Salicylhydroxamic Acid (SHAM) Inhibition of the Dissolved Inorganic Carbon Concentrating Process in Unicellular Green Algae

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ABSTRACT

Rates of photosynthetic O2 evolution, for measuring Kd(CO2 + HCO3-) at pH 7, upon addition of 50 micromolar HCO3- to air-adapted Chlamydomonas, Dunaliella, or Scenedesmus cells, were inhibited up to 90% by the addition of 1.5 to 4.0 millimolar salicylhydroxamic acid (SHAM) to the aqueous medium. The apparent Kd(SHAM) for Chlamydomonas cells was about 2.5 millimolar, but due to low solubility in water effective concentrations would be lower. Salicylhydroxamic acid did not inhibit oxygen evolution or accumulation of bicarbonate by Scenedesmus cells between pH 8 to 11 or by isolated intact chloroplasts from Dunaliella. Thus, salicylhydroxamic acid appears to inhibit CO2 uptake, whereas previous results indicate that vanadate inhibits bicarbonate uptake. These conclusions were confirmed by three test procedures with three air-adapted algae at pH 7. Salicylhydroxamic acid inhibited the cellular accumulation of dissolved inorganic carbon, the rate of photosynthetic O2 evolution dependent on low levels of dissolved inorganic carbon (50 micromolar NaHCO3), and the rate of 14CO2 fixation with 100 micromolar [14C] HCO3-. Salicylhydroxamic acid inhibition of O2 evolution and 14CO2 fixation was reversed by higher levels of NaHCO3. Thus, salicylhydroxamic acid inhibition was apparently not affecting steps of photosynthesis other than CO2 accumulation. Although salicylhydroxamic acid is an inhibitor of alternative respiration in algae, it is not known whether the two processes are related.

Unicellular green algae, Chlamydomonas (4, 21), Chlorella (18), Dunaliella (2, 14), Scenedesmus (11, 23, 28), and cyanobacteria such as Anabaena (29) and Synechococcus (3, 24), when grown with or adapted to low air levels of CO2 in the light, exhibit a Kd(DIC) for photosynthetic O2 evolution with low levels of external DIC by accumulating inorganic carbon inside the cells. This process has been termed a 'dissolved inorganic carbon concentrating mechanism' or 'DIC pump.' When microalgae are grown with elevated levels of CO2 (air supplemented with 1 to 5% CO2), the light-dependent intracellular accumulation of inorganic carbon is suppressed.

During DIC accumulation, either or both CO2 or HCO3-

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2 Abbreviations: Kd(DIC), CO2 plus HCO3- concentration at a given pH at which the rate of DIC dependent photosynthetic O2 evolution is half-maximal; DIC, dissolved inorganic carbon; CA, carbonic anhydrase; PCV, packed cell volume; SHAM, salicylhydroxamic acid.

MATERIALS AND METHODS

Organisms and Growth Conditions

Chlamydomonas reinhardtii 137 cells were grown photoautotrophically in minimal media at 26 ± 2°C with continuous shaking and bubbling with 5% CO2 in air (27). Dunaliella tertiolecta (CSIRO Marine Laboratories, Hobart, Tasmania, Australia) was maintained and cultured on a defined medium (15) with 0.17 m NaCl as described previously (12, 14). The photon flux density was 150 μE m-2 s-1 with a light/dark regime of 16/8 h. The pH of the algal growth medium started around 7 and drifted lower to near 5 during growth of the cultures. While in the exponential growth phase and 1 d before use, cultures were diluted with fresh growth media and aerated only by shaking for 3 to 18 h to develop DIC pumps. Cultures were harvested by centrifugation (1000g x 10 min); the cell pellet was washed once with the fresh growth medium (pH 6.8), and centrifugation was repeated. Finally, the cell pellet was resuspended in a small volume of assay buffer (50
mm Hepes at pH 7.0 or 7.2), kept on ice, and used within 2 h. Chl was estimated on an aliquot after ethanol extraction.

