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Short Communication

Adaptation to CO_2 Level and Changes in the Phosphorylation of Thylakoid Proteins during the Cell Cycle of *Chlamydomonas reinhardtii*[1]

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ABSTRACT

The photosynthetic performance of synchronously grown *Chlamydomonas reinhardtii* alternated rhythmically during the cell cycle. The activity of the "CO_2 concentrating mechanism" including the ability to accumulate CO_2 internally and the activity of carbonic anhydrase peaked after 6 to 9 hours of light and reached minimum after 6 to 9 hours of dark. Consequently, the apparent photosynthetic affinity to extracellular CO_2 alternated rhythmically. At the end of the dark period the cells behaved as if they were adapted to high CO_2 even though they were continuously aerated with air. Results from experiments in which the light or dark periods were extended bear on the interaction between the internal (cell cycle or biological clock) and the external (light) signal. The observed rhythmical alterations in photosynthetic V_{max} may result from changes in PSII activity. The latter may be partly explained by the capacity for phosphorylation of thylakoid proteins, which reached maximum after 9 hours of light and decreased toward the dark period.

The adaptation of green algae from high (5% v/v) to low (air) level of CO_2 involves an elevated capacity to concentrate CO_2 within the cell (1, 9, 17). This has been attributed to the activity of a plasmalemma-located CO_2 transporting system (9). CA[3] activity increases markedly during adaptation to low CO_2 (3, 10). In the case of *Chlamydomonas*, CA is mainly located in the periplasmic space (3, 5). It probably facilitates the supply of CO_2 to the transport mechanism from the HCO_3^- in the medium (9).

The elevated CO_2 concentration at the carboxylating site results in an increased photosynthetic affinity ($K_{½}$) for extracellular CO_2 in cells adapted to low level of CO_2 (1, 9). It has been shown that the adaptation to low CO_2 depends on light and that a product of photosynthesis/photorespiration is most probably involved in its induction (8, 17). On the other hand, since low CO_2 adapted cells accumulate CO_2 internally, it is not clear what serves as the signal for adaptation from low to high CO_2 level.

Studies on the adaptation of algae to various levels of CO_2 were mostly conducted using cultures grown under continuous light. It is known, however, that the photosynthetic performance, particularly V_{max}, the activity of PSII (13, 15, 20), and the level of various enzymes of the Calvin cycle (*cf.* 4) of synchronized green algae are strongly affected by the phase of the cell cycle. While the polypeptides of PSII reaction center are for the most part synthesized during the first hours of the light period, those of the LHC II are made mainly during the 7 to 9 h of the light period (4). The regulation of energy distribution at limiting light intensity, between PSII and PSI is thought to occur via phosphorylation/dephosphorylation of the LHC II (19). The required activities of kinases and phosphatases associated with the thylakoid membranes were detected in various photosynthetic organisms (2, 12, 14, 19). If phosphorylation capacity depended on the cell cycle, it may help to explain the alterations in PSII activity which peak at 7 to 9 h of the light period in synchronized cells. Changes in phosphorylation capacity and hence in PSII activity may also help to explain the alterations in the photosynthetic rate of saturating CO_2 during the cell cycle.

Should adaptation to various levels of CO_2 and hence also capacity to concentrate CO_2 internally depend on the cell cycle, it would provide a powerful tool for the study of molecular mechanisms involved in the adaptation. In this study we investigated the dependence of adaptation to low CO_2 and *in vitro* phosphorylation of thylakoid proteins on the cell cycle in synchronously grown *Chlamydomonas*.

MATERIALS AND METHODS

Chlamydomonas reinhardtii 2137 were grown autotrophically on a 12 h light/dark cycle for at least 4 d at 25°C in 8 L flasks containing the medium described by Ohad *et al.* (11) but without sodium acetate. The culture was continuously aerated with air and stirred with a magnetic stirrer. Light intensity at the surface of the flask was 6 $mW \cdot cm^{-2}$ (400–700 nm). Aliquots were withdrawn from the flask at time intervals, harvested and resuspended in 20 mM Hepes-NaOH (pH 7.5). O_2 exchange, CO_2 uptake, and intracellular C_i pool were determined as in Ref (9). Cells were harvested at various times during the 24 h cycle and placed at −20°C. The CA activity in the sample was then measured as described elsewhere (10). The fluorescence yield in the presence or absence of DCMU was measured as in Ref (6).

