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Short Communication

Photosynthesis and Inorganic Carbon Accumulation in the Acidophilic Alga *Cyanidioschyzon merolae*[1]

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ABSTRACT

The intracellular pH and membrane potential were determined in the acidophilic algae *Cyanidoschyzon merolae* as a function of extracellular pH. The alga appear to be capable of maintaining the intracellular pH at the range of 6.35 to 7.1 over the extracellular pH range of 1.5 to 7.5. The membrane potential increase from −12 millivolts (negative inside) to −71 millivolts and thus $\Delta\tilde{\mu}H^+$ decreased from −300 to −47 millivolts over the same range of extracellular pH. It is suggested that the $\Delta\tilde{\mu}H^+$ may set the upper and lower limits of pH for growth. Photosynthetic performance was also determined as a function of pH. The cells appeared to utilize CO_2 from the medium as the apparent $K_{m(CO_2)}$ was 2 to 3 micromolar CO_2 over the pH range of 1.5 to 7.5. *C. merolae* appear to possess a 'CO_2 concentrating' mechanism.

The growth of photosynthetic organisms living in hot, acidic environments may be limited by their capacity to control their intracellular pH, as well as by the availability of inorganic carbon (C_i) for photosynthesis. The latter is due to CO_2 practically being the only C_i species present at the acid environment and the reduced solubility of CO_2 at elevated temperature. The pH gradient between the cell interior and the acidic medium may favor the accumulation of C_i within the cell to a level determined, at equilibrium, by the pH gradient and the extracellular CO_2 concentration. The intracellular CO_2 level, which is the substrate for the carboxylation reaction, may be smaller than expected at equilibrium due to utilization in photosynthesis and limitations imposed by diffusion and permeability (12). Cyanobacteria and green algae growing under low CO_2 level have been shown to possess a mechanism which enables them to concentrate CO_2 within the cells and thus to perform high photosynthetic rates despite the low CO_2 level (1, 5).

In the present study, we investigated the capacity of the acidiophylic green alga *Cyanidioschyzon merolae* to maintain its intracellular pH as well as the means by which CO_2 is being supplied to the carboxylation site.

MATERIALS AND METHODS

Cells of *Cyanidioschyzon merolae* (3) were grown in the medium described by Enami and Fukuda (4), pH 1.5 at 35°C in 500-ml flasks bubbled wih air. Continuous illumination at 6 $mw \cdot cm^{-2}$ (400–700 nm) was provided. Cells were harvested by centrifugation and resuspended in a medium containing citrate-phosphate buffer adjusted to the desired pH by changing the ratio between citric acid and KH_2PO_4. CO_2-dependent O_2 evolution was measured using an O_2 electrode (Rank Brothers, Bottisham, Cambridge, U.K.). Intracellular pH was determined from the distribution of acetyl (carboxy-^{14}C) salicylic acid at pH values below 4.5 and [^{14}C]-5,5-dimethyloxazoladine-2,4-dione (DMO) at pH values higher than 4.5. The membrane potential, $\Delta\psi$, was calculated from the distribution of the lipophilic cation tetraphenylphosphonium (TPP*) as described elsewhere (6). The intracellular concentration of C_i was determined by the filtering centrifugation technique (5). Cells were centrifuged through a mixture of 1:1 bis(2-ethylhexyl)phthalate and dibutyl phthalate.

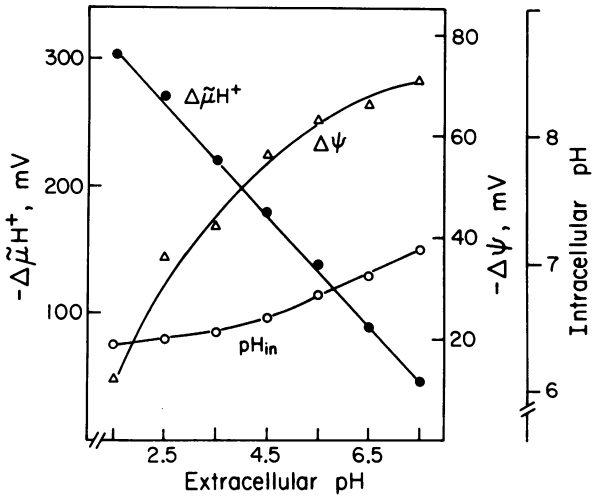

FIG. 1. Intracellular pH, membrane potential ($\Delta\psi$), and electrochemical potential gradient of H^+ ($\Delta\tilde{\mu}H^+$) in *C. merolae* as a function of extracellular pH.

