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Light-induced pH changes by cells of *Chlamydomonas reinhardii:* Dependence on CO_2 uptake

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SUMMARY

Light-induced increase in the pH of a suspension of whole cells of *Chlamydomonas reinhardii* required net photosynthesis. The ratio of CO_2 added:O_2 evolved : H^+ used based on measurement of net changes was 1:1:1.

When a suspension of Chlamydomonas[1] or Dunaliella[2] cells in an unbuffered liquid medium is illuminated the medium becomes more alkaline. In the dark the pH change is reversed. This phenomenon has been termed, "light-induced proton uptake", and mechanistically described as a 'proton pump'[1,2]. Packer *et al.*[3] suggested that these pH changes might be due to the light-induced H^+ uptake activity of chloroplasts which has been observed in preparations from higher plants[4-9]

Earlier work[10] with Elodea and the moss Fontinalis indicated, however, that light-induced pH changes can be due to uptake of CO_2 or HCO_3^- from the surrounding medium. More recently Cummins *et al.*[11] suggested that H^+ movement in Ulva is associated with the movement of HCO_3^-.

In the present study we have examined the effect of CO_2 on the light-induced pH change of a suspension of Chlamydomonas to ascertain whether or not the so-called H^+ movements depend on photosynthetic CO_2 movements.

Chlamydomonas reinhardii ('wild type') was grown autotrophically at 25° in a liquid medium[12] bubbled with air and illuminated continuously with 4000 lux from fluorescent tubes. Cells were harvested by centrifugation, washed twice in CO_2-free distilled water, and suspended at about 9 μl packed cell volume/ml (about 35 μg chlorophyll/ml) in CO_2-free distilled water.

Changes in pH and O_2 concentration were measured at 25° in a closed 3.8 ml Plexiglass vessel using a Radiometer Model GK2641C combination pH electrode and a Clark oxygen electrode (Yellow Springs Instrument Co.), respectively. The output from each electrode was traced simultaneously with two strip chart recorders. The light intensity

($0.7 \cdot 10^5$ ergs/cm^2 per sec) used was saturating for photosynthesis.

Each cell preparation was titrated in dim room light with carbonate-free 3.8 mM NaOH[13] and the titration curve obtained used to calculate the proton changes from observed pH changes.

HCO_3^- was added as $KHCO_3$, and CO_2 as a carbonic acid solution prepared by bubbling distilled water with CO_2 at 20°. Chlorophyll was determined by the method of Arnon[14].

Fig. 1 shows the effect of light and dark on the O_2 concentration and pH of the medium surrounding whole cells of Chlamydomonas. The pH changes were similar to those described previously for this organism[1] and also for *Dunaliella parva*[2]. In the pH curve, the initial lag which was not as pronounced in the O_2 curve, was in part due to the non-linear buffering characteristics of the cells.

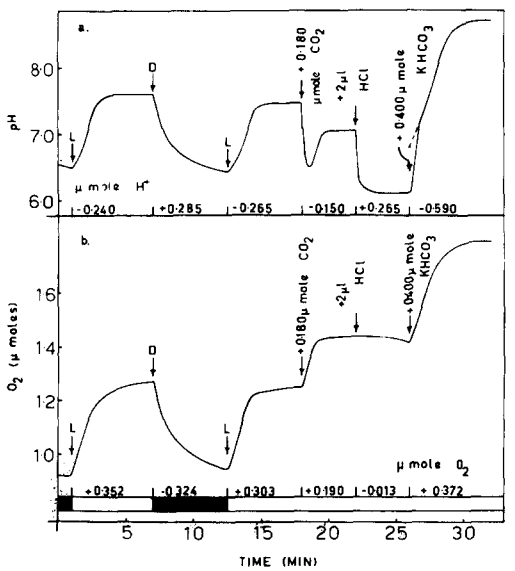

Fig. 1. Changes in pH (a) and O_2 concentration (b) in light and dark with a suspension of *C. reinhardii* cells in distilled water. Values placed horizontally in the body of the graph refer to µmoles O_2 evolved or taken up and to the µmoles H^+ consumed or produced in the suspending medium. The suspension contained 34 µg chlorophyll/ml. L indicates light switched on and D indicates light switched off.

The absence of pH change after 2–3 min illumination has been interpreted[1,2] as a steady state where the rate of light-induced H^+ uptake was balanced by H^+ leakage back into the medium.

The changes in O_2 concentration were very similar in form (Fig. 1) to those of pH. For O_2, however, a steady state was reached when O_2 evolution due to photosynthesis was balanced by O_2 consumption due to respiration.

A relationship between pH and O_2 changes in the light was detected by adding CO_2 after this point was reached (Fig. 1). Net photosynthesis (O_2 evolution) then occurred until a new steady-state O_2 concentration was reached. Corresponding changes in pH were

observed (Fig. 1) following the initial rapid acidification due to hydration of the CO_2 added to the medium. However, addition of H^+ as HCl (Fig. 1; 0.255 μmole H^+) caused no additional light-induced pH rise or O_2 evolution. The same results were obtained if H_2SO_4 was used as the source of added H^+. Addition of acid or CO_2-free KCl had no effect on the subsequent photosynthesis and pH change when $KHCO_3$ was added (Fig. 1).

These results show that the effect of darkness could not be replaced by adding H^+, suggesting that CO_2, produced by respiration, rather than H^+, was necessary for a light-induced pH change.

There was a clear dependence of pH change in the light on the availability and concentration of CO_2 (Fig. 2).

