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Light-saturated photosynthesis – limitation by electron transport or carbon fixation?

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The minimal turnover time, τ, for in vivo electron transport from water to CO_2, was calculated from oxygen flash yields and steady-state light-saturated photosynthetic rates in the marine chlorophyte, *Dunaliella tertiolecta*, cultured at different growth irradiance levels. As cells adapted to lower growth irradiance levels, τ increased from 3.5 to 14.5 ms, in parallel with increases in the contents of chlorophyll *a*, Photosystem II, PQ, cytochrome $b_6 f$, Photosystem I and thylakoid surface density. Thus, at all growth irradiance levels examined, the relative proportion of these membrane-bound electron-transport components remained constant. However, the cellular pool size of ribulose-1,5-bisphosphate carboxylase/oxygenase, determined by radioimmunoassay, was independent of growth irradiance. Hence the ratio of the enzyme to electron-transport chain components varied between 4.8 and 1.2 as a function of growth irradiance levels. The change in this ratio was related quantitatively to the minimal turnover time of electron transport from water to carbon dioxide. Taking into account thylakoid surface density, cellular contents of electron-transport components and diffusion coefficient of plastoquinol, a diffusion time of 2.3 ms was calculated for transport of PQH_2 from Photosystem II to cytochrome $b_6 f$. This rate is 1.5- to 13-times faster than τ. The data strongly suggest that under nutrient saturated conditions the absolute rate of light-saturated photosynthesis is limited by carbon fixation rather than electron transport. It is predicted, however, that in cells grown above 3000 μmol quanta per m^2 per s, electron transport rather than carbon fixation would become the rate-limiting step of light saturated photosynthesis.

Introduction

In all photosynthetic organisms the initial rate of photosynthesis can be increased by increasing the light intensity. At some finite photon flux density, however, photosynthesis becomes light saturated, and a maximal rate (P_{max}) is obtained. Light-saturated photosynthetic rates are independent of the absorption cross section of the photosynthetic apparatus, and are related empirically only to the concentration of photosynthetic units (n) and their minimal turnover time (τ) [12]. Thus:

$$P_{max} = \frac{n}{\tau}$$

In unicellular algae the absolute values of P_{max} vary by an order of magnitude, from 0.2 to over 2.0 μmol O_2 μg per Chl per h [3]. While the

Abbreviations: PS I, Photosystem I; PS II Photosystem II; RC I, reaction center of Photosystem I; RC II, reaction center of Photosystem II; Chl, chlorophyll; PQ, plastoquinone; PQH_2, plastoquinol; PMSF, phenylmethylsulfonyl fluoride.

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absolute values of P_{max} depend on the species in question, within a given species three major factors are known to influence P_{max}: (a) temperature, (b) nutrient status, and (c) growth irradiance regime. There are complex relationships between temperature and light-saturated photosynthetic rates in algae and higher plants. P_{max} obeys an Arrhenius function with a Q_{10} of about 1.8 and shows typical thermal inhibition above a temperature optimum, which varies with growth conditions [4]. In addition, the light intensity required to saturate photosynthesis (I_k) increases as the temperature is raised. For decades algal physiologists have taken the effect of temperature on P_{max} to imply that light-saturated photosynthetic rates are primarily limited by an enzymatic step. The effect of nutrient limitation on P_{max} is somewhat dependent on the nutrient in question. Limitation by inorganic carbon obviously affects P_{max} because it is a substrate of the photosynthetic process. However, if nitrogen is limiting, the synthesis of the proteins and chlorophylls (and hence components of the photosynthetic apparatus) is reduced. Phosphate supply affects the rate of synthesis of nucleic acids [5]. Both nitrogen and phosphate-starved cells have reduced chlorophyll concentrations. In general, nutrient limitation (other than by inorganic carbon) imposes a reduction in the cellular concentration of pigments, photosynthetic units (n), and Calvin cycle enzymes. Consequently, a decrease in the light-saturated photosynthetic rate is found.

