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Influence of spectral range and carbon and nitrogen sources on oxygen evolution and Emerson enhancement in *Chlamydomonas reinhardtii*

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Abstract

Chlamydomonas reinhardtii was grown in medium with different carbon (acetate, CO_2, or both), and nitrogen (ammonium chloride, peptone, urea) sources and under light of different spectral composition. The light-dark cycles were found more suitable for mixotrophic growth than continuous irradiation. Both blue (BR) and red (RR) radiations decreased photosynthetic capacity of mixotrophic cells compared to "white light" (WL). Effect of RR was associated with photon distribution favouring photosystem 1 (PS1) suggesting increased cyclic phosphorylation. Mixotrophic growth in 10 mM NH_4Cl increased photosynthetic oxygen evolution compared to standard concentration of 5 mM NH_4Cl used for growing *C. reinhardtii*. Autotrophic growth stimulated the photosynthetic capacity compared to mixotrophic one. However, higher photosynthetic capacity was achieved for mixotrophic cells by growing them at high NH_4^+/K^+ ratio and high phosphate concentration.

Additional key words: ammonium chloride, CCCP, chlorophyll *a/b*, DCMU, mixotrophy, peptone, phosphates, photosystems 1 and 2, quality of radiation, urea.

Introduction

Chlamydomonas reinhardtii is the first organism, which was used for both site-directed mutagenenesis and detailed biochemical and biophysical characterization of oxygen-evolving photosystem 2 (PS2). It is an ideal model organism for investigation of structure-function relationships in photosynthetic oxygen evolution (Bumann and Oesterhelt 1994, Kerfeld and Krogmann 1998).

PS2 embedded in thylakoid membranes of higher plants, algae, and cyanobacteria uses photons to catalyze a series of electron transfer reactions resulting in splitting of water into molecular oxygen, protons, and electrons. Cytochrome b_6f complex functions in oxygenic photosynthetic membranes as the redox link between the photosynthetic reaction center complexes 2 and 1 and also functions in proton translocation (Cramer *et al.* 1996). Cyanobacteria and algae contain another cytochrome, low-potential c_{549}, which is not found in higher plants (Kerfeld and Krogmann 1998). This cytochrome has a structural role in PS2 and may contribute to anaerobic survival.

Algal photosynthetic apparatus easily adapts to radiation quality as well as to irradiance. Total pigment contents and ratio of various pigments depend on irradiance, light quality, and carbon source (Senger and Fleischhacker 1978). Kowallik and Schürmann (1984) found that O_2 production per total chlorophyll (Chl) amount of *Chlorella vulgaris* cells, grown in (BR) is greater than for those grown in (RR) and indicated that difference was more pronounced on Chl *a* basis. Humbeck and Senger (1984) showed that photosynthetic capacity of *Scenedesmus* cells grown at the same irradiance (2.5 W m^{-2}) was lower in BR than in RR (based on equal Chl content). Sueltemeyer *et al.* (1986) measured O_2 evolution and uptake by *C. reinhardtii* cells grown in ambient (0.034 % CO_2) or 5 % CO_2 enriched air. They concluded that non-cyclic electron flow from water to $NADP^+$ and pseudocyclic electron flow via PS1 to O_2 both significantly contribute to O_2 exchange in the light. They also suggested that the extra O_2 uptake by air-grown algae provides ATP required for the energy dependent CO_2/HCO_3^- concentrating mechanism that is

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Abbreviations: BR - blue radiation; Chl - chlorophyll; E - Emerson enhancement; KA - potassium acetate; P - O_2 evolution rate; PS - photosystem; RR - red radiation; WL - "white light"; α, β - fractions of radiant energy delivered to PS1 and PS2, respectively.
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present in these cells.

Elevated atmospheric CO_2 concentration often reduces stomatal conductance and transpiration and at the same time it stimulates photosynthetic rate and increases radiation use efficiency and water use efficiency (*e.g.* Drake *et al.* 1997). Miura *et al.* (1981) demonstrated that *C. reinhardtii* evolved H_2 repeatedly in an alternating light/dark cycles, and a temporal two-stage biophotolysis proved feasible by use of this green alga. Melis *et al.* (2000) reported that in *C. reinhardtii,* two-stage H_2 production method circumvents the severe O_2 sensitivity of the reversible hydrogenase by temporally separating photosynthetic O_2 and carbon accumulation (stage 1) from the consumption of cellular metabolites and concomitant H_2 production.

The present study is an attempt to manipulate some essential growth conditions to maximize growth of *C. reinhardtii* having high oxygen-evolution rates.

Materials and methods

Vegetative cells of *Chlamydomonas reinhardtii,* 137 C - mating type (+) were grown in a modified medium 2 of Sager and Granick (1953) containing 0.3 % potassium acetate as described by Weeks and Collis (1976). Medium 2 consisted of the following: 0.37 mM $FeCl_3 . 6 H_2O$, 5 mM NH_4Cl, 1.2 mM $MgSO_4 . 7 H_2O$, 1.7 mM Na citrate . 2 H_2O, 0.36 mM $CaCl_2$, 22 mM K-acetate (KA) . 3 H_2O, 10 mM K_2HPO_4, 10 mM NaH_2PO_4, and 1 cm^3 of micronutrients (H_3BO_3, $MnSO_4$, $(NH_4)_6Mo_7O_{24}$, $ZnSO_4$, $CuSO_4$, Na_2WO_4, KI, $Co(NO_3)$, $NiCl_2$, $V_2O_4SO_4$, and $NaSeO_4$).

