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Effect of Oxygen and Temperature on the Efficiency of Photosynthetic Carbon Assimilation in Two Microscopic Algae[1]

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ABSTRACT

The CO_2 compensation points of *Coccochloris peniocystis*, a blue-green alga and *Chlamydomonas reinhardtii*, a green alga, were determined at pH 8.0 in a closed system by a gas chromatographic technique. The compensation point of *Chlamydomonas* increased markedly with temperature, rising from 0.79 microliter per liter CO_2 at 15 C to 2.5 microliters per liter CO_2 at 35 C. In contrast, the compensation point of *Coccochloris* at 20 C was 0.71 microliter per liter CO_2 and rose to only 0.95 microliter per liter CO_2 at 40 C.

The compensation point of the green alga was significantly reduced at low O_2 concentrations (1 to 2%) when measured over the temperature range of 15 to 35 C. The compensation point of the blue-green alga, over the temperature range of 20 to 40 C, was unaffected by lowering the O_2 concentration.

The whole cell CO_2 affinity of *Chlamydomonas* decreased substantially with increasing temperature at 21% O_2 whereas little change was observed over the same temperature regime when the CO_2 affinity was determined at O_2 concentrations of 1 to 2%. The CO_2 affinity of *Coccochloris* did not decrease significantly with either increasing temperature or O_2 concentration.

These results suggest that while photorespiration is undetectable in *Coccochloris* some photorespiratory CO_2 release occurs in *Chlamydomonas*.

The Γ[2] of freshwater algae, when measured by a variety of techniques, have been found to be uniformly low. Some unicellular green algae appear to have Γ values lower than 10 μl l^{-1} (3, 13, 14, 23) and a wider range of algae exhibit this phenomenon when the Γ is determined at an alkaline pH (3). Although the measured Γ for algae appear to be lower than those found in C_3 plants, this does not indicate the absence of photorespiration. A high rate of photorespiratory carbon efflux in equilibrium with a high rate of carbon influx would result in the alga having a low compensation point though photorespiration is present. Another indication of whether or not photorespiration occurs can be obtained by the examination of the effect of temperature on Γ. It has been shown that in leaves of C_3 plants the rate of photorespiration and Γ increase with increasing temperature (12, 15, 18, 20). Laing *et al.* (22) have attributed these changes to a decrease in the soybean leaf affinity for CO_2 and an increased affinity for O_2 with increasing temperature. The purified enzyme, RuBP carboxylase (EC 4.1.1.39), when isolated from soybean leaf tissue showed a substantial increase in the K_m CO_2 whereas little change was noted in the affinity of the enzyme for O_2.

An increase in the internal O_2/CO_2 solubility ratio has also been proposed as an explanation for the change in the rate of photorespiration at elevated temperatures (21). However, other studies indicate that the change in the photorespiratory rate could not be correlated with the increase in the O_2/CO_2 solubility ratio which occurs with an increase in temperature (15).

In this paper we report studies on the effect of temperature and O_2 concentration on Γ and CO_2 affinity of a unicellular green and a unicellular blue-green alga.

MATERIALS AND METHODS

Axenic cultures of *Coccochloris peniocystis* Kutz. (1548) and *Chlamydomonas reinhardtii* Dangeard (90) were obtained from the culture collection of algae at the University of Texas, Austin (culture collection numbers in parentheses). The algae were cultured axenically in medium continuously bubbled with air and harvested as described previously (7). The algal cells were resuspended in 50 mM K-phosphate (pH 8.0) which had been previously flushed with either CO_2-free N_2 or air as a means of obtaining low (1–2%), and air-saturated (21%) levels of dissolved O_2.

For the determination of Γ, the algal suspension was placed in a temperature-controlled cylindrical glass incubation vessel (16 × 5 cm), the algae maintained in suspension with a magnetic stirrer and a layer of degassed mineral oil 3 cm deep placed on the surface to prevent gas exchange with the air. The suspension was then illuminated (2.5×10^4 μw cm^{-2}) with a 150-w flood light and the depletion of DIC in the medium monitored by removing, at timed intervals, 3.0-ml samples of algal suspension through a septum-stoppered sampling port in the incubation vessel. The DIC concentration of suspension samples was determined, after separation from the algal cells, by the gas-chromatographic technique described previously (3). The free CO_2 concentration at pH 8.0 was calculated from the DIC concentration using the equations of Buch (5).