In contrast to the effects of temperature and nutrients, the effect of growth irradiance on P_{max} is curious. Cells grown at high irradiance levels have less cellular chlorophyll, generally fewer photosynthetic units and a higher maximal photosynthetic rate per unit chlorophyll than cells grown at low irradiance levels [1,6–8]. It would appear that the average turnover time of the photosynthetic apparatus, τ, varies with growth irradiance. Cells with fewer photosynthetic units have faster turnover times and vice versa [1,9]. The change in turnover time of the photosynthetic apparatus suggests that under nutrient replete conditions photosynthetic electron flow between PS II and PS I, and/or the rate of carbon fixation varies with growth irradiance level.

We hypothesized that changes in τ under nutrient-saturated growth conditions may be reflected by a change in the pool size of one or more components in the photosynthetic apparatus. We report here on the effects of growth irradiance levels on pool sizes of a number of components of the photosynthetic electron-transport chain as well as ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) in the marine chlorophyte, *Dunaliella tertiolecta*. We relate here changes in the pool size of the electron-transport components and Rubisco to the minimal turnover time of the photosynthetic apparatus, in order to identify the likeliest component limiting P_{max}. Our results suggest that, at light saturation, electron transport from water to carbon dioxide is limited by the concentration of Rubisco per electron-transport chain.

Materials and Methods

D. tertiolecta (Woods Hole clone DUN) was maintained at 18 °C under steady-state growth conditions in 3.2 l turbidostats with natural sea water enriched with f/2 nutrients as previously described [10]. Light was provided continuously by banks of VHO fluorescent tubes and cells were grown at five irradiance levels: 80, 200, 700, 1200 and 1900 μmol quanta per m^2 per s. The cultures were kept optically thin ((3–5) 10^5 cells/ml) to minimize self shading. Growth irradiance levels were measured in the turbidostats with a Biospherical QSL100 scalar quantum sensor (400–700 nm). At all growth irradiance levels nutrient concentrations were saturating. The pH of the sea water was buffered at 7.8 and the cells were continuously bubbled with air. During steady-state growth at each light level the cultures were sampled for pigment content, oxygen flash yields, and photosynthetic characteristics. In addition, large volume samples (about 2.0 l) were concentrated by continuous centrifugation, flash frozen in liquid N_2 and stored at −60 °C for analysis of electron-transport components and chloroplast proteins.

Photosynthesis pigments were extracted by homogenizing samples filtered on glass fiber filters in 90% acetone. Corrected spectra were recorded on an Aminco DW-2a spectrophotometer and chlorophylls *a* and *b* were calculated using the equations of Jeffrey and Humphrey [11]. Caro-

tenoid concentrations were calculated using an extinction coefficient of 2500 $mM^{-1} \cdot cm^{-1}$ at 480 nm [12].

Oxygen flash yields were measured with a Rank Brothers electrode using single turnover flashes provided by three synchronized GenRad Strobos-lave 1539A xenon flash bulbs. Oxygen evolution was measured at flash frequencies of 10, 20, 30 and 40 s^{-1} and flash yields were calculated from linear regression analysis of flash frequency and the corresponding rate of oxygen production. Care was taken to ensure that flashes were saturating and the addition of a weak far-red (720 nm) background source had no effect on calculated yields. Oxygen flash yields were normalized to chlorophyll concentrations (PSU_{O_2}, the so-called Emerson and Arnold numbers, see Ref. 9). The cellular concentration of RC II was calculated from cellular chlorophyll concentrations and the flash yields assuming that each oxygen produced required four light-driven one-electron oxidation steps, each with a quantum yield of unity [1].

Photosynthesis rates in continuous light were measured with a YSI 5331 Clark-type polarographic oxygen electrode in a specially designed thermostated PVC chamber [10]. The chamber has an optical path of 1 cm through the sample and is designed with a flat face to ensure even illumination of the sample. A collimated beam was provided from a 150 W tungsten halogen source filtered through a 'hot' dichroic mirror to remove infrared. Irradiance levels up to 3000 μmol quanta per m^2 per s could be achieved and light was attenuated to desired intensities with neutral density filters. Fresh, unconcentrated samples were taken from the turbidostat and gently degassed with N_2 to between 80 and 90% of O_2 saturation. Dark respiration was measured prior to illumination in the chamber. The samples were exposed to 12 sequentially increasing irradiance levels, each lasting 10 min, until no further increase in the rate of oxygen production was observed. This rate was entirely independent of additional $NaHCO_3$ and we have no evidence that photosynthetic rates were limited by the availability of inorganic carbon. The light-saturated gross photosynthetic rate (P_{max}) was calculated from the light-saturated net photosynthetic rate and the dark respiration measured prior to illumination.