Effect of spectral quality on O_2-evolution rates and Emerson enhancement (E) of cells grown under 14-h photoperiod: Cells were grown mixotrophically in potassium acetate medium with 3-N-morpholino-2-hydroxy propane sulfonic acid (MOPSO) at pH 6.8 for 8 d under equal irradiance of BR, RR, and WL (15 W m^{-2}) by enclosing each culture flask into a plastic filter (either blue or red). The rates of photosynthetic oxygen evolved from the whole cells were measured using a bare platinum electrode. The oxygen electrode used in this research was modified from that used by Joliot and Joliot (1968). The oxygen electrode consists of two compartments: the lower one is a bare platinum electrode (Pt disc 6 mm in diameter) upon which algae are deposited. The platinum is the cathode while a large-surface of silver ring, encircling the platinum cathode, is acting as the anode. The anode is about 3 mm from the edge of the platinum. Constituents of the experimental medium depended on the type of the experiment. However, the following constituents were constant in most of the experimental media: *a*) 20 mM MOPSO, *b*) 0.6 mM $MgSO_4$, *c*) 0.2 mM $CaCl_2$, and *d*) 1 M KCl. The measurement of oxygen evolution rate (P) was carried out in two steps as described by Canaani and Malkin (1984) and Dowidar (1990). Firstly, algal cells were induced into state 1 by irradiating them for 10 min by continuous 710 nm and modulated 650 nm. The P from this step was measured and defined as P_a. Secondly, the 710 nm beam was turned off for few seconds and the P was measured only at 650 nm alone and was defined as P_b. The 710/650 nm ratio was kept constant at the irradiances applied. Both Pa and Pb were computed per Chl amount [relative].

Emerson enhancement (E) was calculated as the ratio of the O_2 evolution rate P_a (710 + 650 nm) to P_b (650 nm). In the equation $E = \beta/\alpha$, α and β are fractions of the absorbed photon distribution to PS1 and PS2 respectively, and $\alpha + \beta = 1$ (Canaani and Malkin 1984).

Effect of carbon source on O_2 evolution rates and E: Cells were grown in two media buffered either with MOPSO or phosphate (10 mM K_2HPO_4 and 10 mM NaH_2PO_4). Cells were grown either photoautotrophically in an inorganic medium 2 bubbled with CO_2 (0.033 or 5 % CO_2 in air) or mixotrophically in 22 mM KA. Culture media containing KA were bubbled with CO_2-free air (air passed through KOH) to sweep any CO_2 produced in the medium. A third type of culture was buffered either with MOPSO or phosphate and grown in KA bubbled either with 0.033 or 5 % CO_2. Different partial pressures of CO_2 were prepared in the laboratory by mixing different volumes of 25 % CO_2 in air and pure air from *AIRCO* (Philadelphia, USA). The P and E were measured in above mentioned experimental media. The concentration of K_2CO_3 depended on the type of growing cells. Those grown in 0.033 and 5 % CO_2 contained 30 μM and 5 mM of K_2CO_3, respectively. CO_2 (0.033 or 5 %) was bubbled through the corresponding experimental medium during the experiment. Each experimental medium was adjusted at pH 6.8, after a steady state reached during preparation. In addition, P and E was measured after addition of 200 μM and after 10 min another 200 μM of KA to each experimental medium or in experimental medium containing 400 μM KA. 14-h photoperiod and WL was used for growing cells; otherwise a different radiation quality was mentioned.

Effect of uncouplers, nitrogen source, pH, NH_4^+/K^+ ratio, and Ca^{2+} on O_2 evolution rate: Three uncouplers were studied, two of which are inhibitors of photophosphorylation [3,4-dichlorophenyl-1,1-dimethyl urea (DCMU) and carbonyl cyanide m-chlorophenyl-hydrazone (CCCP)], and the third is an inhibitor of mitochondrial phosphorylation [dinitrophenol (DPN)]. The DCMU and CCCP were used in a concentration of

10 μM, while 200 μM DPN was used.

The following N sources in the experimental medium were studied: *1*) 6 mM urea, *2*) 0.5 % peptone, *3*) 0.2 mM NH_4Cl, *4*) 5 mM NH_4Cl, and *5*) 10 mM NH_4Cl. P and E were measured after 10 min of adding the N source.

C. reinhardtii cells were grown mixotrophically in NH_4 acetate (NH_4A) or KA media containing the nutrients listed above and buffered with MOPSO for NH_4A and with 3-N-morpholino-2-ethane sulfonic acid (MES), MOPSO, and phosphate buffer for KA media. The pH was adjusted to 6.2, 6.8 and 7.4 for MOPSO and phosphate and only to 6.8 for MES.

Cells were grown mixotrophically in three media containing different NH_4^+/K^+ ratios: 0.23 (control), 22, 27 in an attempt to improve their photosynthetic capacity. Each medium was buffered either with MOPSO or phosphate.

Both P_a and P_b were also measured in the presence or absence of Ca^{2+}. Exclusion of Ca^{2+} from the experimental medium was achieved by adding 0.1 mM ethylene glycol trichloroacetic acid (EGTA) to each medium.

Total Chl content was determined spectrophotometrically in 80 % acetone as described by Arnon (1949).