For the phosphorylation determination, cells were broken by French press (0.028 $kg \cdot m^{-2}$), and thylakoids were isolated by centrifugation (100,000*g*, 5 min) on a cushion of 2 M sucrose in 50 mM Tris-HCl (pH 8.0) as previously described (14). Phosphorylation *in vitro* of thylakoids proteins was carried out essentially

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[3] Abbreviations: CA, carbonic anhydrase; C_i, inorganic carbon; LHC II, light harvesting chlorophyll *a*, *b*, complex of PSII.

as described by Owens and Ohad (12). Thylakoids were incubated 5 min in light or dark at 25°C in 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, and 0.15 Ci/mmol [^{32}P]ATP (Amersham International Ltd.). The reaction was terminated by centrifugation (Eppendorf microcentrifuge) and resuspended in electrophoresing sample buffer (12). Chl measurement, lithium dodecylsulfate PAGE, and autoradiography were carried out as described earlier (14).

RESULTS AND DISCUSSION

The photosynthetic performance of *C. reinhardtii* synchronously grown on low CO_2 was strongly affected by the time at which it was determined (Figs. 1, 2). Photosynthetic V_{max} (at saturating CO_2 concentration), the photosynthetic affinity for extracellular C_i ($K_{½}$), the photosynthetic rate at limiting C_i level (V_{10}), the ability to accumulate C_i internally, and CA activity alternated rhythmically over a 24 h period. Cell cycle dependent alterations in photosynthetic activity have been reported previously (13, 15, 20) and were attributed to changes in PSII activity (13) and the level of ribulose bisphosphate carboxylase/oxygenase (4). We also observed large changes in PSII activity as determined by the rate of 2,6-dichlorophenol indophenol reduction and variable fluorescence. PSII activity and variable fluorescence reached maximum after 6 to 8 h light and minimum after 6 to 9 h of dark (not shown but see Refs. 13, 15). Figure 3 presents an autoradiograph of the phosphorylation *in vitro* of various polypeptides of the thylakoid membranes as a function of time in synchronously grown *Chlamydomonas*. It also presents the polypeptide pattern as detected following Coomassie blue staining of the gel (lane G in Fig. 3). Only one lane is presented

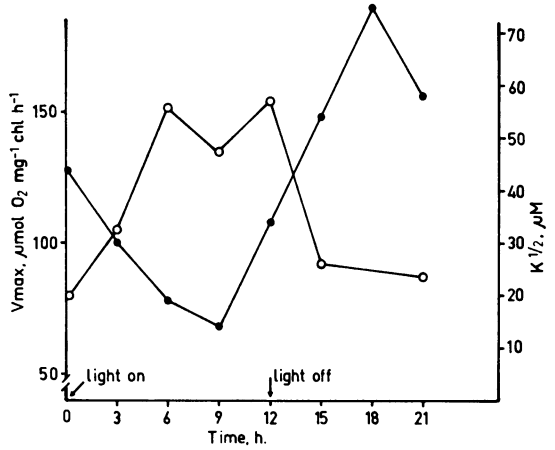

FIG. 1. Photosynthetic V_{max} (O) and apparent photosynthetic affinity to extracellular C_i ($K_{½}$, ●) in synchronously grown *C. reinhardtii* as a function of time. Cells were taken at different times during the cell cycle, centrifuged, and resuspended in 20 mM Hepes-NaOH (pH 7.5). The dependence of photosynthetic rate on CO_2 concentration was measured at 25°C in the O_2 electrode chamber. Light intensity was 8 mW·cm^{-2} (400–700 nm).

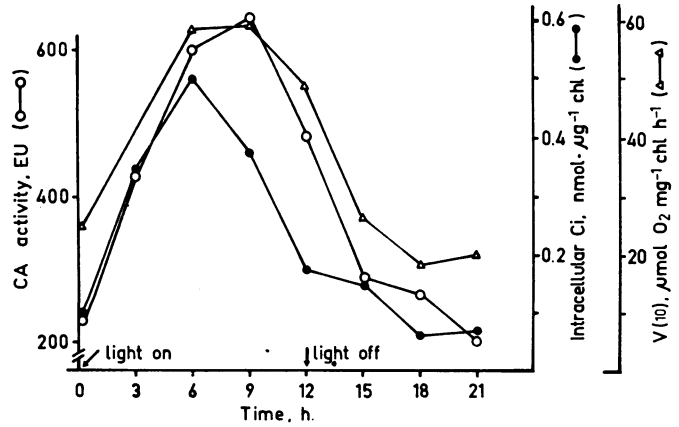

FIG. 2. The dependence on time of CA activity, photosynthetic rate at limiting C_i ($V_{(10)}$) and intracellular level of C_i of synchronously grown *C. reinhardtii*. $V_{(10)}$ was determined in the presence of 10 µM C_i. Intracellular C_i was determined following exposure of the cells to 50 µM $^{14}C_i$ for 10 s. Other conditions as in Figure 1.