*Scenedesmus obliquus* from N. I. Bishop (Dept. of Botany, Oregon State University, Corvallis, OR) were maintained on slants prepared with *Scenedesmus* medium (6) containing 0.5% glucose, 0.25% yeast extract, and 2% agar (NYG) (5). Algae were grown photoautotrophically in *Scenedesmus* medium (6) in thermostated cylinders (800 mL) at 32°C with continuous bubbling with 5% CO₂ in air. The cylinders were illuminated continuously from both sides by two banks of fluorescent lights that provided the algae an intensity of 100 μE·m⁻²·s⁻¹. The following procedure was used to activate the DIC pumps in *Scenedesmus* where endogenous DIC, particularly at alkaline pH, would slow down the rate of activation. Cultures were harvested by centrifugation (1000g × 10 min) in midexponential phase of growth, the cell pellet was washed once with water, and centrifugation was repeated. The final cell pellet was resuspended to a volume of 5 μL PCV mL⁻¹ in 20 mM phosphate buffer at pH 7.2 or dibasic sodium phosphate at pH 9.0. Five μL PCV was equal to 25 μg equivalent of Chl. Cells were shaken without aeration for 3 to 4 h in light at either pH 7.2 or 9.0 to develop the DIC pumps. The phosphate buffer at pH 7.2 prevented a rise in pH from alkalization of the medium during DIC uptake. When starting at pH 9.0 with Na₂HPO₄, the pH did not increase to over 10. Before tests, an aliquot was centrifuged briefly in a microfuge, and the cells were resuspended in the same volume of fresh phosphate at the indicated pH of 7.2 or 9.0. Hepes buffers were prepared fresh daily in boiled glass distilled water, and the pH was adjusted with crystals of AR grade KOH in an effort to keep the DIC to a minimum (28). The phosphate buffer at pH 7.2 was prepared by dissolving monobasic sodium phosphate in boiled water and adjusting the pH with dibasic sodium phosphate. All solutions were continuously sparged with N₂ until used.

Intact chloroplasts from *Dunaliella* were isolated as described in reference (12) and inorganic carbon uptake measured as in (14, 20).

Photosynthetic Oxygen Evolution

Photosynthetic CO₂-dependent oxygen evolution was measured with a Clark-type oxygen electrode (Rank Brothers, Cambridge, UK). Harvested cells were diluted from a concentrated suspension to 25 μg Chl·mL⁻¹ in 50 mM Hepes or 20 mM phosphate buffered at pH 7.0 or 7.5 for *Chlamydomonas* or *Dunaliella*. *Scenedesmus* cells were assayed in 20 mM phosphate buffer at pH 7.2 or 20 mM Na₂HPO₄ at pH 9.0.

Cell suspensions were then illuminated with 800 μE·m⁻²·s⁻¹ light at 25°C in a thermostated O₂ electrode chamber until the endogenous DIC was consumed by the cells, as judged by the cessation of O₂ evolution (Fig. 1, ref. 28). Rates of CO₂-dependent O₂ evolution were then measured after addition of known amounts of NaHCO₃. As an indication of DIC pump activity, the rate of photosynthetic O₂ evolution from the addition of DIC was used to calculate Kₐₛ(DIC) as described previously (21). Where indicated, SHAM was added to the reaction mixture from a stock solution of 0.45 mM in ethanol. Because SHAM has low solubility in water, the effective concentration in solution would be lower than the amount added. The dilution factor for the SHAM stock was at least 100-fold and the carry over of 1% or less ethanol did not effect the rate of O₂ evolution. Nevertheless, 1% ethanol was added to controls without for comparative purposes. All experiments were run at least in duplicate, and all results for each alga were confirmed with cultures grown at three different periods.

¹⁴CO₂ Fixation

The ¹⁴CO₂ fixation was measured in a 2 mL cell suspension in 25 mM Hepes-KOH buffer (pH 7.5) containing cells equivalent to 40 μg Chl. The algal suspension was continuously stirred in a thermostated cuvette (25°C) and illuminated with 800 μE·m⁻²·s⁻¹ from a Kodak projector. The reaction was initiated by adding an aliquot from a stock solution of H⁺⁴CO₃⁻ (0.5 mCi/mmol, Research Products International, USA) to provide 2 mM and every 5 min an additional aliquot was added to give 1 mM bicarbonate. Aliquots of 0.45 μL SHAM in ethanol was added to the cell suspension 1 min prior to addition of H⁺⁴CO₃⁻. To the control algal suspension, an equal amount of ethanol was added. After 5, 10, or 15 min, the fixation was terminated by adding 1 mL hot aqueous methanol 80% (v/v). Radioactivity was determined in 50 μL aliquot after releasing unfixed inorganic carbon by adding 0.45 mL acetic acid (0.5 N) and warming the vials at 80°C for 2 h.

