{-1}$.

**FIG. 2.** Eadie-Hofstee plot of photosynthesis rate versus [Na$^+$] for a [DIC] of 32 μM. Photosynthesis was measured at 30°C (pH 8.02) and light was supplied at 250 μE·m$^{-2}$·s$^{-1}$ PAR. The [Chl] was 7.1 μg·mL$^{-1}$. Phases 1 and 2, which yield the low (410 μM) and high (4.2 mM) $K_s$(Na$^+$) respectively, are indicated.

**FIG. 3.** Effect of [DIC] on (A) low and (B) high $K_s$(Na$^+$). Experiments were conducted between pH 8.02 and 8.09, at 30°C, and at a light intensity of 250 μE·m$^{-2}$·s$^{-1}$ PAR. The [Chl] ranged between 4.5 and 8.5 μg·mL$^{-1}$.

**FIG. 4.** Effect of Li$^+$ on the rate of Na$^+$-stimulated photosynthesis (O$_2$ evolution). Photosynthesis was measured at 25 μM DIC and various [Na$^+$] in the presence of 0 (○); 1 mM (○); 2.5 mM (●), and 5 mM (▲) LiCl. Experiments were conducted at pH 8.05, 30°C and light was supplied at 250 μE·m$^{-2}$·s$^{-1}$ PAR. The [Chl] was 9 μg·mL$^{-1}$.
competitive inhibitor of Na\(^+\) with respect to photosynthetic O\(_2\) evolution (Fig. 4) and the inhibitory effect of Li\(^+\) on Na\(^+\)-stimulated photosynthesis was substantially overcome by increasing the [Na\(^+\)] (Fig. 4). Lithium, at concentrations up to 5 mM, had no effect on photosynthesis in the absence of Na\(^+\) (data not shown). The high \(K_a(\text{Na}^+)\) increased from 6.1 mM at 0 mM Li\(^+\) to 18 mM at 2.5 mM Li\(^+\) and 73 mM at 5 mM Li\(^+\) (Fig. 4). The \(K_i(\text{Li}^+)\) for Na\(^+\)-stimulated photosynthesis calculated from the data in Figure 4 was 1.9 mM ± 1.4 (SD). The effect of Li\(^+\) on the high \(K_a(\text{Na}^+)\) could not be evaluated due to the high [Na\(^+\)] required to overcome the Li\(^+\) inhibition of photosynthesis.

The increased rate of photosynthetic O\(_2\) evolution (Fig. 1) and carbon fixation (Fig. 5B) brought about by 5 mM Na\(^+\) paralleled increases in the intracellular [DIC] (Fig. 5A). The increase in the intracellular [DIC] in the presence of Na\(^+\) was due largely to increased HCO\(_3^-\) transport, rather than CO\(_2\) transport, as the rate of CO\(_2\) supply to the cells, from HCO\(_3^-\) dehydration (17), was about 13-fold lower than the observed rate of photosynthesis (calculation not shown). The addition of Li\(^+\) to the cell suspensions resulted in a substantial decrease in the rate of Na\(^+\)-stimulated carbon fixation as well as a decrease in the intracellular concentrations of DIC (Fig. 5). The inhibition of Na\(^+\)-stimulated HCO\(_3^-\) transport by Li\(^+\) in conjunction with the observation that Li\(^+\) competitively inhibited Na\(^+\)-stimulated photosynthesis (Fig. 4) suggests that Na\(^+\) and Li\(^+\) act at a common site which directly affects the cells ability to actively transport HCO\(_3^-\).

Effect of Na\(^+\) CA and Li\(^+\) on CO\(_2\) Transport. CO\(_2\) transport by *Synechococcus* was stimulated by micromolar concentrations of Na\(_2\)SO\(_4\), but not by similar concentrations of K\(_2\)SO\(_4\) or LiCl (Fig. 6). Micromolar concentration of NaCl also enhanced CO\(_2\) uptake indicating that the effect was specific to Na\(^+\) (not shown). However, micromolar levels of Na\(^+\) which stimulated HCO\(_3^-\) transport (Figs. 1 and 5) did not significantly increase either the initial or steady state rate of CO\(_2\) uptake above that elicited by 100 \(\mu\)M Na\(^+\) (Fig. 6). Lithium concentrations (20 mM) sufficient to inhibit HCO\(_3^-\) transport (Fig. 5) also had little effect on the initial or steady state rate of CO\(_2\) uptake (Fig. 6).