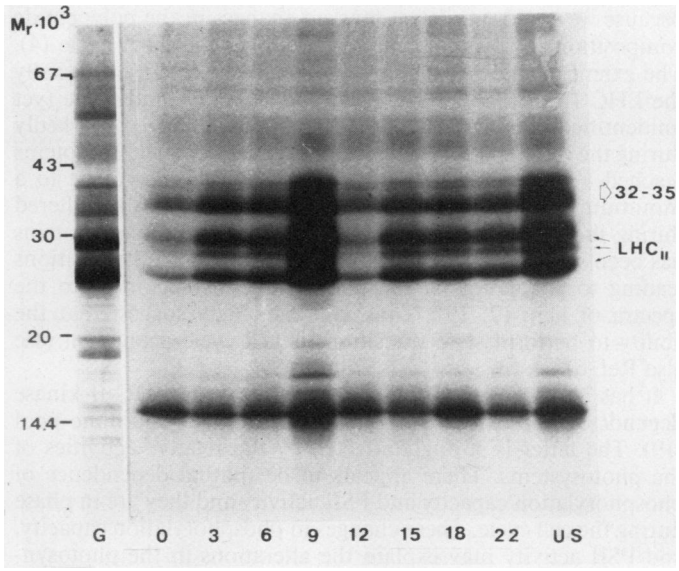

FIG. 3. *In vitro* phosphorylation of thylakoid polypeptides isolated at different times during the cell cycle of *C. reinhardtii*. Light on was at 0 h, light off at 12 h. Thylakoid preparation was treated with [^{32}P]ATP for 5 min in the light. We could not detect protein phosphorylation in the dark in all the samples. G, Coomassie brilliant blue R staining of the gel, showing the polypeptide composition of the thylakoid membrane; US, a sample from unsynchronized culture.

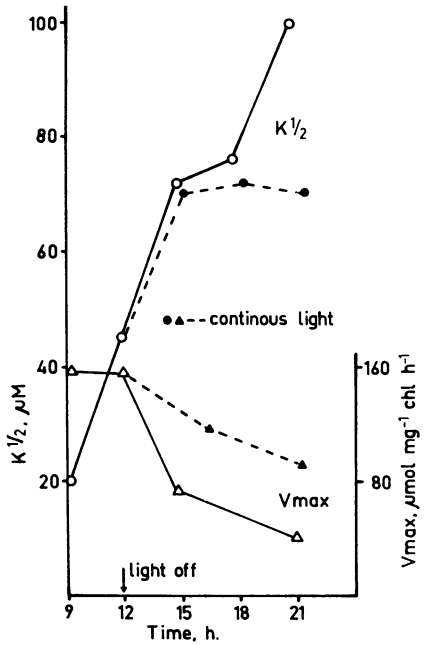

FIG. 4. The $K_{½}$ and V_{max} from synchronously grown culture as a function of time. Cells were kept either under the normal light/dark regime or under continuous light.

because we could not detect marked changes in the polypeptide composition of thylakoid membranes over the 24 h cycle (4). The extent of phosphorylation of thylakoid proteins, especially the LHC II polypeptides (25, 29, and 30 kDa), and some (yet unidentified polypeptides of 32 to 35 kDa, changed markedly during the cell cycle. *In vitro* phosphorylation of LHC II proteins reached a maximum after 8 to 9 h of light. It decreased to a minimum toward the end of the light period and was not altered during the dark period. Phosphorylation of thylakoid proteins has been shown to be involved in the State II-State I transitions leading to adaptation of the photosynthetic machinery to the spectra of light (2, 19). Thus, our data may suggest that the ability to perform state transitions is cell cycle dependent (see also Ref. 6).

It has been suggested that the activity of the LHC II kinase depends on the extent of reduction of the plastoquinone pool (19). The latter is strongly affected by the relative activities of the photosystems. There appears to be mutual dependence of phosphorylation capacity and PSII activity and they are in phase during the cell cycle. These changes in phosphorylation capacity, and PSII activity may explain the alterations in the photosynthetic rate at saturating CO_2 level (V_{max} in Fig. 1). It is unlikely, however, that the affinity for extracellular C_i (Fig. 1) and the photosynthetic rate at limiting (10 μM) C_i level ($V_{(10)}$, Fig. 2) are affected by the phosphorylation and PSII activities.