Inorganic Carbon Uptake

DIC uptake by algal cells was estimated by silicone oil filtration (4, 14, 20). Assays were performed in light of 400 μE·m⁻²·s⁻¹ at 25°C in 400 μL microfuge tubes in a Beckman Microfuge II. Cell suspension was placed in a microfuge tube over a gradient of 25 μL of 1 M glycine and 0.75% (w/v) SDS at the bottom, overlaid with 75 μL of a silicone oil mixture (four parts of Wäcker AR-200 to one part of Wäcker AR-20 silicone oil from SWS Silicones, Adrian, MI). A large evaporating dish filled with cold water was placed on the top of the open microfuge with the loaded tubes so that heat from the illumination did not warm the cell suspension or change the viscosity of the silicone oil. After preillumination for 1 min, DIC uptake was initiated by the addition of 5 μL of NaH⁴CO₃ (Research Products International; specific radioactivity 45 mCi·mmol⁻¹ for *Chlamydomonas*, and 35 mCi·mmol⁻¹ for *Scenedesmus*), and the incubation was terminated after 30 to 60 s by turning on the microfuge. Where appropriate, 1.5, 2, or 4 mM SHAM was added to the cell suspension at the beginning of the 1 min preillumination. The DIC uptake was calculated by estimating the cell volume using ⁴C-sorbitol and H₂O as previously described (21). The final concentration of NaHCO₃ in the medium was about 150 μM. Inorganic carbon accumulation was calculated by subtracting the acid stable radioactivity from the total ¹⁴C uptake.

RESULTS

Effect of SHAM on Photosynthetic Oxygen Evolution

After cultures grown on high CO₂ were adapted in air for 24 h, cells of both *Chlamydomonas* and *Dunaliella* at pH 7.0...
had a well developed DIC pump for a $K_{c,3}$(DIC) with 1 to 10 μM DIC. When these algae were supplied with 50 to 75 μM HCO$_3^-$ at pH 7.0 or 7.5, oxygen was evolved at a maximal rate of about 90 to 95 μmol O$_2$-mg$^{-1}$ Chl$^{-1}$ (Table I; Fig. 1), until the DIC concentration was depleted to about 5 to 10 μM. When 3 mM SHAM was added to the culture along with 75 μM HCO$_3^-$, the rate of photosynthetic oxygen evolution was inhibited about 70% for Chlamydomonas and 60% for Dunaliella, but oxygen evolution would continue at this reduced rate for a longer period until most of the DIC was used up and DIC concentration became rate limiting. The addition of an excess amount of HCO$_3^-$ (0.5–2 mM) at the third arrow in Figures 1 and 2 reversed the SHAM inhibition of photosynthetic oxygen evolution (Table I). As explained later, the apparent $K_{c,3}$(SHAM) was around 2.5 mM (Fig. 3A), but higher concentrations of SHAM were not used to prevent inhibition of photosynthesis. This reversal of SHAM inhibition of photosynthetic oxygen evolution by a high level of HCO$_3^-$ suggests that SHAM inhibited the DIC pumps but not photosynthetic CO$_2$ reduction (Fig. 3B). With a higher concentration of DIC at pH 7 to 7.5 enough CO$_2$ would be available to diffuse into the cell to sustain maximum photosynthetic oxygen evolution.

Since both these algae belong to the same family and appear to have similar DIC-concentrating mechanism (2, 14), the effect of SHAM on Scenedesmus adapted to air at two different pH was also tested. Scenedesmus adapted at acidic pH

![Figure 1. Photosynthetic oxygen evolution by air-adapted C. reinhardtii or D. tertiolecta with a DIC pump. Cells containing 50 μg Chl were mixed with 2 mL 50 mM Heps buffer at pH 7.0 or 7.5 in the cuvette of an O$_2$ electrode in the light. First the cells were allowed to deplete the endogeneous DIC in the medium. A low level of 75 μM NaHCO$_3^-$ was added (the first arrow) for 1 min to establish $V_{max}$ and then 3 mM SHAM (final concentration) and additional 75 μM NaHCO$_3^-$ (the second arrow) was added. After measuring SHAM inhibition, $V_{max}$ was reestablished with high HCO$_3^-$ (0.5 mM).](image)