The minimal turnover time, τ, for whole-chain photosynthetic electron flow from water to carbon dioxide was calculated from oxygen flash yields (PSU_{O_2}) and light-saturated photosynthetic rates:

$$\tau = \frac{1}{PSU_{O_2} P_{max}}$$

P-700 was measured in Triton X-100 extracts of whole cells by light-induced oxidation according to general procedure of Marsho and Kok [13]. Cells were harvested by centrifugation and disrupted by sonication in 1.5 ml of 50 μM Tris-HCl (pH 8.0) containing 0.01% (v/v) Triton X-100 at 0–4 °C. The suspensions were clarified by centrifugation, and the extracts were adjusted to Chl *a* concentrations of 5–10 μM. Chl *a* concentrations were determined using an extinction coefficient of 60 $mM^{-1} \cdot cm^{-1}$ at 677 nm. The reversible, light-induced oxidation of P-700 was measured using the dual-wavelength mode of an Aminco DW-2c spectrophotometer. Sodium ascorbate and methyl viologen were added to final concentrations of about 10 mM and 100 μM, respectively, and the sample was allowed to equilibrate in the dark for 2 min. Absorption changes (ΔA) at 697 nm (P-700) were measured relative to an isosbestic wavelength of 720 nm. Actinic illumination of 5 s duration was provided by a focused 150-W tungsten-halogen source filtered through two Corning 5543 filters ($\lambda_{max} = 420$ nm). The photomultiplier was protected by a single Corning 2030 blocking filter. The actinic illumination was sufficient to saturate the P-700 signal at Chl *a* concentrations less than 12 mM. ΔA was calculated as the difference between the baseline A (reduced P-700) and the fully oxidized A measured after the rapid fluorescence decay at the end of actinic illumination. (Background fluorescence was minimized by placing the cuvette in the secondary sample position.) P-700 concentrations were calculated using an A difference coefficient of 64 $mM^{-1} \cdot cm^{-1}$ [3].

Plastoquinone was extracted from thylakoids in 90% acetone and separated from chlorophylls by partitioning into petroleum ether (b.p. 35–60 °C). The petroleum ether was washed three times with 95% MeOH to remove residual lipids and the extract was dried at 30 °C on a rotary evaporator. The dried extract was redissolved in a minimal

volume of absolute ethanol. Absorbance was determined from 230–380 nm on Aminco DW2c spectrophotometer. PQ in the reference cell was reduced with $NaBH_4$ and the difference spectra were obtained. The spectra were corrected for base line variations. Absorption changes resulting from the reduction of PQ by $NaBH_4$ were measured at 262 using 232 and 309 nm as the isosbestic wavelengths. PQH_2 was calculated using an extinction coefficient of 15 $mM^{-1} \cdot cm^{-1}$ [14].

Cytochrome *f* was measured in Triton X-100 extracts of whole cell by a spectrophotometric method [15]. Cells were harvested by centrifugation and disrupted by sonication in 3 ml of 60 mM potassium phosphate buffer (pH 6.5), containing 1 mM $MgCl_2$/1 mM $MnCl_2$/2 mM EDTA/1% (v/v) Triton X-100. The suspensions were clarified by centrifugation, and the extracts were adjusted to Chl *a* concentration of 5–10 μM. The concentration of reduced samples using ferricyanide and ascorbate. The maximum absorption difference at 554 nm was measured relative to isosbestic wavelengths of 543 and 560 nm. Cytochrome *f* was calculated assuming an extinction coefficient of 17.5 $mM^{-1} \cdot cm^{-1}$ [15].