Results and discussion

Effect of radiation quality during growth on O_2 evolution rates and E: Cells of *C. reinhardtii* were grown mixotrophically in potassium acetate (KA) under equal irradiances of BR (400 - 510 nm), RR (> 600 nm), and WL (control) before being suspended in the experimental medium. BR-cells had significantly lower ($P < 0.01$) Chl *a/b* than those grown under RR (Fig. 1, Table 1). This difference was due to high Chl *b* concentration in cells grown under BR and high Chl *a* concentration in those grown under RR. The mean values of P_a and P_b are significantly lower ($P < 0.01$) in cells grown under BR than those under RR or WL. On the contrary, mean values of E and the fraction of photons delivered to PS2 (β) measured from cells grown under BR were significantly higher ($P < 0.01$) than those under RR and were significantly lower than those of cells grown under WL. The fraction of radiant energy delivered to PS1 (α) followed a pattern opposite to that observed for β. Hence the photosynthetic capacity of BR-cells was lower than that under RR and both cells had lower capacity than the control ones.

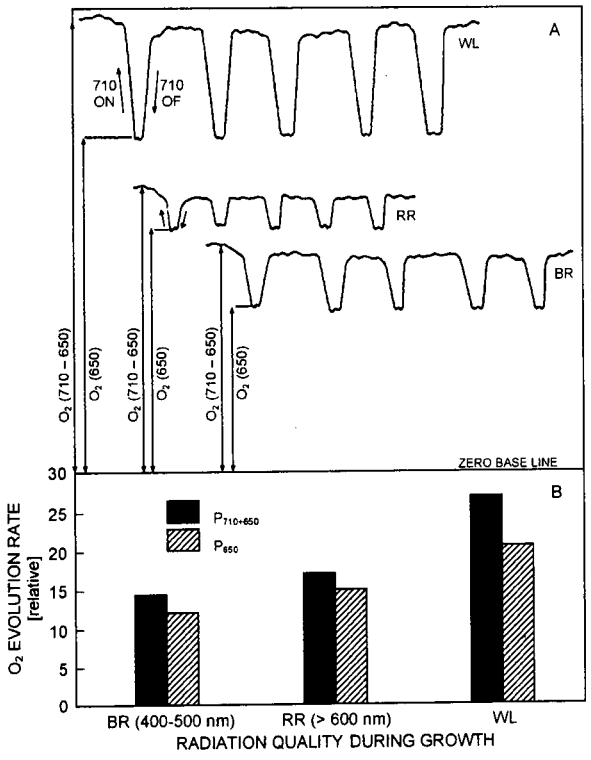

Fig. 1. *A* - Chart recorder tracing of oxygen-evolution rates, indicating Emerson enhancement, measured from cells grown mixotrophically under blue (400 - 510 nm) or red (> 600 nm) radiation and "white light". *B* - Effect of radiation quality, blue (400 - 510 nm) or red (> 600 nm) radiation and "white light" on oxygen-evolution rates (710 + 650 nm) and (650 nm), indicating Emerson enhancement, measured in cells grown mixotrophically.

The adaptation of *C. reinhardtii* 137C+ strain to BR and RR agrees with the adaptation of other algae to those radiation qualities. Such adaptation was reported for autotrophically grown strain 90 of *C. reinhardtii* and *Chlorella pyrenoidosa* (Hess and Tolbert 1967), for *Chlorella vulgaris* (Kowallik and Schürman 1984) and for *Scenedesmus obliquus* cells (Humbeck and Senger 1984). The decrease of Chl *a/b* ratio during growth in BR might be expected considering that the absorption band for Chl *b* is approximately 50 % greater than the absorption for Chl *a* between 400 and 500 nm (Fujita and Hattori 1962). Kowallik and Schürman (1984) reported that high Chl *b* concentration is necessary for an efficient function of PS2. Despite the high Chl *b* content and high value of photons transferred to PS2 (suggesting high photosynthetic capacity) of BR-cells their photosynthetic capacity was lower than those grown under RR. One possible explanation for this major change is that BR-stimulated O_2 uptake (Pickett and French 1967) may result in the consumption of some photosynthetically-evolved O_2 leading to an apparently lower photosynthetic rate. The effect of BR on the stimulation of oxygen uptake was further reported by Miyachi *et al.* (1978, 1980) in *C. vulgaris*. On the other hand, the value of α absorbed by RR-grown cells may result in an increased cyclic flow of PS1. Consequently, electron flow from PS2 may slow down oxygen evolution of these cells when compared to those grown under WL (lower α

value), even though RR contains both wavelengths required for the stimulation of PS2 and PS1. The high value of α fraction of cells grown under RR (suggesting an increased cyclic electron flow) may also account for the lower E value of such cells. This fast function of PS1 may resemble state 2 of cells grown under RR when compared to those grown under both WL and BR. The situation with the BR cells is somewhat complicated. Cells grown under BR may be characterized by high E and high oxygen rate (Canaani and Malkin 1984). The BR-simulated O_2 uptake, mentioned earlier, may explain the reason for non-fulfillment of the second character of state 1. This latter speculation is more probable to account for reduced O_2 yield of cells grown under BR than to speculate about high energy transfer from PS2 to PS1.

Cells grown under RR (Wilhelm *et al.* 1985) may hinder energy transfer from PS2 to PS1. This in turn may result in the distribution of photons in favour of PS2, higher β of BR-grown cells than those grown under RR, leading to higher oxygen yield. The latter may not be detected due to the BR-stimulated oxygen uptake. The lower oxygen yield of *C. reinhardtii* cells grown under BR, compared to those grown under RR, agrees with the photosynthetic rates of *Scenedesmus* cells grown under BR (Humbeck and Senger 1984) and with fluorescence results of Kowallik and Schürman (1984). The latter authors, however, did not interpret their high fluorescence of PS2 to indicate low photosynthetic capacity of cells grown under BR.