The intracellular pool of DIC was also measured by mass spectrometry (Fig. 7; 20) under conditions where pool formation was predominantly due to CO\(_2\) transport (i.e. 100 \(\mu\)M NaCl, Fig. 6; 25 \(\mu\)g mL\(^{-1}\) CA; 18). In this case, photosynthesis was inhibited by iodoacetamide. Consequently, the disappearance of CO\(_2\) from the medium upon illumination represented uptake into the intracellular pool rather than uptake plus fixation. Upon illumination (Fig. 7A) the cells rapidly removed nearly all the CO\(_2\) from the medium as was the case for noninhibited cells (Fig. 6). Addition of CA to the suspension caused a significant increase in the [CO\(_2\)] indicating that the ratio of [CO\(_2\)] to [HCO\(_3^-\)] in the medium was far removed from equilibrium, due initially to the relative uptake of CO\(_2\) (12, 20). Similar results have been previously reported for both iodoacetamide poisoned and non-poisoned cells (12, 20). Following the initial increase in [CO\(_2\)] (Fig. 7A), however, CA-dependent CO\(_2\) uptake occurred giving rise to a second steady-state [CO\(_2\)]. In the presence of CA, the measured [CO\(_2\)] was directly proportional to the [DIC] in the medium. The difference between the DIC remaining in the medium and that which was originally present represents DIC taken up into the intracellular pool (20). In the example shown (Fig. 7A), CA-stimulated CO\(_2\) uptake generated a steady state intracellular DIC pool of 46.9 mM, which was quantitatively released from the cells upon darkening. Due to the 2 to 3 s response time of the mass spectrometer, the initial phase of CA-dependent CO\(_2\) uptake was not completely resolved. Consequently, the [DIC] in the medium and the intracellular DIC pool present just at the point in time when CA was added can not be accurately determined. An estimate of the CA-independent DIC pool, however, can be made by extrapolating back along the CA-dependent CO\(_2\) uptake curve. This procedure indicated that the CA-independent pool was small (less than 10 mM), in agreement with measurements made using the silicone fluid centrifugation technique (Fig. 5). In the latter case (minus CA), the rate of CO\(_2\) supply to the cells would limit the rate of CO\(_2\) transport and thus the magnitude of the intracellular DIC pool. Using the silicone fluid centrifugation method, we have previously observed a CA-dependent increase in intracellular [DIC] (18). Our present findings confirm this result.

The ability of the cells to form an intracellular pool through CA-dependent CO\(_2\) transport in the absence and presence of Li\(^+\) (20 mM) was also assessed using the mass spectrometer technique, as shown in Figure 7B.

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Fig. 6. Effect of Na⁺ on CO₂ transport by Synechococcus. Cells (7.7 µg Chl. mL⁻¹) were incubated in the dark, at pH 8, for several minutes in the presence of 50 µM K₂CO₃ and the indicated combination of salts. CO₂ uptake was initiated by turning on the lights (L) (600 µE. m⁻². s⁻¹ PAR). The medium, in addition to 25 mM BTP/23.5 mM HCl; A, no addition; B, 50 µM K₂SO₄; C, 50 µM LiCl; D, 50 µM K₂SO₄ + 50 µM Na₂SO₄; E, 50 µM K₂SO₄ + 50 µM Na₂SO₄ + 25 mM NaCl; F, 50 µM Na₂SO₄ + 20 mM LiCl. The initial rate of CO₂ disappearance from the medium, in µmol CO₂·mg⁻¹ Chl·h⁻¹, was A, 21.5; B, 22.0; C, 27.0; D, 52.3; E, 52.9; F, 52.9.