The cellular content of Rubisco was determined by immunoassay of soluble cell protein after separation by SDS-polyacrylamide gel electrophoresis. About $5 \cdot 10^8$ cells were spun down and resuspended in 1 ml of an homogenizing buffer (100 mM Na_2CO_3, 100 mM dithiothreitol and 200 μM PMSF). The suspension was sonicated for 90 s at 4°C with a Kontes microprobe sonicator. SDS and glycerol were added to give final concentrations of 0.5% and 10%, respectively, and a Chl/SDS ratio of 15:1. The sample was gently stirred at room temperature for 10 min and centrifuged at $20000 \times g$ for 10 min. Proteins in the supernatant were denatured by boiling for 2 min, 35 μl aliquots were separated by electrophoresis on a 10% polyacrylamide gel slab using the buffers of Laemmli [16], and the proteins were transferred to nitrocellulose by electrophoresis as described [17]. The large subunit of Rubisco was detected by successive incubation of the nitrocellulose with polyclonal antibodies raised against pea large subunit and ^{125}I-labeled protein A. Autoradiograms were scanned to provide quantitation. Rubisco isolated for *D. tertiolecta* was used to establish a quantitative calibration. A linear relationship was established between the volume of cell extract loaded onto gels and the product of (autoradiographic peak area) and (band width).

Rubisco activity was measured in crude protein extracts following sonication of cells in 100 mM Tris-HCl (pH 8.1), containing 1 mM EDTA and 1 mM dithiothreitol. Activity was measured in a 1 ml mixture containing 100 mM Tris-HCl (pH 8.1)/0.1 mM EDTA/8 mM $MgCl_2$/0.5 mM reduced glutathione/20 mM $NaH^{14}CO_3$ (0.04 μCl/μmol)/1 mM RuBP/0.1 ml enzyme extract (about 0.5 mg protein/ml). After 10 min preincubation with $NaH^{14}CO_2$, the reaction was initiated by the addition of RuBP and terminated after 10 min by the addition of 1 ml 24%(w/v) trichloroacetic acid [18]. The solution was evaporated to dryness and the residue dissolved in 1 ml of warm water. Aliquots of the water-soluble material were measured for radioactivity by scintillation counting.

For electron microscopy, cells grown at 1750 and 100 μmol quanta per m^2 per s were spun down at $500 \times g$, fixed in 2% glutaraldehyde in 0.05 M cacodylate buffer, postfixed with 2% osmium tetroxide, dehydrated through an ethanol series and embedded in Epon. Ultrathin sections were cut with a diamond knife on an ultramicrotom and poststained with 2% uranylacetate. Samples were examined using a Philips 300 transmission electron microscope. Specific volume and surface density of ultrastructures were estimated from morphotometric measurements of electron micrographs, using a coherent test system [19].

Results

The cellular concentration of photosynthetic pigments in *D. tertiolecta* decreased with increasing growth irradiance levels (Fig. 1). In addition, Chl *b* and carotenoids changed disproportionally relative to Chl *a*, as cells adapted to photon flux densities.

The effect of growth irradiance on the cellular concentrations of PS II and the 'size' of the photosynthetic unit are shown in Fig. 2. The average size of the combined antenna complex for oxygen production (PSU_{O_2}) was reduced by 30% as growth irradiance was increased from 80 to 700 μmol

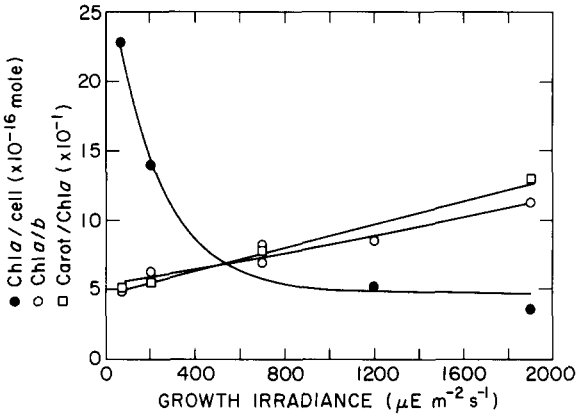

Fig. 1. Cellular pigmentation of *D. tertiolecta* adapted to various growth irradiance levels.