The $K_{1/2}$ and $V_{(10)}$ are strongly affected by the activity of the CO_2 concentrating mechanism (1, 9).

The daily changes in the apparent photosynthetic affinity may be attributed to the alterations in the ability to accumulate C_i internally (Fig. 2). CA activity which is thought to participate in the CO_2 concentrating mechanism by facilitating the supply of CO_2 (from bicarbonate) to the CO_2 carrier (9), also alternated rhythmically in a manner similar to that of $K_{1/2}$, $V_{(10)}$, V_{max}, and PSII (Fig. 2).

Figure 4 depicts data from experiments which were designed to test whether the alternations in the various activities (Figs. 1 and 2) are due to an endogenous signal (cell cycle or biological clock) or due to the light/dark cycle. The apparent photosynthetic affinity to extracellular C_i decreased ($K_{1/2}$ increased) toward the end of the light period and reached a minimum at the end of the dark period. This pattern was not affected by maintaining the cells under continuous light instead of entering the dark period. The same behavior was also observed for photosynthetic V_{max} (Fig. 4) and variable fluorescence (not shown). It is thus suggested that the decreasing apparent photosynthetic affinity to extracellular CO_2, toward the dark period, is due to an internal rhythm (biological clock or cell cycle) and not a result of the light regime as such. To distinguish between a response to a biological clock *versus* cell cycle we grew *Chlamydomonas* cells under the same light/dark cycle to the stationary phase. Cells

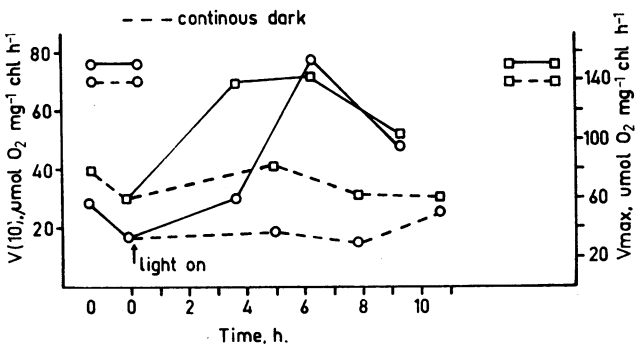

FIG. 5. The photosynthetic V_{max} and $V_{(10)}$ in synchronously grown cells as affected by time. Cells were kept under the normal light/dark regime or under continuous dark.

taken from the stationary phase were very active as far as C_i accumulation or photosynthetic V_{max} are concerned; however, they failed to exhibit the rhythmical alterations in $K_{1/2}$ and V_{max} (not shown). These data may indicate that it is the phase of the cell cycle which governs the response and not a biological clock directly as suggested in the case of *Gonyaulax* (13). It is possible, however, that the biological clock governs the cell cycle. Thus, at this time we cannot rule out the possibility that the biological clock is affecting the cell cycle and hence also the adaptation to CO_2 level.

It has been reported that light is required for the adaptation of nonsynchronously grown green algae and of cyanobacteria to low level of CO_2 (1, 7, 17). This dependence on light has been attributed to the effect of light on the level of photosynthesis/photorespiration metabolite which may be involved in the sensing of the low CO_2 environment (8, 17). We examined whether light is also required in the case of synchronously grown cultures. The photosynthetic V_{max}, the rate of photosynthesis at limiting concentrations of C_i ($V_{(10)}$), and CA activity increased following the onset of the light period (Figs. 1 and 2). This rise in V_{max}, $V_{(10)}$, and CA activity were light dependent as it was not observed when the cells were kept under an extended dark period instead of entering the light period (Fig. 5, not shown for CA). These data strongly indicate that light is required for the adaptation of the cells to the low Co_2 conditions. It is not clear, however, based on the data presented here, whether the dependence on light is due to the lack of formation of the photosynthesis/photorespiration product or due to the arrest of the cell cycle in the absence of the light (18). In the latter case the dependence on light would indicate that the cells must undergo a certain phase of the cell cycle to form the proteins involved in the adaptation to low CO_2 (7).

The activity of the "Co_2 concentrating mechanism" decreased toward the end of the light period (when synchronously grown *Chlamydomonas* cells approach mitosis, 15). At the end of the dark period the cells exhibited photosynthetic performance characteristics which resemble those observed in high CO_2 grown cells even though they were aerated continuously with low CO_2 concentration (air). Thus, contrary to the adaptation from high to low CO_2 level which requires the presence of low CO_2 signal (8, 17), adaptation to high CO_2 may occur at a certain phase of the cell cycle regardless of the CO_2 concentration present or the light/dark regime (Figs. 2 and 4).

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