![Figure 2. Photosynthetic oxygen evolution by S. obliquus adapted in air for 3 to 4 h in the light at either pH 7.2 or 9.0 in 20 mm sodium phosphate buffer to develop a DIC pump. Cell suspensions with approximately 25 μg Chl per mL were shaken continuously in air and light for 3 to 4 h to develop DIC pumps. An aliquot of 2 mL of suspension was pelleted by centrifugation for about 10 s in a microfuge and the pellet was resuspended in 2 mL of phosphate buffer presaged with nitrogen and put in the O$_2$ electrode. First the cells were allowed to deplete endogenous DIC, and then the rate of O$_2$ evolution with 100 μM HCO$_3^-$ was measured until depleted. Then the inhibited rate was measured with 1.5 mm SHAM and 100 μM HCO$_3^-$, $V_{max}$ was restored with excess HCO$_3^-$ (1 or 2 mM).](image)
has predominantly a DIC pump for external CO$_2$, whereas at alkaline pH DIC uptake is predominantly by a bicarbonate pump (11, 28). SHAM (1.5 mM) inhibited about 70% of the photosynthetic oxygen evolution at pH 7.2 by *Scenedesmus* cells that had been adapted at pH 7.2 to low CO$_2$ conditions (Fig. 2). These results were similar to those with *Chlamydomonas* and *Dunaliella*. However, with *Scenedesmus*, which were air-adapted at pH 9 to develop a plasmalemma HCO$_3^-$ transporter (28), photosynthetic O$_2$ evolution was only inhibited by SHAM about 28% at pH 7.2 or 14% at pH 9.0 (Table 1; Fig. 2). Inhibition of photosynthetic O$_2$ evolution by SHAM was reversed by 1 to 2 mM HCO$_3^-$ for the cells adapted at pH 7.2 and tested at pH 7.2 or adapted at pH 9.0 and tested at pH 7.2. Cells air-adapted at pH 9.0 and tested at pH 9.0 had a high rate of HCO$_3^-$-dependent O$_2$ evolution that was only slightly inhibited by SHAM. It appears that SHAM inhibited a DIC pump which utilizes external CO$_2$, but did not inhibit an external HCO$_3^-$ transporter at pH 9.

Concentration curves for SHAM inhibition of photosynthesis, respiration, and DIC uptake are only of experimental value. SHAM is relatively insoluble in water, and addition of 1 to 5 mM SHAM from a stock solution of 0.45 mM in ethanol is an operational measurement; the amount of effective SHAM that remained in aqueous solution (not in suspension) is difficult to assess, but it is less than that added. In evaluating the relative SHAM concentration, we have compared its effect on photosynthesis (Fig. 3B) and respiration at similar concentrations (13). With CO$_2$-grown *Chlamydomonas* the addition of up to 5 mM SHAM did not inhibit photosynthetic oxygen evolution (Fig. 3A). Both in CO$_2$-grown and air-adapted cells with high DIC levels, the inhibition of $^{14}$CO$_2$-fixation by addition of 5 mM SHAM was only about 10% (Fig. 3B). With air-adapted cells at pH 7 or 7.5 on limiting DIC, O$_2$ evolution was progressively inhibited by increasing concentration of SHAM with a $K_1$ of about 2.5 mM SHAM. This inhibition was reversed by high DIC concentrations (Fig. 1; Table 1). These results suggest that SHAM did not inhibit the photosynthetic capability of the cells, but rather the SHAM inhibition of photosynthesis was due to reduced DIC uptake. Nevertheless, to avoid other possible effects from SHAM, only concentrations of 2 to 3 mM were added with *Chlamydomonas* or *Dunaliella* cells, although these levels did not completely inhibit the CO$_2$ pump. In these experiments, similarly, concentrations of SHAM partially inhibited respiration by *Chlamydomonas* (13), also with a $K_1$ of about 2.5 mM. With *Scenedesmus*, concentrations of SHAM above 2 mM became inhibitory to photosynthetic oxygen evolution even at high DIC levels, and the cells became brownish in color (results not shown). Below 2 mM SHAM, O$_2$ evolution by *Scenedesmus* with excess DIC was not significantly inhibited. Thus, only 1.5 mM SHAM was used in experiments with *Scenedesmus*. SHAM (1–5 mM) did not inhibit isolated periplasmic CA from *Chlamydomonas*, nor did it inhibit the CA activity measured with the whole cells (results not shown).

Up to 1 mM n-Propylgallate did not inhibit the DIC pump in *Chlamydomonas* or CO$_2$ fixation. Tetraethyl thiuram disulfide (disulfiram) at 10 mM inhibited CO$_2$ fixation and could not be tested as a DIC pump inhibitor. Although these toxic compounds inhibit the alternative respiration, they are not considered to be as specific inhibitors as SHAM.