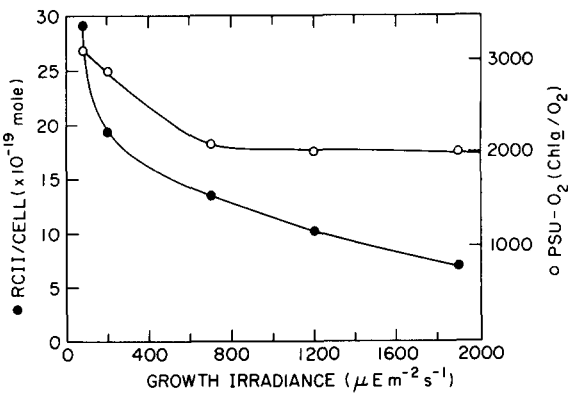

Fig. 2. Effect of growth irradiance level on photosynthetic unit size (PSU_{O_2}) and cellular content of PS II reaction centers.

quanta per m^2 per s, but only small changes in the 'size' of PSU_{O_2} were observed at higher photon flux densities. The cellular content of PS II reaction centers decreased with increased growth irradiance, showing a 75% decrease between 80 and 1900 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Fig. 2). Similar changes were observed for PS I (Table I). In this species the cellular content of PS II and PS I reaction centers changed simultaneously and a constant ratio of about 1.4 PS I/PS II was found for cultures grown under a wide range of growth irradiance levels (Table 1). In the terminology currently used in the literature, *D. tertiolecta* adapts to changes in growth irradiance level primarily by changing the number and not the 'size' of photosynthetic units.

Changes in the cellular contents of pigment protein complexes can be related to changes in chloroplast morphology and ultrastructure. Transmission electron micrographs of cells grown at 1750 μmol quanta per m^2 per s (HL cells) and cells grown at 100 μmol quanta per m^2 per s (LL cells) are shown in Fig. 3. The chloroplasts in HL cells are characterized by a high quantity of starch globules and by the low density of thylakoid membranes. In contrast, in chloroplasts of LL cells the photosynthetic membranes are very dense and organized in stacked groups of 4–6 membranes. Morphometric measurements of the electron micrographs (Table II) show that the chloroplast-specific volume did not significantly change as cells adapted to higher growth irradiance levels; however, the specific volume of the pyranoid and its accompanying starch globules increased. Con-

TABLE I

EFFECT OF GROWTH IRRADIANCE LEVEL ON THE STOICHIOMETRY OF PHOTOSYNTHETIC ELECTRON-TRANSPORT COMPONENTS IN *DUNALIELLA TERTIOLECTA*

Cyt, cytochrome.

Growth irradiance (μmol quanta per m^2 per s)	Cellular concentration ($\times 10^{-19}$ mol or $6 \cdot 10^5$ molecules)					Molar ratio
	RC II	PQ	Cyt *f*	RC I	Rubisco	RC II:PQ:Cyt *f*:RC I:Rubisco
80	29.0	915	48.7	40.0	34.0	1: 32: 1.7: 1.4: 1.2
200	19.2	670	34.4	33.3	33.8	1: 35: 1.8: 1.7: 1.8
700	13.4	540	17.6	17.5	34.6	1: 40: 1.3: 1.3: 2.6
1200	10.2	–	16.9	12.0	29.0	1: –: 1.7: 1.2: 2.8
1900	6.9	233	12.1	9.2	31.6	1: 34: 1.8: 1.3: 4.6