The third major change observed as a result of *C. reinhardtii* adaptation to different radiation qualities is the reduced total productivity of cultures grown under BR compared to those grown under RR and WL. This result is consistent with the work of Kubín *et al.* (1983) who found that *C. vulgaris* cells grown under BR (400 - 510 nm) had the slowest production rates and consequently the lowest total dry mass, compared to those grown under green radiation or WL.

Effect of carbon source on O_2-evolution rate and E: Both P_a and P_b measured in cells grown under different carbon sources can be arranged in descending order as follows (Fig. 2*A*, Table 2): P of cells grown in 5 % CO_2 > air > air + 200 or 400 μM KA > 5 % CO_2 + 200 or 400 μM KA > *P* of cells grown in KA. Values of E and β followed a different pattern from that observed for P. Generally, α usually followed the opposite pattern to β based on the mathematical assumption used by Canaani and Malkin (1984) (α + β = 1). The difference between mean values of E or β under different carbon sources was highly significant.

The present data agree with those of Wiessner (1969) who found that photosynthetic capacities of autotrophically grown *Chlamydobotrys stellata*, *Chlamydomonas*

Table 1. Effect of radiation quality during growth on Chl *a*/*b* ratio, O_2 evolution rate [mmol(O_2) g^{-1}(Chl) s^{-1}], Emerson enhancement (E), and fractions of radiant energy delivered to PS1 (α) or PS2 (β) of *C. reinhardtii* cells grown mixotrophically under 14-h photoperiod. BR - blue radiation, RR - red radiation, WL - "white" light. Means ± SE. In all cases $P < 0.01$.

	Chl *a*/*b*	O_2 (710+650 nm)	O_2 (650 nm)	E	β	α
BR	1.81 ± 0.10	14.10 ± 0.40	11.74 ± 0.38	1.200 ± 0.004	0.545 ± 0.0010	0.455 ± 0.0010
RR	3.48 ± 0.06	16.62 ± 0.21	14.39 ± 0.25	1.148 ± 0.003	0.535 ± 0.0010	0.465 ± 0.0010
WL	2.24 ± 0.05	26.17 ± 0.30	29.83 ± 0.70	1.319 ± 0.004	0.569 ± 0.0007	0.431 ± 0.0007
F	204	9002	300	591	441	441
$LSD_{0.05}$	0.195	0.940	1.430	0.012	0.003	0.003

Table 2. Effect of KA on Chl *a*/*b* ratio, O_2 evolution rate [mmol(O_2) g^{-1}(Chl) s^{-1}], Emerson enhancement (E), and fractions of radiant energy delivered to PS1 (α) or PS2 (β) of *C. reinhardtii* cells grown in air and 5 % CO_2 under 14-h photoperiod. Means ± SE. In all cases $P < 0.01$.

	Chl *a*/*b*	O_2 (710+650 nm)	O_2 (650 nm)	E	β	α
Air	2.03 ± 0.10	46.68 ± 0.50	38.33 ± 0.12	1.27 ± 0.007	0.560 ± 0.0005	0.440 ± 0.0005
Air + 200 μM KA	-	38.89 ± 0.20	30.38 ± 0.09	1.28 ± 0.006	0.561 ± 0.0005	0.439 ± 0.0005
Air + 400 μM KA	-	38.98 ± 0.33	30.46 ± 0.27	1.28 ± 0.008	0.561 ± 0.0006	0.439 ± 0.0006
5 % CO_2	2.00 ± 0.05	58.53 ± 0.70	44.67 ± 0.42	1.31 ± 0.010	0.567 ± 0.0010	0.433 ± 0.0010
5 % CO_2 200 μM KA	-	31.11 ± 0.20	25.71 ± 0.05	1.21 ± 0.006	0.547 ± 0.0004	0.453 ± 0.0004
5 % CO_2 200 μM KA	-	31.30 ± 0.60	25.79 ± 0.18	1.21 ± 0.010	0.548 ± 0.0010	0.452 ± 0.0010
KA	2.24 ± 0.07	26.10 ± 0.31	18.75 ± 0.11	1.32 ± 0.004	0.569 ± 0.0004	0.431 ± 0.0004
F	43	1462	1628	251	270	270
$LSD_{0.05}$	0.060	0.990	0.690	0.013	0.003	0.003

mundana, *C. vulgaris*, and *Scenedesmus obliquus* are higher than those of the same mixophically grown cells. He concluded that mixotrophic growth promotes acetate assimilation and retards photosynthetic capacity. This reported retardation of photosynthetic capacity, which was also observed from O_2 evolution rate of mixotrophically grown *C. reinhardtii*, could be due to two factors; Firstly, some of the photosynthetically evolved oxygen may be used up for the reoxidation of reduced pyridine nucleotides, resulting from acetate assimilation. Secondly, the high acetate concentration usually used for growing algae, including *C. reinhardtii*, may inhibit the oxygen evolution system (OES) (Saygin *et al.* 1986).

We found that cells grown in air had 16.83 % lower photosynthetic capacity (P_a) compared to the CO_2 grown ones. Higher P of CO_2-grown cells was reported for *Chlamydomonas segnis* by Badour and Kim (1986) and for *C. reinhardtii* by Spalding *et al.* (1984). The latter researchers found that photosynthetic capacity of air grown cells is 18.27 % lower than that of CO_2 grown cells.