**Effect of SHAM on $^{14}$C-Inorganic Carbon Uptake and $^{14}$CO$_2$-Fixation**

The effect of SHAM on DIC-uptake by air-adapted cells of *Chlamydomonas* was measured by incubating the cells for 0.5 to 2 min with SHAM and low concentrations of $[^{14}C]$HCO$_3^-$ (150 mM HCO$_3^-$), which required a DIC pump for rapid accumulation. Total DIC uptake would be the sum of $^{14}$C fixation and DIC accumulation in the cells. DIC accumulation was calculated by subtracting acid stable radioactivity from the total $^{14}$C carbon uptake. With our experimental conditions, total DIC accumulation was maximized after 1 to 2 min (Fig. 4) and varied between 5 to 8 mM. Consequently, most rates of DIC accumulation were measured over only 60 or 120 s. In *Chlamydomonas* 2 mM SHAM inhibited by 70 and 80% the $^{14}$C accumulation in 1 min at pH 7.0 (Fig. 4) or 7.5 (data not shown). Higher concentrations of SHAM (4 mM) inhibited uptake more than 90% at both pH. In 1 to 2 min of photosynthesis, which did not reach $V_{max}$, the total $^{14}$CO$_2$ fixation at pH 7.0 or 7.5 was inhibited 45% with 2 mM SHAM and about 90% with 4 mM SHAM. Most of the inhibition of $^{14}$CO$_2$ fixation was due to reduced carbon accumulation and not due to impaired fixation ability (Fig. 3), because the rate of CO$_2$ fixation approached $V_{max}$ with higher NaHCO$_3$ concentrations.

With *Scenedesmus*, the DIC accumulation was inhibited about 50 to 60% by 1.5 mM SHAM in the cells adapted to air at pH 7.2 and tested at pH 7.2 (Fig. 5A) or about 80% when tested at pH 9.0 (Fig. 5B). These cells air-adapted at pH 7.2 had a well developed CO$_2$ pump, but the HCO$_3^-$ pump was less developed. The *Scenedesmus* cells, adapted at pH 9.0 to activate the HCO$_3^-$ pump, when tested at pH 7.2 were inhib-
Figure 4. Inorganic carbon uptake by the air-adapted cells of C. reinhardtii. The \(^{14}\)C uptake was done at pH 7.0 in Hepes buffer (50 mM); similar results were obtained at pH 7.5. Control (○), 2 mM SHAM (■), 4 mM SHAM (△). Cells were preincubated in light with SHAM for 1 min before initiating the \(^{14}\)C uptake. DIC uptake was stopped by removal of the cells upon turning on the microfuge.

Figure 5. Inorganic carbon accumulation by the air adapted cells of S. obliquus. Cells were air adapted to form a DIC pump at pH 7.2 for 3 to 4 h, and \(^{14}\)C uptake was measured at pH 7.2 (A) or 9.0 (B). Cells were also air adapted at pH 9.0, and \(^{14}\)C accumulation was measured at pH 7.2 (C) and pH 9.0 (D). Control (○); + 1.5 mM SHAM (●).

cells was inhibited only 20% by SHAM. SHAM inhibition of \(^{14}\)CO\(_2\) fixation at pH 7 would appear to be due to SHAM inhibition to a decrease in DIC accumulation. At alkaline pH 9.0, SHAM neither inhibited DIC accumulation nor \(^{14}\)CO\(_2\)-fixation.

Bicarbonate Uptake by Chloroplasts Isolated from Dunaliella was not Inhibited by SHAM

Isolated intact chloroplasts from air-adapted Chlamydomonas (20) and Dunaliella (14) cells, but not CO\(_2\)-grown cells, accumulate DIC. When tested with Dunaliella chloroplasts, this accumulation from the medium was vanadate sensitive, as if an ATPase was involved (14), and thus we proposed that the chloroplast DIC pump involved an ATP/HCO\(_3^-\) transporter. The chloroplast DIC uptake process was not inhibited by SHAM (Fig. 6), whereas SHAM did inhibit CO\(_2\) uptake by whole Dunaliella cells at pH 7.0 and 7.5 (Table I) similarly to Chlamydomonas and Scenedesmus. These results are consistent with SHAM inhibition of CO\(_2\) uptake but not HCO\(_3^-\) uptake by the whole cells or chloroplasts.

DISCUSSION

Salicylhydroxamic acid (SHAM) at 1 to 2 mM inhibited DIC accumulation by three algae, Chlamydomonas, Scen-
Cells were air-adapted at indicated pH for 24 h for Chlamydomonas and 4 h for Scenedesmus to develop DIC pumps. CO₂ fixation was the rate observed in the first minute test period after 1 min of preillumination with or without SHAM. The added [¹⁴C]HCO₃⁻ concentration was 150 μM, but due to lower light intensity (400 μE·m⁻²·s⁻¹) these rates were less than Vₘₐₓ.