To determine the apparent K_m CO_2 of the algae, photosynthetic rates at varying HCO_3^- concentrations were measured at a light intensity of 2.5×10^4 μw cm^{-2}, with a thermostatted Clark-type O_2 electrode (Hansatech Ltd., Kings Lynn, Norfolk, U.K.) calibrated as previously described (3). Dark respiration rates were also determined with the O_2 electrode.

Chl was determined after extraction with methanol or 90% acetone (3).

RESULTS

Effect of Temperature on Γ. The unicellular algae, when incubated in a closed system, reached equilibrium with the external carbon concentration in 15–20 min after the onset of illumination. The inorganic carbon concentration at equilibrium ranged from 35 to 125 μl l^{-1} DIC depending upon the alga, temperature and

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[2] Abbreviations: Γ: CO_2 compensation point; RuBP: ribulose-1,5-bisphosphate; DIC: dissolved inorganic carbon; PEP: phosphoenolpyruvate.

O_2 concentration. As all experiments were performed at pH 8.0, the "free" CO_2 concentration which represents Γ can be calculated for each DIC equilibrium concentration. Expressed in this manner Γ ranged from 0.75 to 2.5 $\mu l\ l^{-1}$ CO_2, again depending upon the alga, the temperature and O_2 concentration.

Although the two algae appear to be similar, in that Γ values are very low, it is in the response of Γ to increasing temperature that significant differences between *Coccochloris* and *Chlamydomonas* become apparent (Fig. 1). Over the temperature range studied and at 21% O_2, the green alga Γ approximately doubles for every 10 C rise, increasing from 0.79 ± 0.07 $\mu l\ l^{-1}$ CO_2 at 15 C to 2.51 ± 0.14 $\mu l\ l^{-1}$ at 35 C. In comparison, Γ of *Coccochloris*, at the same dissolved O_2 concentration and over the temperature range of 20–40 C, increases less than 26%, rising from 0.71 ± 0.04 $\mu l\ l^{-1}$ CO_2 at 20 C to 0.95 ± 0.07 $\mu l\ l^{-1}$ CO_2 at 40 C.

The O_2 sensitivity of Γ for both algae at each experimental temperature was also examined. *Coccochloris*, which exhibited only a minor increase of Γ with increasing temperature, was not affected by the lowering of the O_2 concentration to 1–2% (Fig. 1). The results obtained for *Chlamydomonas*, however, indicated that Γ, particularly at the higher temperatures is significantly reduced by lowering the O_2 concentration to well below air levels (Fig. 1).

Dark respiration rates of these algae were also determined over the temperature range 15–40 C. The respiration rate of *Chlamydomonas* increased over the entire temperature range, however, the greatest rate of change occurred from 25 to 35 C (Table I). Over this temperature interval of 25–35 C, Γ, when determined under low O_2 concentrations, increased most rapidly. In contrast to the green alga, dark respiration rates of *Coccochloris* were lower and increased linearly with increasing temperature (Table I).

Effect of Temperature on Apparent CO_2 Affinity. The increase in Γ with temperature could be partially accounted for by an increased rate of O_2-sensitive photorespiration in *Chlamydomonas*. The affinity of these cells for CO_2 was, therefore, measured and compared to the CO_2 affinity of *Coccochloris*.

In *Coccochloris* the apparent K_m CO_2 remained constant at 0.14 μM with increasing temperature at both O_2 concentrations (Fig. 2). A marked change, however, was observed in the affinity of *Chlamydomonas* for CO_2 (Fig. 3), the apparent K_m CO_2 rising from 0.55 μM at 15 C to 1.10 μM at 35 C. This change in affinity