The carbon source affects Chl *a*/*b* ratio significantly ($P < 0.01$) (Table 2). The difference between mean values of Chl *a*/*b* in cells grown autotrophically and mixotrophically was highly significant, when both types of cultures are buffered either with MOPSO or phosphate. Mean values of Chl *a*/*b* determined from 5 % CO_2+KA cultures buffered either with MOPSO or phosphate were significantly higher ($P < 0.05$) than those of 5 % CO_2 and KA cultures buffered with the corresponding buffer.

Mean values of Chl/DM (chlorophyll per dry mass unit) determined in mixotrophic cultures buffered either with MOPSO or phosphate were significantly lower ($P < 0.05$) than those of air and 5 % CO_2 cultures. Mean values of Chl/DM determined in 5 % CO_2+KA were significantly lower than those of air and 5 % CO_2 cultures. Generally, mixotrophic cultures have lower Chl/DM than those of air and 5 % CO_2 autotrophic cultures. Presence of acetate and 5 % CO_2 decreased Chl/DM of the 5 % CO_2 cultures but did not result in any significant decrease in cells grown in air. Higher photosynthetic capacity of cells grown autotrophically is associated with higher Chl *b* concentration and higher total Chl content. The high concentration of Chl *b* may be required to secure an efficient function of PS2 (Kowallik and Schürmann 1984). High content of Chl (*a*+*b*) may be required for an efficient capturing of photons necessary for higher photosynthetic capacity.

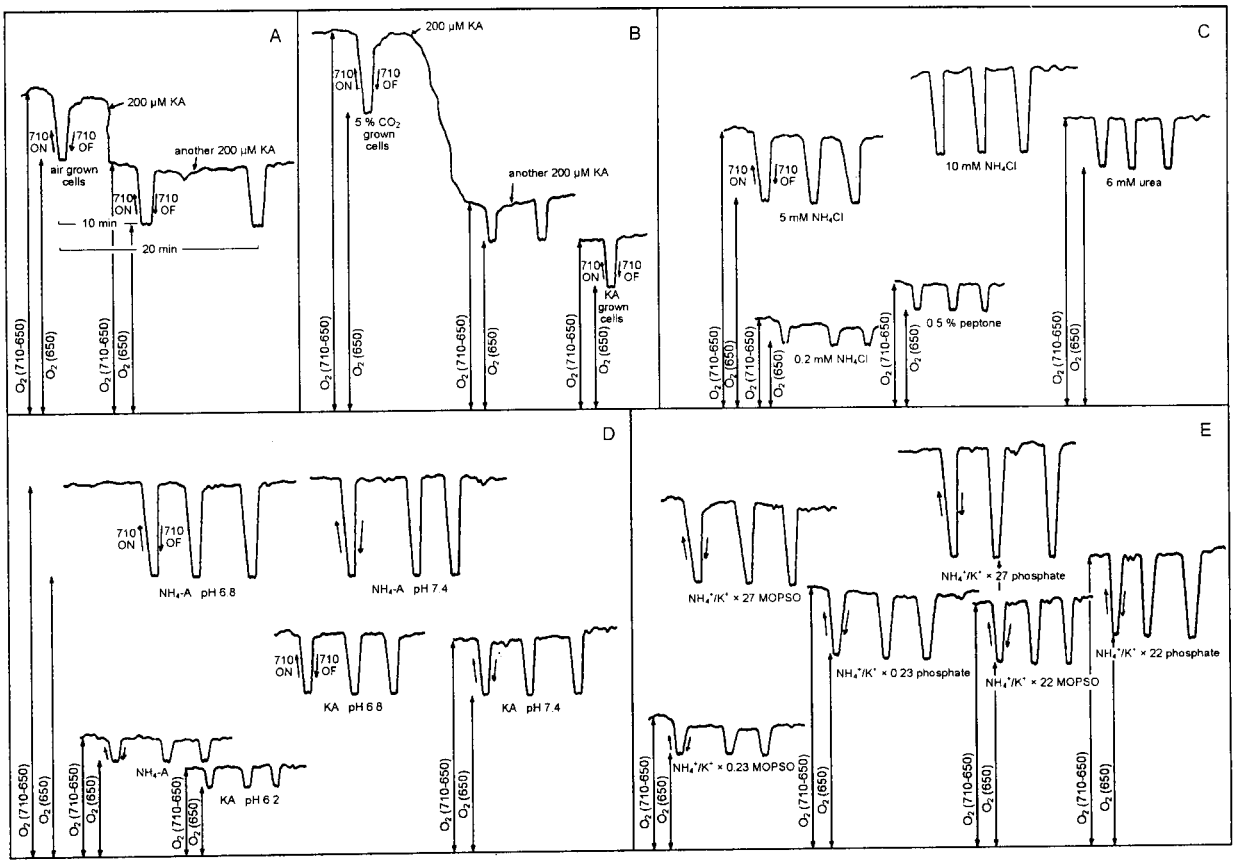

Fig. 2. Chart recorder tracing of oxygen-evolution rates, indicating Emerson enhancement, measured from cells grown under various treatments: *A* - air, potassium acetate (KA); *B* - 5 % CO_2, KA; *C* - mixotrophy, KA; *D* - mixotrophy, ammonium acetate (NH_4A) or KA, pH 6.2-7.4; *E* - mixotrophy, different NH_4^+/K^+ MOPSO or phosphate, pH 6.8. Sensitivity on the lock-in 10 (*C*), 20 (*A*, *B*, *D*), or 30 (*E*) mV. Chart speed 0.167 mm s^{-1}, applied voltage 800 mV, $I_{710/650} = 3$, frequency of the chopper 8 Hz.