TABLE II

EFFECT OF GROWTH IRRADIANCE ON CHLOROPLASTS ULTRASTRUCTURE IN *DUNALIELLA TERTIOLECTA*

	Low light 100 μmol quanta per m^2 per s ($n = 31$)	High light 1750 μmol quanta per m^2 per s ($n = 14$)
Chloroplast-specific volume	0.495 ± 0.047	0.477 ± 0.048
Pyranoid + starch--specific volume	0.164 ± 0.026	0.218 ± 0.024
Chloroplast-active fraction-specific volume	0.331 ± 0.031	0.259 ± 0.026
Thylakoid surface density (cm^2/cm^3)	$(4.38 \pm 1.31) \cdot 10^5$	$(2.17 \pm 0.47) \cdot 10^5$

sequently, the volume of the chloroplast occupied by thylakoids, and presumably available for the photosynthetic reactions, is reduced in HL cells. For heuristic purposes we call this volume the 'chloroplast-active fraction', defined as the volume of the chloroplast minus the volume of the pyrenoid and its accompanied starch globules (Table II). Surface density of the LL cell thylakoids (membrane unit area per chloroplast unit volume) is twofold greater than in HL cells. Since the reaction center content is fivefold greater (Fig. 2, Table I), the estimated membrane area occupied by an electron-transport chain is larger in HL cells than in LL cells.

Growth irradiance levels profoundly influenced light-saturated photosynthetic rates (Fig. 4). On a cellular basis P_{max} was constant over a wide range of growth irradiance levels. However, when P_{max} is normalized to Chl *a* or to PS II reaction centers, it increases linearly with growth irradiance (Fig. 4). These variations in P_{max} suggest that τ varies with growth irradiance levels. The calculated turnover time changed fourfold, from 3.6 ms to 14.6 ms. Changes in τ were directly proportional to the cellular content of either Chl *a* or reaction centers (Fig. 5). Thus, in *D. tertiolecta*, the maximal rate of electron transport from water to carbon dioxide decreases with an increasing number of photosynthetic units.

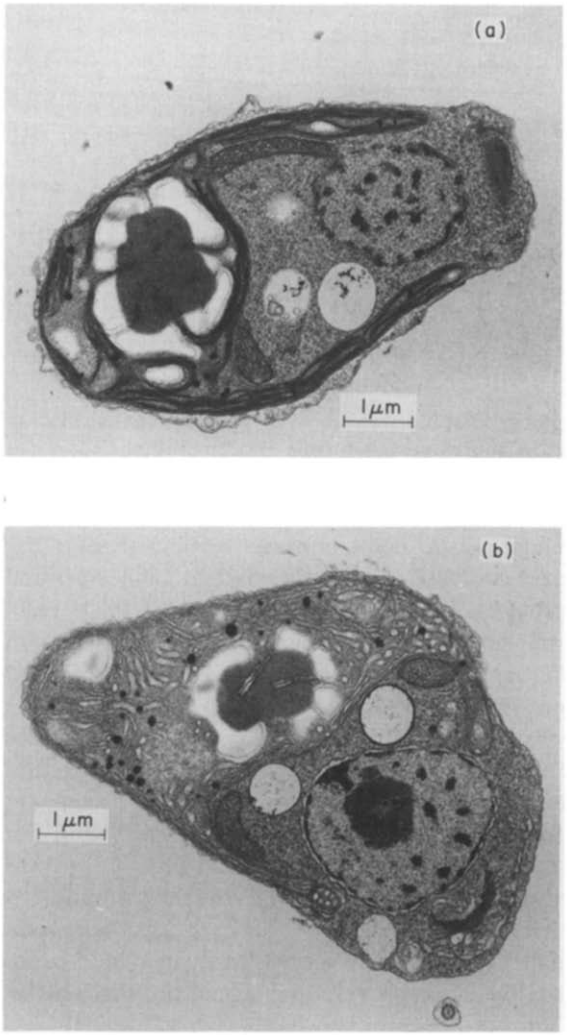

Fig. 3. Transmission electron micrographs of thin sections through *D. tertiolecta* grown at (a) 100 (LL) and (b) 1750 (HL) μmol quanta per m^2 per s.