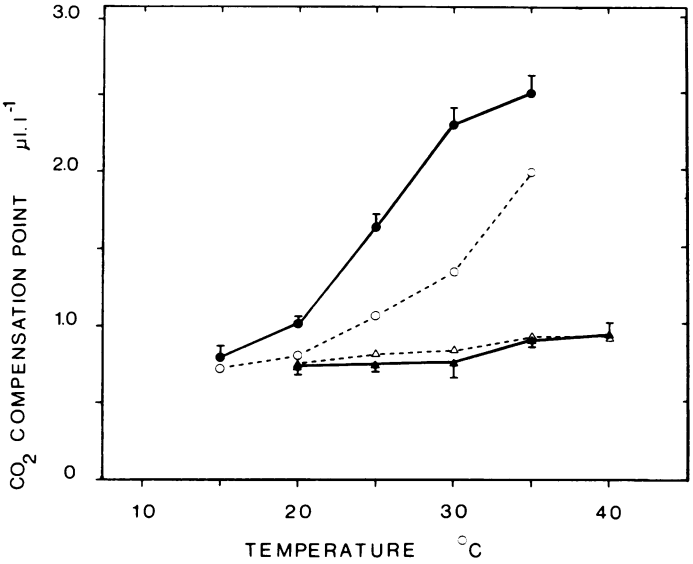


FIG. 1. Effect of temperature on the Γ of *Chlamydomonas* at 21% O_2 (●—●—●) and 1–2% O_2 (○——○); and *Coccochloris* at 21% O_2 (▲—▲—▲) and 1–2% O_2 (△—△—△). Vertical bars represent the SE of the mean.

Table I. *Effect of Temperature on Rate of Dark Respiration of Coccochloris and Chlamydomonas*

Coccochloris		*Chlamydomonas*	
Temperature	O_2 Uptake	Temperature	O_2 Uptake
C	*μmol mg Chl*$^{-1}$ *h*$^{-1}$	*C*	*μmol mg Chl*$^{-1}$ *h*$^{-1}$
20	15.2 ± 2.6	15	17.3 ± 2.9
30	24.4 ± 3.7	25	26.7 ± 3.5
40	32.3 ± 4.4	35	54.9 ± 3.9