Effect of uncouplers, nitrogen source, pH, NH_4^+/K^+ ratio and Ca^{2+} on O_2 evolution rate: Both photosynthetic uncouplers, CCCP and DCMU, abolished P_a in 15 min. The mitochondrial uncoupler DNP did not result in any significant effect on P_a, P_b, E, β, and α at the three carbon sources used (Table 3). The source of ATP required for acetate metabolism in *C. reinhardtii* was photosynthetic phosphorylation, specifically noncyclic, and not mitochondrial. This conclusion was based on results showing that both CCCP, an inhibitor of Hill reaction (Izawa 1977), and DCMU inhibited O_2 production from photoassimilation of acetate, while DNP did not.

Different nitrogen sources affected Chl *a/b* ratio (Table 4). Mean Chl *a/b* determined from cells grown in medium containing 0.2 mM NH_4Cl was significantly higher ($P > 0.05$) than those from media containing 6 mM urea, 10 mM NH_4Cl and control. Mean values of Chl *a/b* ratio from the latter three media were not significantly different. Mean value of Chl *a/b* determined from cells grown in 5 % peptone medium was not significantly different from that of 0.2 mM NH_4Cl medium. The higher mean values of Chl *a/b* determined for cells grown in 0.2 mM NH_4Cl and 5 % peptone are due to lower concentrations of Chl *b* compared to those of cells grown in control medium, 10 mM NH_4Cl, and 6 mM urea. The high concentration of Chl *b* determined from cells grown in the latter three media was associated with their high *P*. The difference between mean values of P_a measured from cells grown in different nitrogen sources was highly significant. Mean values of P_a measured in cells grown in media containing the N sources mentioned earlier can be arranged in a descending order, according to their media, as follows: 10 mM NH_4Cl > 6 mM urea > 5 mM NH_4Cl > 0.5 % peptone > 0.2 mM NH_4Cl. Mean values of P_b followed a pattern similar to that for P_a. The increased photosynthetic capacity with 10 mM NH_4Cl may be due to role of ammonia in the stimulation of electron flow (Izawa 1977). Since no phosphorylation will occur without electron flow, the increased NH_4Cl from 5 to 10 mM may enhance ATP production required for KA activation. Consequently, acetate incorporation will be enhanced leading to higher photosynthetic capacity. The

Table 3. Effect of uncouplers (0.01 mM CCCP, 0.01 mM DCMU, 0.2 mM DNP) on O_2 evolution rate [mmol(O_2) g^{-1}(Chl) s^{-1}], Emerson enhancement (E), and fractions of radiant energy delivered to PS1 (α) or PS2 (β) of *C. reinhardtii* cells grown in KA, 5 % CO_2 and air under 14-h photoperiod. Means ± SE. In all cases $P < 0.01$.

Parameter	Uncoupler	KA	5 % CO_2	Air	*F*	$LSD_{0.05}$
Chl *a/b*		2.24 ± 0.070	2.00 ± 0.100	2.03 ± 0.05	42.63	0.06
O_2 (710+650 nm)	before CCCP	26.11 ± 0.300	58.53 ± 0.043	48.71 ± 0.60	14.62	0.99
	after CCCP	0	0	0		
	before DCMU	26.12 ± 0.310	58.06 ± 0.690	48.76 ± 0.52	1600	0.99
	after DCMU	0	0	0		
	before DNP	26.07 ± 0.310	58.53 ± 0.520	48.78 ± 0.38	1600	0.99
	after DNP	26.07 ± 0.310	58.53 ± 0.520	48.78 ± 0.68	1452	0.99
O_2 (650 nm)	before DNP	19.75 ± 0.310	44.68 ± 0.240	38.33 ± 0.41	1628	0.69
	after DNP	19.75 ± 0.310	44.68 ± 0.240	40.22 ± 0.41	1628	0.69
E	before DNP	1.319 ± 0.007	1.31 ± 0.009	1.27 ± 0.005	251	0.013
	after DNP	1.319 ± 0.010	1.31 ± 0.008	1.27 ± 0.004	251	0.013
β	after DNP	0.569 ± 0.001	0.567 ± 0.0006	0.56 ± 0.0005	270	0.003
α	after DNP	0.431 ± 0.001	0.433 ± 0.0006	0.44 ± 0.0005	270	0.003

Table 4. Effect of nitrogen source on Chl *a/b* ratio, O_2 evolution rate [mmol(O_2) g^{-1}(Chl) s^{-1}], Emerson enhancement (E), and fractions of radiant energy delivered to PS1 (α) or PS2 (β) of *C. reinhardtii* cells grown in KA under 14-h photoperiod. Means ± SE.