RESULTS AND DISCUSSION

The average intracellular pH increased from 6.35 at an extracellular pH of 1.5 to 7.1 at pH 7.5 (Fig. 1). It may thus be concluded that *C. merolae* possesses the capacity to maintain its internal pH within rather narrow limits (0.75 pH units) while the extracellular pH changed by 6 pH units. The Δ pH decreased from −4.85 (alkaline inside) to 0.4 (acid inside). At the same time the $\Delta\psi$ hyperpolarized from −12 mv at pH 1.5 to −71 mv at pH 7.5. This hyperpolarization was too small to result in maintenance of the free energy difference for H^+ ($\Delta\tilde{\mu}H^+$) and the latter decreased from −300 mv at pH 1.5 to −47 mv at pH 7.5.

At pH 1.5, the $\Delta\tilde{\mu}H^+$ may cause a considerable influx of H^+ depending on the passive permeability for H^+ and the activity of

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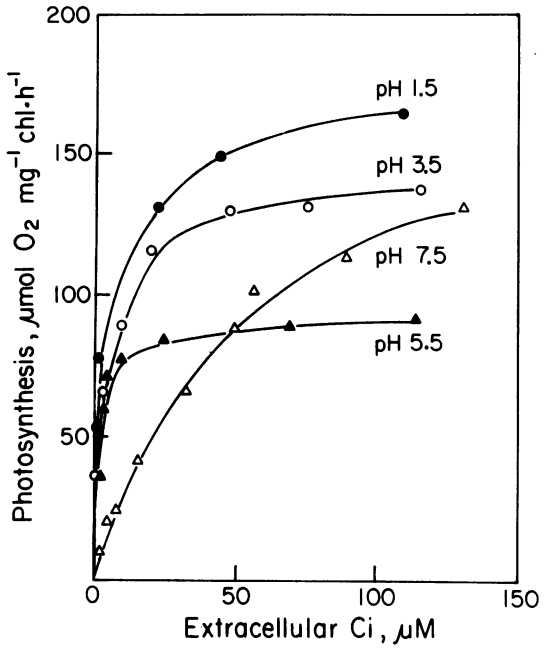

FIG. 2. Dependence of photosynthetic O_2 evolution on the concentration of C_i in the medium at various pH values. Light intensity was 6 mw·cm^{-2} (400–700 nm), 30°C.

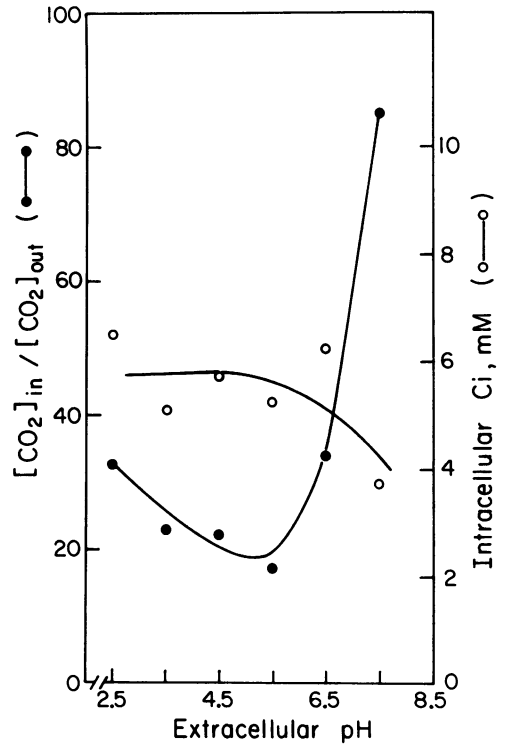

FIG. 3. The intracellular C_i pool and the calculated accumulation of CO_2 as a function of intracellular pH. Cells were exposed to $^{14}C_i$ concentration of 65 μM for 1 min. Other conditions as in Figure 2.

H^+ symport mechanisms (see 11). Protons arriving at the inner side of the plasmalemma must be extruded to avoid acidification of the cytoplasm, presumably by means of an H^+-ATPase (7). The stoichiometry of H^+ pumped per ATP at pH 1.5, can not exceed 1 (since the amount of energy available by dissociation of ATP to ADP will not be large enough to translocate more than 1 H^+ per ATP, against a $\Delta\tilde{\mu}H^+$ of −300 mv (9). Thus, the energy requirement for pumping H^+ outward may set the lower limit of external pH at which growth is possible. The $\Delta\tilde{\mu}H^+$ may also set the upper limit of external pH for growth, as at alkaline pH values, $\Delta\tilde{\mu}H^+$, the driving force for H^+ symport processes, reaches values close to zero (Fig. 1).