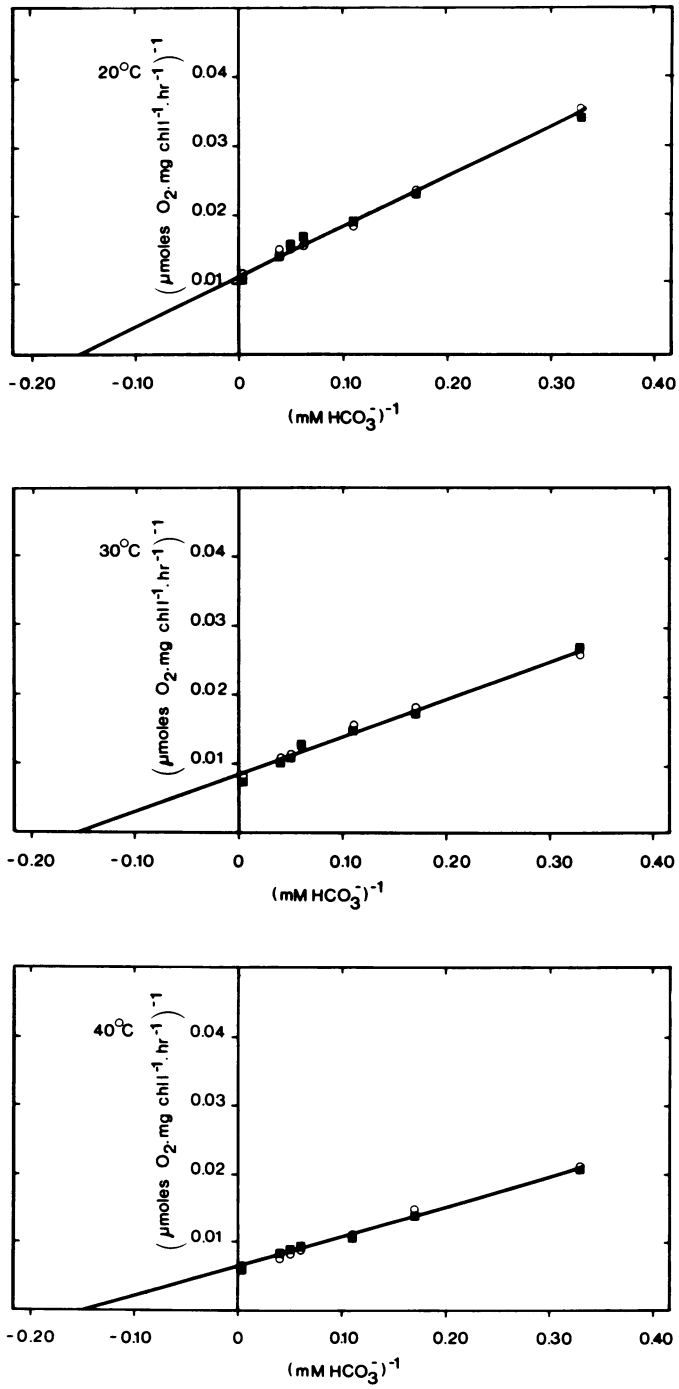
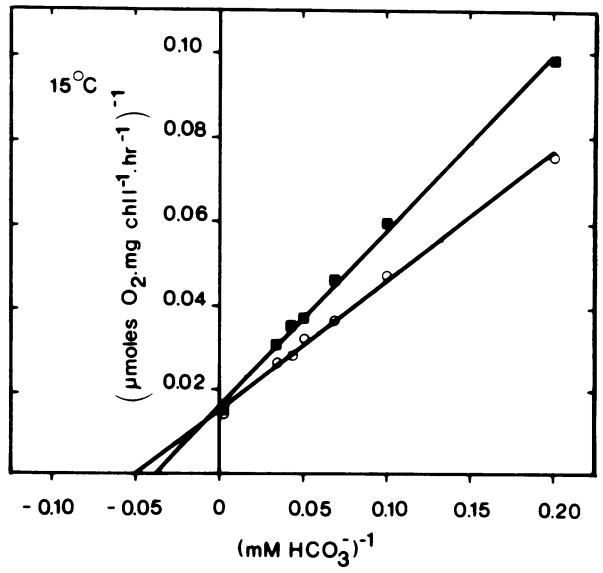
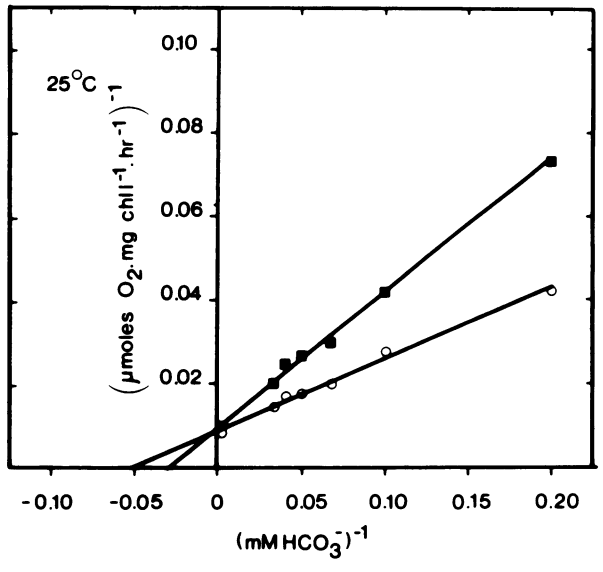
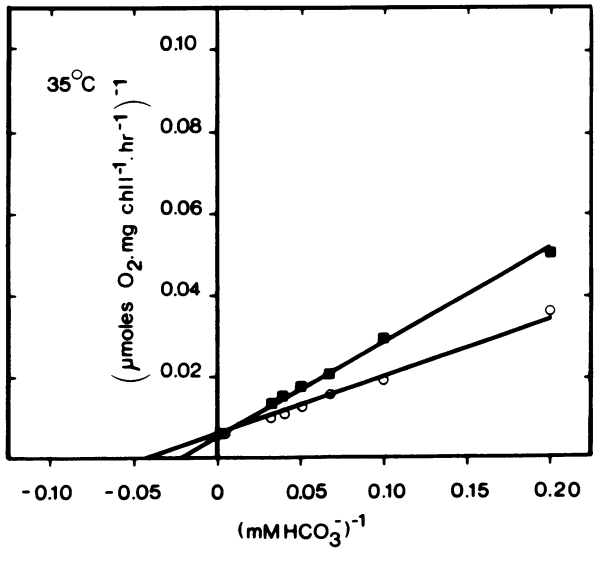


FIG. 2. Double reciprocal plots of the rate of photosynthetic O_2 evolution in *Coccochloris* as a function of $NaHCO_3$ concentration under 21% O_2 (■—■—■) and 1–2% O_2 (○—○—○) at three different temperatures.







for the substrate is O_2 dependent as the apparent K_m CO_2 of this alga (0.44 μM) was unaffected by increased temperature when the cells were incubated under low O_2 concentration (Fig. 3). The increase in the apparent K_m CO_2 with increasing temperature in *Chlamydomonas* when incubated at 21% O_2 could result in an increase in the rate of photorespiration with a concomitant increase in Γ.