	Chl *a/b*	O_2 (710+650 nm)	O_2 (650 nm)	E	β	α
NH_4Cl 0.2 mM	2.50 ± 0.15	9.24 ± 0.20	7.86 ± 0.17	1.20 ± 0.005	0.546 ± 0.0010	0.454 ± 0.0010
NH_4Cl 5.0 mM	2.25 ± 0.05	26.13 ± 0.29	19.77 ± 0.13	1.32 ± 0.003	0.569 ± 0.0005	0.431 ± 0.0005
NH_4Cl 10.0 mM	2.13 ± 0.12	31.58 ± 0.30	23.19 ± 0.58	1.36 ± 0.003	0.577 ± 0.0006	0.423 ± 0.0006
Peptone 0.5 %	2.40 ± 0.08	12.76 ± 0.24	10.87 ± 0.10	1.20 ± 0.007	0.545 ± 0.0010	0.455 ± 0.0010
Urea 6 mM	2.22 ± 0.09	26.54 ± 0.40	21.93 ± 0.09	1.21 ± 0.003	0.548 ± 0.0006	0.452 ± 0.0006
F	3.63	472.68	780.27	345.00	344. 89	344.89
P	< 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
$LSD_{0.05}$	0.237	0.400	0.840	0.013	0.003	0.003

opposite was true with 0.2 mM NH_4Cl. The lower photosynthetic capacity of cells grown in 6 mM urea may be attributed to increased cyclic phosphorylation of PS1 in such cells. This increased cyclic phosphorylation may be a requirement for high ATP concentration which was found in *C. reinhardtii* (Pineda and Cardenas 1985) as an energy-dependent urea uptake. This suggested increased cyclic phosphorylation is a third example demonstrating how the two photosystems of this organism adapt to growth conditions. In cells grown in 10 mM NH_4Cl, the high total Chl concentration may ensure an efficient capturing of photons required for their high photosynthetic capacity. This in turn will lead to higher production of ATP and NADPH.

The E followed a different pattern from that observed for both P_a and P_b. Mean E measured in cells grown in 10 mM NH_4Cl medium was the highest followed by that of control. Mean values of E measured in cells grown in 6 mM urea, 5 % peptone, and 0.2 mM NH_4Cl media were not significantly different but they were lower than those measured in the control and the 10 mM NH_4Cl medium. Mean values of β followed the same pattern observed for E, $E = \beta/\alpha$, while α followed the opposite pattern. The difference between mean values of α or β calculated for cells grown in media containing different N sources was highly significant. Thus the initial radiant energy distribution favouring PS2 (reflected in mean value of β) was significantly higher in cells grown in 10 mM NH_4Cl and control compared to those in cells grown in 6 mM urea, 0.5 % peptone, and 0.2 mM NH_4Cl media.

On the contrary, radiant energy distribution favoring PS1 (reflected in mean value of α) was higher in cells grown in 6 mM urea, 0.5 % peptone, and 0.2 mM NH_4Cl media, compared to those in 10 mM NH_4Cl and control. The distribution of radiant energy favouring either one of the two photosystems may be a prerequisite for the production of specific proportion of the light reaction intermediates. The latter are required for specific carbon assimilation products necessary for growth.

At the pH range from 6.2 to7.4 P_a, P_b, E and β, followed the same pattern (Fig. 2B, Table 5) while α followed the opposite pattern. Radiant energy distribution favouring PS2, as manifested in β value, was higher in cells grown in NH_4A than in KA medium, but mean values of β calculated for cells grown either in NH_4A or KA at pH 6.8 did not differ from those at pH 7.4, but they were higher than those at pH 6.2. Rate of electron transport depends on both the internal and external pH (Rottenberg 1975). The rate of electron transport may be slower at pH 6.3 resulting in slower ATP production. In turn, this may lead to decreased acetate incorporation leading to low P. The low P observed in *C. reinhardtii* at low pH value agrees with results of Coleman and Colman (1981) with *Coccochloris*. Mean values of α can be arranged in a descending order according to their media as follows: pH 6.2 KA > pH 6.2 NH_4A > pH 6.8 or pH 7.4 KA > pH 6.8 or pH 7.4 NH_4A.

Cells grown in media containing NH_4^+/K^+ ratio 0.23 (control) and buffered either with MOPSO or phosphate had significantly higher ($P < 0.01$) Chl *a/b* ratio than those grown in media with this ratio 22 or 27 (Table 6). Mean values of Chl *a/b* determined in cells grown in media with 22 or 27 ratio were not significantly different. The lower mean values of Chl *a/b*, indicating higher concentrations of Chl *b*, coincided with higher P of cells grown in media with NH_4^+/K^+ ratio of 22 and 27 compared to control (Fig. 2*E*). The P_a, P_b, E and β followed the same pattern but α behaved differently. Cells grown in media with NH_4^+/K^+ ratio of 0.23 had significantly lower ($P < 0.01$) mean values of P_a than that with 22 ratio and especially of 27 ratio (Table 6). Mean values of P_b can be arranged in a descending order according to their NH_4^+/K^+ ratio as follows: 27 and phosphate > 27 and MOPSO > 22 and phosphate > 22 and MOPSO > 0.23 and phosphate > 0.23 and MOPSO. Mean values of E or β calculated for cells grown in $NH_4^+/K^+ = 0.23$ medium buffered with MOPSO were significantly lower ($P < 0.01$) than those in the 22 and 27 media. Mean values of E or β calculated for cells grown in cultures buffered with phosphate can be arranged descending according to their NH_4^+/K^+ ratio as follows: $NH_4^+/K^+ = 27 > NH_4^+/K^+ = 22 > NH_4^+/K^+ = 0.23$.

Table 5. Effect of pH on O_2 evolution rate [mmol(O_2) g^{-1}(Chl) s^{-1}], Emerson enhancement (E), and fractions of radiant energy delivered to PS1 (α) or PS2 (β) of *C. reinhardtii* cells grown in NH_4A or KA under 14-h photoperiod. Means ± SE. In all cases $P < 0.01$.