Table II. Effect of SHAM on the [¹⁴C]CO₂ Fixation (Acid Stable Radioactivity) by Chlamydomonas and Scenedesmus

<table>
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* Assays with Scenedesmus plus 2 and 4 mM SHAM were not run due to SHAM toxicity; these concentrations of added SHAM did not inhibit photosynthesis by Chlamydomonas with 2 mM NaHCO₃.

Figure 6. Lack of SHAM inhibition of bicarbonate uptake by Dunaliella chloroplasts. These chloroplasts were isolated and tested for DIC uptake as previously described (14). 150 μM [¹⁴C]NaHCO₃ and 5 mM SHAM were used. Chloroplasts from air-adapted cells (●, ○); chloroplasts from CO₂-grown cells (△, ▲). Closed symbols, control without SHAM; open symbols, with 5 mM SHAM.

desmus, and Dunaliella at pH 7 where CO₂ was the primary DIC species being removed from the medium. SHAM did not inhibit HCO₃⁻ uptake by Scenedesmus cells in pH 9 medium or by Dunaliella chloroplasts isolated from air-adapted cells. However, vanadate, an ATPase inhibitor, inhibited HCO₃⁻ uptake at pH 9 by Scenedesmus (28) or by isolated chloroplasts (14). Thus, SHAM and vanadate appear to be two inhibitors of DIC uptake that differentiate between CO₂ uptake and HCO₃⁻ uptake. Interpreting our results is complicated by the fact that both pumps coexist and overlap between pH 6 and 8.3 where DIC is a mixture of CO₂ and HCO₃⁻. In addition, most solutions generally have initially enough dissolved CO₂ even at pH 8.3 to sustain considerable O₂ evolution until the CO₂ is reduced below Kₘ,₅(CO₂). These problems were managed by using solutions nearly DIC free and by removing the endogenous DIC in a preillumination period of 1 to 2 min until O₂ evolution ceased (see procedure and Fig. 1 of ref. 28). Nevertheless, further additions, such as a SHAM solution, carried in additional CO₂, which in turn had to be depleted. Another problem was that the CO₂ pump in Scenedesmus seems to be more active or dominated over the HCO₃⁻ pump as long as there were significant levels of CO₂.

SHAM has been used extensively as an inhibitor of the mitochondrial alternative oxidase in plants (9, 26). SHAM also inhibits respiration in algae, where the location and mechanism of its action are not characterized (13). SHAM inhibition of active CO₂ accumulation by algae could be due to a role of the alternative oxidase system in CO₂ uptake or to a nonrelated SHAM inhibition of a respiratory component of the DIC pumps. The need for energy for DIC pumps by algae is widely recognized (16, 24). The function of the alternative oxidase in mitochondria is not known other than to dissipate excess energy without generating ATP. Since the operation of DIC pumps requires energy, and since SHAM inhibition of the alternative oxidase in the mitochondria should not inhibit ATP generation, it seems unlikely that SHAM inhibition of the CO₂ pump should be due to reduced mitochondrial alternative oxidase activity. Thus, other hy-
hypothesis are needed to explain SHAM inhibition of the CO₂ pump. This type of respiration, involving quinol oxidation, might be in other membranes to create ion exchange gradients. The large increase in pH of the algal medium when CO₂ is taken up indicates a requirement for neutralization of the OH⁻ when HCO₃⁻ is converted to CO₂ and OH⁻. This conversion is usually facilitated by periplasmic CA (19), and indeed acetazolamide, a CA inhibitor, reduced both CO₂ uptake and alkalization of a neutral medium (results not shown). However, SHAM did not inhibit the partially isolated periplasmic CA. In nature during photosynthesis the large volume of bathing medium may wash away the hydroxyl ion or the OH⁻ may be used to convert a HCO₃⁻ to CO₂, which is precipitated as CaCO₃ (7). Within the periplasmic space, there may also be a respiratory requirement for a H⁺ to titrate HCO₃⁻ to CO₂ and water or to neutralize the OH⁻, and in some manner SHAM is inhibiting this process.

ACKNOWLEDGMENTS

We thank Dr. David Husic for tests on SHAM inhibition of isolated periplasmic CA and CA activity exhibited by whole cells of Chlamydomonas.

LITERATURE CITED