DISCUSSION

The presence of photorespiration in algae has not been demonstrated unequivocally although these organisms produce glycolate in photosynthesis and some green algae have been shown to possess a functional glycolate pathway (25). Various rates of CO_2 release in light have been demonstrated in a range of algae which suggested that photorespiration occurred in some species (7). Other workers, however, have failed to detect photorespiration in a similar range of species (23). The present study provides evidence that some photorespiratory CO_2 release occurs in the green alga *Chlamydomonas* although it is undetectable in the blue-green alga *Coccochloris*.

The response of Γ of the green alga to both O_2 and temperature indicates that photorespiration occurs in *Chlamydomonas* and that the rate of photorespiration increases with temperature and with increasing O_2 concentration (Fig. 1). Similar relationships between temperature and Γ, and O_2 concentration and Γ have been shown in a wide range of C_3 higher plants (6, 17, 18, 20). Other work has shown that photorespiratory rate increases with an increase in temperature which would result in higher values of Γ (15, 19, 22). The absolute values of Γ for *Chlamydomonas* are very much lower than those of C_3 plants, but the degree of change in Γ with increasing temperature at 21% O_2 is very similar to that observed in higher C_3 plants. The rise in Γ with an increase in temperature in *Chlamydomonas* at low O_2 concentration may be accounted for by an increase in the rate of dark respiration in the light (Table I). It has been suggested that dark respiration may be a second component of Γ and one that is O_2-insensitive (26, 27).

An increase in the rate of photorespiration with rising temperature in *Chlamydomonas* is reflected in the decreased affinity of the cell for CO_2. At 21% O_2 the apparent K_m CO_2 increases with temperature whereas at low O_2 concentration it remains constant. Such a result is consistent with the findings that the ratio of the apparent K_m oxygenase/K_m carboxylase of the leaves of C_3 plants and of isolated RuBP carboxylase increases with temperature (1, 22). Such a stimulation of RuBP oxygenase activity would cause the formation of additional photorespiratory substrate and the subsequent increase in the rate of release of CO_2 would result in an elevation of Γ.

In contrast to *Chlamydomonas*, the blue-green alga *Coccochloris* does not demonstrate any effect of O_2 concentration and temperature on Γ and the apparent K_m CO_2 remains constant with changes both in temperature and O_2 concentration. The physiological responses of the alga to increased temperature and to O_2 concentration are identical to those observed in higher plants which possess the C_4 pathway of CO_2 fixation and consequently display a restricted rate of photorespiration (4, 8, 16). These results suggest that RuBP carboxylase in the blue-green alga is protected from the effects of O_2 or that CO_2 produced in photorespiration is efficiently refixed. It has been reported that blue-green algae have some metabolic similarities to C_4 plants in that they have high levels of PEP carboxylase (10) and that photosynthetic CO_2

FIG. 3. Double reciprocal plots of the rate of photosynthetic O_2 evolution in *Chlamydomonas* as a function of $NaHCO_3$ concentration under 21% O_2 (■—■—■) and 1-2% O_2 (○—○—○) at three different temperatures.

fixation is inhibited by malonate, an inhibitor of PEP carboxylase (11).

Alternatively, photorespiration in the blue-green alga might be precluded and severely limited in *Chlamydomonas* by the protection of RuBP carboxylase as a result of a build up of a high concentration of inorganic carbon in the cell by an active transport of bicarbonate. Evidence that HCO_3^- is taken up during photosynthesis by *Coccochloris* has been provided by Miller and Colman (24) and interesting studies by Badger *et al.* (2) have demonstrated an accumulation of inorganic carbon during photosynthesis in the blue-green alga *Anabaena variablis* and in *C. reinhardtii* which is attributable to bicarbonate transport. Some indication of an active accumulation of inorganic carbon by algal cells is also provided in the present study, since the apparent K_m CO_2 of both algae studied (Figs. 2 and 3) are both considerably lower than the K_m CO_2 of 71 and 17.5 μM reported, respectively, for RuBP carboxylase isolated from a blue-green alga (9) and a higher plant (1). The results of Badger *et al.* (2) also indicate that the inorganic carbon pool accumulated in *Anabaena* was much greater than that in *Chlamydomonas* suggesting that there is a disparity in the efficiency of bicarbonate transport between the two algae. The difference in the physiological response to O_2 between *Coccochloris* and *Chlamydomonas* reported in this study may, therefore, reflect a difference in the efficiency of the bicarbonate transport systems of the two algae.

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