A representative autoradiogram of a Western blot of the large subunit of Rubisco determined for *D. tertiolecta* at five different growth irradiance levels is shown in Fig. 6. The gel was loaded in each lane with the protein extracted from a constant number of cells. The autoradiogram indicates that the cellular concentration of large subunit is essentially constant over the entire range of growth irradiance levels. From Western blots with purified Rubisco from *D. tertiolecta* we calculated

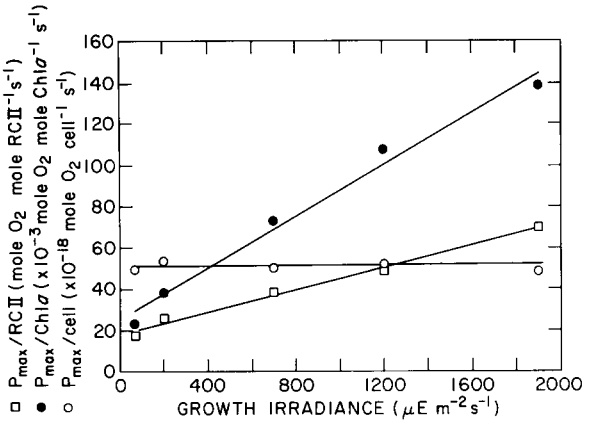

Fig. 4. Photosynthetic capacity – P_{max} in *D. tertiolecta* adapted to various growth irradiance levels. P_{max} is expressed per Chl *a*, PS II reaction center and cell.

a cellular concentration of $2 \cdot 10^6$ molecules of Rubisco, regardless of growth irradiance.

Accompanying the measurement of large subunit by the Western blot technique we measured the in vitro activity of Rubisco. This assay was performed on whole-cell extracts (Table III). Enzyme activity, normalized either to cell number or to Rubisco content, is again essentially constant over the range of irradiance levels, averaging $40 \cdot 10^{-18}$ mol CO_2 per cell per s. From Western blots calibrated with purified Rubisco from *D. tertiolecta*, we calculated an average specific activity of 12.3 mol CO_2 mol per enzyme per s. The ratio of Rubisco activity to saturated light photosynthesis rate was fairly constant, averaging 0.79 mol CO_2/mol O_2, which is remarkably close to the expected photosynthetic quotient of nitrate fed algal cultures [20].

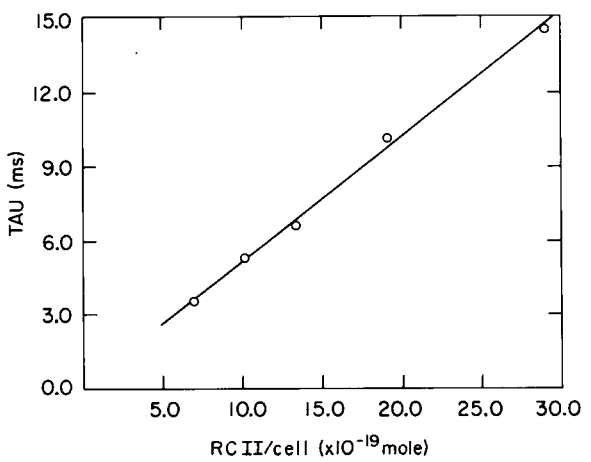

Fig. 5. Relationship between the minimum turnover time of the photosynthetic apparatus (τ) and the cellular concentration of PS II reaction centers in *D. tertiolecta* grown at various irradiance levels.

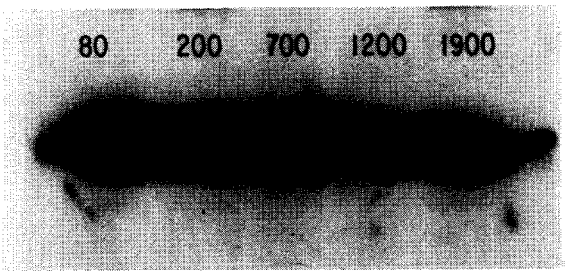

Fig. 6. Autoradiogram of Rubisco large subunit separated electrophoretically from *D. tertiolecta* whole cell extracts. Numbers indicate growth irradiance in μmol quanta per m^2 per s.