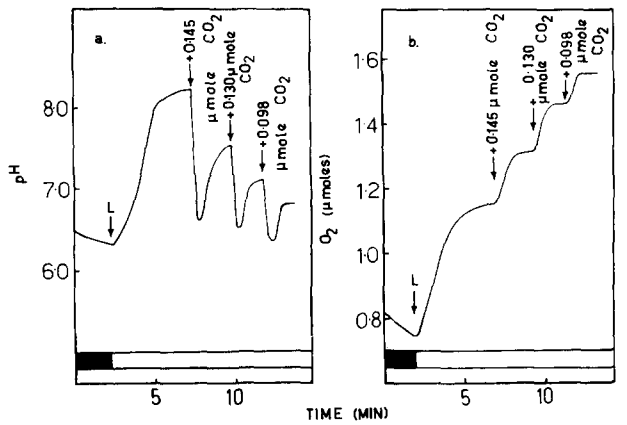

Fig. 2. Effect of CO_2 on pH (a) and O_2 concentration (b) changes by a suspension of *C. reinhardii* cells in distilled water (34 μg chlorophyll/ml). L indicates light switched on.

The equivalence of the O_2 and H^+ changes was found by comparing the net change in each following the light or dark periods used (see Fig. 1). In the light for 1 mole O_2 evolved due to photosynthesis, 1 mole H^+ was removed from the medium (Table I). Similarly, in the dark for 1 mole O_2 consumed in respiration 1 mole H^+ was formed in the medium. These results were obtained in a number of experiments showing widely differing extents of pH change ranging from 0.15 to 0.40 μmole H^+ lost from the medium in the

TABLE I

RELATIONSHIP BETWEEN NET CHANGES IN H^+ AND O_2 CONCENTRATIONS IN THE LIGHT AND DARK

C. reinhardii cells were suspended in distilled water at 25°.

	Number of experiments	*μmoles H^+/μmoles O_2*
Light	12	1.02 ± 0.03★
Dark	4	1.01 ± 0.05★

★Mean ± S.E.

light and 0.26 to 0.49 μmole H^+ formed in the medium in the dark. Further, since the CO_2/O_2 ratio in the light was unity (Table II) this suggests that for 1 mole CO_2 fixed by the cells 1 mole H^+ was consumed.

TABLE II

RELATIONSHIP BETWEEN CO_2 ADDED AND O_2 EVOLVED IN THE LIGHT BY *C. reinhardii*

The cells were suspended in distilled water at 25°. CO_2 was added as a carbonic acid solution prepared by bubbling distilled water with CO_2 gas.

Expt.	*Total CO_2 added (μmole)*	*Total O_2 evolved (μmole)*	*CO_2/O_2*
1	0.098	0.099	0.99
2	0.145	0.156	0.93
3	0.180	0.190	0.95
4	0.325	0.243	1.34
5	0.326	0.303	0.93
6	0.400	0.372	1.05
		Mean ± S.E.	1.04 ± 0.06

The equivalence found above was not obtained after the addition of HCO_3^- (Fig. 1) because of the initial pH change due to dehydration of HCO_3^- in the medium. When allowance was made for this effect the extent of the light-induced pH change (−0.390 μmole H^+) was closely related to the amount of O_2 evolved (0.372 μmole).

The maximum rates of net O_2 evolution (333 ± 12 (S.E.) μmoles/h per mg chlorophyll) observed were the same whether HCO_3^- or CO_2 was added and in each case corresponded to maximum rates of net pH change equivalent to 325 ± 15 (S.E.) μmoles H^+/h per mg chlorophyll.

The foregoing results show a strict dependence of pH change on CO_2 and are not in agreement with those of Shuldiner and Ohad[1] or Ben-Amotz and Ginsberg[2] who found no difference between air-saturated and CO_2-free media. It is possible, however, that the 'CO_2-free' systems used by these workers were not in fact free of the CO_2 which would be produced by respiration. With whole cells, at least, the most reliable method for testing the effect of CO_2 would appear to be by addition at the compensation point (Figs. 1 and 2).

There are a number of reactions of CO_2 and H^+ which singly or together might explain the observed relationship between pH change, photosynthesis and respiration.

(1) $HCO_3^- + H^+ \rightleftharpoons CO_2 + H_2O$. In this reaction CO_2 would be removed in the light by photosynthesis and evolved in the dark by respiration.

(2) $HCO_3^- + H^+$ move together into the cell in the light and out in the dark.

(3) HCO_3^- moves into the cell in the light and a base moves out and removes a H^+ from the medium.

The first possibility is the simplest which would account for the observed results. It does, however, require the exclusive movement of CO_2. On the other hand the second and third reactions depend on the movement of HCO_3^- and not CO_2. Cummins *et al.*[11]

have suggested that in Ulva a proton moves with HCO_3^- thus conserving chanrge in a co-transport system. This would be possible in organisms which utilise HCO_3^- readily. However, bicarbonate utilisation in photosynthesis has not been clearly established in Chlamydomonas[15].

The buffering capacity of chloroplast preparations can be up to 2-fold greater in light than in the dark[16]. In our experiments with whole cells the buffering capacity when a steady-state concentration of O_2 was reached was similar in dim or strong light but satisfactory measurements could not be made in darkness because of rapid CO_2 changes in the medium. However, our conclusions were made from net changes of H^+ and not, as was the case in previous studies with whole cells[1,2] or chloroplasts[4-9], from calculated rates based on assumptions regarding changes in the dark. Thus differences in buffering capacity due to light would not affect the calculated ratios.

At present it is not possible to say that light-induced pH changes by whole cells are wholly or partly due to an influx of protons which might participate in the formation of a proton gradient[17,18] and as a consequence of this the relationship between these whole cell phenomena and the light-induced pH changes shown by chloroplasts[4,9] is not yet obvious.

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