Fig. 7. Measurement of the intracellular DIC pool by mass spectrometry. Synechococcus cells (10 µg Chl·mL⁻¹) were preincubated in 25 mM BTP/23.5 mM HCl buffer (pH 8.0) in the light (600 µE·m⁻²·s⁻¹) for 4 min in the presence of 100 µM NaCl and 3.3 mM iodoacetamide. Upon darkening, K₂CO₃ was added to give a final [DIC] of 74 µM. CO₂ transport was initiated by turning on the light (L). A. Time course of CO₂ depletion of the medium and the effect of CA (25 µg·mL⁻¹, 62.5 Wilbur-Anderson units) and darkness (D) upon the extracellular [CO₂]. The addition of CA, even in the absence of cells, caused a 10% increase in the m/z = 44 signal, presumably due to relaxation of unstirred layer effects around the membrane inlet. This enhancement has been subtracted from the actual chart recorder tracing, B–C. Time course of CO₂ depletion of the medium, already containing 25 µg·mL⁻¹ CA, in the absence (B) or presence (C) of 20 mM LiCl. Since CA maintains equilibrium between CO₂ and HCO₃⁻, the measured [CO₂] was directly proportional to the [DIC] in solution. The DIC present in the intracellular pool at steady state could be calculated as the difference between that originally present and that present at steady state. An intracellular volume of 75 µL·mg⁻¹ Chl was assumed. The same cell suspension was used for experiments reported in B to C and [DIC] was 74 µM. Due to increased ionic strength (20 mM LiCl), however, the equilibrium proportion of CO₂ in solution decreased from 1.56% (B) to 1.39% (C), an 11% decrease. This difference accounts for the initial difference in [CO₂] between B and C. Note the difference in the time scales between A and B and C.

DISCUSSION

A complex relationship exists between the effects of [Na⁺] and [DIC] on photosynthesis of the cyanobacterium Synechococcus UTEX 625 (Figs. 1–3). Analysis (Fig. 2) of the rate of photosynthesis as a function of [Na⁺] indicated two phases to the response curve, suggesting two distinct effects of Na⁺ on photosynthesis. In addition, the [Na⁺] required for one-half the maximum rate of photosynthesis decreased with increased [DIC] (Fig. 3). Increased [DIC] also resulted in an increase in the fraction of photosynthesis which was apparently independent of added Na⁺ (Fig. 1).

Biphasic Response. Over the range of [DIC] used in the present experiments, photosynthesis was limited by the intracellular pool of DIC (CO₂) (Figs. 1 and 5) (9, 14), which serves as the immediate substrate for photosynthetic carboxylation (7, 14, 18). Thus, factors which affect the generation and maintenance of this pool might be expected to contribute to the complex interaction between [Na⁺] and [DIC] in photosynthesis. Indeed, both HCO₃⁻ transport (Fig. 5) and CO₂ transport (Fig. 6) were found to be stimulated by Na⁺, in agreement with our previous studies (11, 19–21). Similar results have also been reported for Anabaena variabilis (1, 2, 15, 25). However, the Na⁺ requirement for CO₂ transport was distinctly different from that found for HCO₃⁻ transport (Figs. 5 and 6), being in the micromolar (<1000) rather than millimolar (1–50) range (11, 19, 20, 22). These two levels of Na⁺ approximately corresponded to those found for the low and high Kₛ(Na⁺) for photosynthesis (Figs. 2 and 3). Given the effect of Na⁺ on DIC transport (Figs. 5 and 6) and the precursor-product relationship between DIC transport and the intracellular DIC pool and between the pool and photosynthesis (14, 18), it seems reasonable to assume that the biphasic response (Figs. 2 and 3) was due, in part, to Na⁺-dependent contributions from...
both CO₂ and HCO₃⁻ transport to the intracellular DIC pool and hence to photosynthesis. In a previous study, we have shown that both CO₂ and HCO₃⁻ were simultaneously and continuously transported by *Synechococcus* UTEX 625 under DIC-limiting conditions for photosynthesis (12).