The extreme pH values are those conditions under which the biological system may be limited by the driving force for transport processes. At moderate conditions, on the other hand, the capacity for transport processes and accumulation of the transported substrate is mainly kinetically limited (see 11). Thus, organisms which can grow at extreme pH values such as *C. merolae* may be most suitable for studies on the regulation of the H^+ pump by the $\Delta\tilde{\mu}H^+$ (see 9, 11).

As stated in the introduction, another factor limiting photosynthesis and growth at acidic, hot environments is the availability of CO_2. The V_{max} of photosynthesis, at saturating light and CO_2, was highest at pH 1.5, decreased as the pH was raised to 5.5, and increased again as the pH was raised to 7.5 (Fig. 2). The apparent $K_{m(C_i)}$ was 2 to 4 μM C_i at pH values of 1.5 to 5.5 and 30 μM C_i at pH 7.5. The apparent $K_{m(CO_2)}$ calculated from Figure 2 is 2 to 3 μM CO_2 over the entire range of pH. These data might be taken as an indication that CO_2 is probably the C_i species taken up from the medium. The observed $K_{m(CO_2)}$ in *C. merolae* is an order of magnitude lower than that reported for *Cyanidium caldarium*, another acidiophylic alga (10). The kinetic parameters of ribulose 1,5-bisphosphate carboxylase were not determined in *C. merolae*. The very low apparent $K_{m(CO_2)}$, however, suggested that *C. merolae* might be capable of accumulating C_i within the cell as is the case in other green algae (1) and cyanobacteria (5). The intracellular level of C_i was measured in experiments in which $^{14}C_i$ was supplied for 1 min. The accumulation ratio $((CO_2)_{in}/(CO_2)_{out})$ (Fig. 3) was calculated assuming that the different C_i species (CO_2 and HCO_3^-) in the medium and within the cells were at equilibrium. Figure 2 suggested that *C. merolae* utilizes CO_2 and not HCO_3^- from the medium. Furthermore, experiments in which $^{14}CO_2$ or $H^{14}CO_3^-$ were provided showed that CO_2 was taken up faster than HCO_3^-. The intracellular C_i pool was 3.1 or 0.75 mM when C_i (65 μM) was provided as CO_2 or HCO_3^- (respectively) for 10 s to *C. merolae* cells at pH 7.5 (not shown). It is thus possible that CO_2 is the species which crosses the plasmalemma of *C. merolae* (see 8). Therefore, if the different C_i species within the cells are not at equilibrium, the accumulation ratios of CO_2 (Fig. 3) may be underestimated.

On the other hand, the data in Figure 3 were calculated assuming an average intracellular pH at each extracellular pH (Fig. 1) and equal distribution of the C_i species across the cell compartments. These assumptions may not be valid, particularly in the light, since the chloroplast stroma may be more alkaline than the cytoplasm. The C_i transporting system may be located in the chloroplast envelope, *i.e.* the C_i pool is mainly confined to the chloroplast (2, 8). Thus, is of interest to estimate the accumulation ratio on the following assumptions: the chloroplasts occupy about half of the cell volume (Seckbach personal communication); the stromal pH in the light is 8.0; the cytoplasmic pH is 6.2 (to yield the average intracellular pH of 7.1 at extracellular pH 7.5); CO_2 equilibrates rapidly across the plasmalemma; the C_i translocating system is located in the chloroplast envelope, *i.e.* the cells accumulate C_i across the chloroplast envelope only (2). The CO_2 accumulation ratio across the chloroplast envelope calculated on the above assumptions is 24 as opposed to 85 depicted in Figure 3, for extracellular pH of 7.5. It is concluded that even though the exact CO_2 accumulation ratio cannot be assessed at present, *C. merolae* exhibits a CO_2 concentrating mechanism. This capacity to accumulate CO_2 within the cells results in the low apparent $K_{m(CO_2)}$ (Fig. 2) and enables the cells to cope with the very low level of C_i present at

hot and acidic environments.

Photosynthetic V_{max} (Fig. 2) and accumulation ratio of CO_2 (Fig. 3) were minimal at pH 5.5. While the latter cannot be the reason for the former, because photosynthetic V_{max} is measured at saturating CO_2, the reduction of both parameters at pH 5.5 could result from a common, as yet unknown, cause.

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