Treatment		O_2 (710+650 nm)	O_2 (650 nm)	E	β	α
pH 6.2	NH_4A	13.74 ± 0.05	11.78 ± 0.10	1.166 ± 0.007	0.538 ± 0.0010	0.462 ± 0.0010
	KA	9.73 ± 0.03	8.59 ± 0.06	1.132 ± 0.006	0.531 ± 0.0010	0.469 ± 0.0010
pH 6.8	NH_4A	46.59 ± 2.20	33.65 ± 0.08	1.385 ± 0.002	0.581 ± 0.0005	0.419 ± 0.0005
	KA	26.16 ± 0.40	19.83 ± 0.25	1.319 ± 0.004	0.569 ± 0.0009	0.431 ± 0.0009
pH 7.4	NH_4A	46.67 ± 0.83	33.17 ± 0.53	1.380 ± 0.003	0.579 ± 0.0006	0.421 ± 0.0006
	KA	25.96 ± 0.92	19.71 ± 0.65	1.317 ± 0.007	0.568 ± 0.0010	0.432 ± 0.0010
F		857	849	745	457	457
$LSD_{0.05}$		1.21	1.10	0.015	0.003	0.003

Table 6. Effect of ammonium/potassium ratio on Chl *a*/*b* ratio, O_2 evolution rate [mmol(O_2) g^{-1}(Chl) s^{-1}], Emerson enhancement (E), and fractions of radiant energy delivered to PS1 (α) or PS2 (β) of *C. reinhardtii* cells grown in KA under 14-h photoperiod. Means ± SE. In all cases $P < 0.01$.

NH_4^+/K^+	Chl *a*/*b*	O_2 (710+650 nm)	O_2 (650 nm)	E	β	α
0.23 MOPSO	2.25 ± 0.053	26.01 ± 0.39	19.71 ± 0.41	1.32 ± 0.010	0.569 ± 0.0004	0.431 ± 0.0004
0.23 phosphate	2.10 ± 0.057	49.68 ± 0.43	35.73 ± 0.50	1.39 ± 0.004	0.582 ± 0.0005	0.418 ± 0.0005
22.00 MOPSO	1.89 ± 0.066	46.66 ± 0.60	33.30 ± 0.20	1.39 ± 0.008	0.582 ± 0.0007	0.418 ± 0.0007
22.00 phosphate	1.81 ± 0.100	53.57 ± 0.63	37.02 ± 0.38	1.41 ± 0.010	0.585 ± 0.0004	0.415 ± 0.0005
27.00 MOPSO	1.86 ± 0.050	71.81 ± 0.23	50.92 ± 0.30	1.41 ± 0.009	0.585 ± 0.0005	0.415 ± 0.0005
27.00 phosphate	1.80 ± 0.070	77.97 ± 0.53	54.52 ± 0.23	1.43 ± 0.006	0.589 ± 0.0010	0.411 ± 0.0010
F	7.57	839	699.5	748	820.5	820.5
$LSD_{0.05}$	0.198	1.15	0.40	0.019	0.002	0.002

Cells grown in medium containing NH_4^+/K^+ ratio of 27, buffered with phosphate, had the highest mean values of β and lowest α. However, cells grown in medium containing NH_4^+/K^+ ratio of 0.23 buffered with MOPSO had the highest mean values of α and lowest values of β. Thus the growth of *C. reinhardtii* in high NH_4^+/K^+ ratio resulted in 3.5 % increase in radiant energy distribution favoring PS2 compared to control (5 mM NH_4Cl). This increase paralleled their high Chl *b* content and O_2 evolution rate. The high Chl *b* concentration associated with high NH_4^+/K^+ ratio may ensure an efficient photosynthetic capacity (Kowallik and Schürmann 1984).

Also one of the factors that may account for such high photosynthetic capacity is the stimulatory effect of NH_4 on electron flow from PS2 to PS1. The latter may speed up electron flow and consequently high ATP production in the presence of high inorganic P. This may result in enhanced acetate activation and incorporation, leading to higher photosynthetic capacity.

High total Chl/DM detected with high phosphate and high NH_4^+/K^+ ratio may assure efficiency of cells in capturing radiant energy, which appears to be distributed in favour of PS2 (high β). This may result in higher P providing higher concentrations of ATP and NADPH.

Mean values of P_a determined before adding EGTA were 27.05, 50.10 and 60.20 mmol(O_2) g^{-1}(Chl) s^{-1} for mixotrophic, air, and 5 % CO_2 grown cells. The latter two types of cells were used as control. Values of P_a were abolished completely, after 10 min of adding EGTA. Many researchers suggest a role for Ca^{2+} in the O_2 evolving part of PS2. In this regard, Ono and Inoue (1983) showed that when the chloroplasts were treated with EGTA prior the reactivation attempts, Mn^{2+} alone could not affect recovery. Barr *et al.* (1983) identified a calcium-selective site of action between PS1 and PS2. This effect involved binding of calcium to (presumably) protein carboxyl group. Brand and Becker (1984) reported that calcium may function directly in several aspects of photo-synthesis. Some evidence supports a calcium function in the water-splitting complex, and other evidence indicates a reaction center function in PS2. Calcium in reaction center 2 may be tightly bound in chloroplasts. The latter reviewers revealed that many reports suggest that Ca^{2+} may relate to the function of a 23 kD protein on the oxidizing side of PS2.

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