**Low $K_m$(Na⁺).** Under CO₂-limiting conditions, active CO₂ transport alone supported low rates of photosynthesis at pH 8 (12). Carbonic anhydrase, which enhanced the rate of CO₂ supply to the cells, permitted a dramatic increase in the rate of Na⁺-dependent CO₂ transport (100 μM Na⁺), resulting in a substantial increase in the steady state level of the DIC pool (Fig. 7; 18). In cells in which the Calvin cycle was not inhibited, CA addition was also found to substantially increase the rate of photosynthesis under CO₂-limiting conditions (18). From these results, it was evident that *Synechococcus* UTEX 625 had a high capacity for CO₂ transport which was limited by the low rate of CO₂ supply to the cells. At present, there is no evidence in support of a direct interaction between added CA and the DIC transport systems.

Enhancement of the rate of CO₂ transport by micromolar levels of Na⁺ would also be expected to increase the intracellular DIC pool and the rate of photosynthesis, as in the case with CA. In the absence of other contributing factors, the Na⁺-photosynthesis dose-response curve would yield a low $K_m$(Na⁺) which reflected the Na⁺-requirement for transport. However, two additional factors must be considered. At pH 8, the rate of photosynthesis would be limited by the CO₂ supply rate rather than CO₂ transport capacity. This means that the CO₂-dependent portion of the Na⁺ versus photosynthesis curve would be prematurely truncated at a point which was dependent upon [DIC]. Second, Na⁺-dependent HCO₃⁻ transport would also contribute some extent to the steady state level of the intracellular DIC pool and, therefore, to the rate of photosynthesis. Thus, the initial increases in the rate of photosynthesis with increased [Na⁺], from which the low $K_m$(Na⁺) was derived (Fig. 2), cannot be considered to be solely a function of Na⁺-stimulated CO₂ transport. In addition, the requirement for Na⁺ in intracellular pH regulation (22) plus other unidentified actions would further complicate the relationship between CO₂-dependent photosynthesis and low concentrations Na⁺. Consequently, the value of the low $K_m$(Na⁺) has no specific meaning with regard to CO₂ transport per se. Rather, this value is a reflection of the component processes which directly or indirectly influence photosynthesis through a Na⁺-dependent mechanism, CO₂ transport being one of the more significant processes.

**High $K_m$(Na⁺).** In light of the previous discussion, it is unlikely that the high $K_m$(Na⁺) for photosynthesis reflected only the Na⁺-requirement for HCO₃⁻ transport. However, at low [DIC] where CO₂ transport supported less than 10% of the observed rate of photosynthesis, Na⁺-dependent HCO₃⁻ transport was certainly the predominant factor in the makeup of the high $K_m$(Na⁺) (Fig. 5). The 11-fold decline in the $K_m$(Na⁺), with increased [DIC], in large part, reflected a decrease in the contribution of HCO₃⁻ transport to the intracellular DIC pool relative to that of CO₂ transport. This conclusion is supported by the observation that CO₂ transport occurred at or near the maximum theoretical rate of CO₂ supply (pH 8) at all [DIC] up to 200 μM, as indicated by the maintenance of a nonequilibrium, steady state [CO₂] near zero (e.g. Fig. 6) (12). The possibility that increased [HCO₃⁻] caused a decrease in the $K_m$(Na⁺) for the HCO₃⁻ transport system was not likely because a sigmoidal response of photosynthesis versus [HCO₃⁻], at fixed [Na⁺], was not observed.

**Na⁺-Independent Photosynthesis.** CO₂ transport occurred in the absence of added Na⁺, although at a reduced rate (Fig. 6). It is difficult, however, to determine whether this uptake was truly Na⁺-independent or the result of contaminant Na⁺. Leaching of Na⁺ from the washed cells may result in extracellular [Na⁺] as high as 70 μM (11). It seems likely, therefore, that the low level of CO₂ transport which occurred in the absence of added Na⁺ was the source of carbon for that part of total photosynthesis which was apparently Na⁺-independent (Fig. 1). The Na⁺-independent rates of photosynthesis (Fig. 1) can be fully accounted for by CO₂ uptake alone (calculations not shown) (17), in accord with the above conclusions.

Millimolar levels of Na⁺ are not absolutely required by *Synechococcus* to achieve high rates of photosynthesis. The Na⁺-requirement can be relaxed in air-grown cells by high [DIC] (Figs. 1 and 3) (11, 22) or by low pH (22) or eliminated by growth on high CO₂ concentrations (19), by DIC-sufficient growth conditions (19) or by growth in standing culture (11). It has been suggested that the relief of the millimolar requirement for Na⁺ in photosynthesis by low pH is due to H⁺ substitution for the Na⁺ in the HCO₃⁻ transport system (1, 2). This conclusion was deduced solely on the basis of measurements of the rate of DIC transport at pH 7 (1). At pH values below 8, however, it is extremely difficult to make a clear and positive distinction between CO₂ and/or HCO₃⁻ transport as the source of intracellular DIC (17). An alternative, but not mutually exclusive, explanation for the low pH effect is that CO₂ transport substitutes for HCO₃⁻ transport, thereby reducing the Na⁺-requirement for transport and photosynthesis from the millimolar to the micromolar level. The high capacity for CO₂ transport possessed by *Synechococcus* would not be limited by low pH. Since both Na⁺ is rate and, therefore, the cells would be able to generate the large intracellular pool of DIC necessary for high rates of CO₂ fixation. In fact, high rates of CO₂ supply from the environment to the cells appears to be a common characteristic of all the above noted conditions which affect the millimolar requirement for Na⁺ in photosynthesis, except in the case of standing culture cells (11). An additional common feature is that all these cell types also possess the ability to actively transport CO₂ and in all but standing culture cells have been shown to require low levels of Na⁺ for full CO₂ transport activity. This suggests that CO₂ transport is a constitutive component of the photosynthetic apparatus of *Synechococcus* and that the millimolar requirement for Na⁺ in HCO₃⁻ transport (and photosynthesis) is a response to overcome a limiting condition for photosynthesis.

**Relationship between CO₂ and HCO₃⁻ Transport.** The observation that Na⁺ stimulated both CO₂ and HCO₃⁻ transport has been taken as evidence (1, 2) in support of a model (23, 25) that suggests that CO₂ and HCO₃⁻ are actively transported by a common system. It was proposed for *Anabaena* (23, 25) that extracellular CO₂ was hydrated by a membrane-bound CA-like moiety or by CA itself (1) and then transferred, as HCO₃⁻, directly to the HCO₃⁻ transporter which passed it across the membrane. Extracellular HCO₃⁻ was taken up directly from the medium by the same HCO₃⁻ transporter (23, 25). Our finding (Figs. 5 and 6; 20) that CO₂ and HCO₃⁻ transport have substantially different requirements for Na⁺, however, suggests that two separate transport systems may be involved in DIC transport in *Synechococcus*. This proposal is also supported by the following observations. First, a substantial rate of CA-dependent CO₂ transport (Fig. 7A) and photosynthesis (18) occurred at a [Na⁺] which was insufficient to support a significant rate of HCO₃⁻ transport. Second, Li⁺, which competitively inhibited Na⁺-stimulated photosynthesis and HCO₃⁻ transport had no apparent effect on the rate of CO₂ transport and only a small effect on the intracellular DIC pool generated by CA-dependent CO₂ transport (Figs. 6 and 7). In contrast, Li⁺ drastically reduced the level of the intracellular DIC pool generated by Na⁺-dependent HCO₃⁻ transport (Fig. 5). This, a completely functional or operating HCO₃⁻ transport system was not essential to the transport of CO₂. Furthermore, we have shown that steady state CO₂ transport was unaffected by the initiation (by Na⁺ addition) of HCO₃⁻ transport or by steady state HCO₃⁻ transport (12). In a system in which CO₂ transport was directly coupled to the HCO₃⁻
transporter it would be expected that the transport of HCO$_3^-$ from the medium would reduce the rate of CO$_2$ transport (23, 25). Additional studies are presently underway to further clarify the relationship between CO$_2$ and HCO$_3^-$ transport in Synechococcus UTEX 625.

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