The cellular concentration of several photosynthetic electron-transport components and Rubisco are given in Table I. The cellular concentrations of all the membrane-associated electron-transport components changed in proportion to one another as growth irradiance changes, while the cellular Rubisco content remained relatively constant. Thus, the stoichiometric relationship among the measured thylakoid-associated components does not change, averaging 1 RC II : 34 PQ : 1.6 cytochrome *f* : 1.4 RC I. We shall refer to this relation-

TABLE III

EFFECT OF GROWTH IRRADIANCE ON IN VITRO RUBISCO ACTIVITY IN *DUNALIELLA TERTIOLECTA*

I (1)	Rubisco activity		Rubisco/P_{max}
	(2)	(3)	(4)
80	46.5	13.7	0.93
200	33.0	9.8	0.62
700	41.1	11.9	0.82
1200	40.0	13.8	0.77
1900	38.6	12.2	0.80

(1) μmol quanta per m^2 per s.
(2) 10^{-18} mol CO_2 per cell per s.
(3) mol CO_2 per mol enzyme per s.
(4) mol CO_2 per mol O_2.

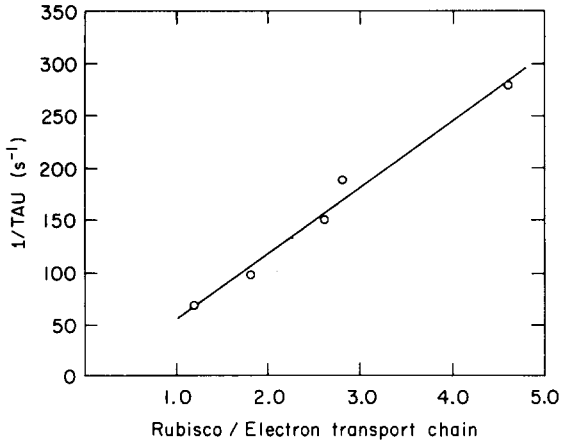

Fig. 7. Relationship between the ratio of Ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco) to electron-transport chain and the maximal turnover rate of the photosynthetic apparatus.

ship, normalized per RC II, as an electron-transport 'chain'.

The molar ratio of Rubisco/electron-transport chain increased from 1.2 to 4.6 as growth irradiance increased from 80 to 1900 μmol quanta per m^2 per s. The ratio of Rubisco/electron-transport chain was linearly correlated with changes in the maximal rate of electron transport from water to carbon dioxide, $1/\tau$, over the entire range of growth irradiance levels examined (Fig. 7).

Discussion

The data presented here show that the maximal rate of photosynthetic electron flow from water to carbon dioxide in *Dunaliella tertiolecta*, grown under nutrient replete conditions, increases as cells adapt to higher growth irradiance levels. Under the conditions of these experiments, the measured rate of light-saturated photosynthesis is intrinsically limited by reactions within a complete, fully functional photosynthetic apparatus, and not by secondary factors such as CO_2 availability, the rate of nitrogen assimilation, and so on. Similar results have been reported for *Chlorella pyrenoidosa* [1], and a variety of marine unicellular algae, all grown in turbidostat cultures [3,7,8,10]. In all cases the calculated turnover time of each photosynthetic unit is inversely related to the cellular concentration of such units.

Changes in τ are related to changes either in the rate constant of a rate-limiting reaction or in the pool size of a rate-limiting component. The time for oxidation of Q_A is 400–450 μs and is apparently independent of the number of PS II reaction centers which are closed [21]. This turnover time is about an order of magnitude smaller than τ, and suggests that reactions on the donor side of PS II and the oxidation of Q_A are not normally rate limiting. There is evidence that the diffusion and oxidation of PQH_2 is a rate-limiting step in intersystem electron flow [21,22]. If the diffusion of PQH_2 were limiting the overall rate of photosynthetic electron flow between water and carbon dioxide, we might expect changes in τ to be correlated with changes in either PQ or cytochrome *f* relative to PS II. This is not the case in *D. tertiolecta*. On the contrary, the synthesis of all of membrane-associated electron-transport components is highly coordinated and there is a basically constant stoichiometric relationship between all of the components measured. In this species an electron-transport chain has a statistical meaning and can be operationally defined as the (fixed) stoichiometric relationship between electron transport components normalized to one PS II reaction center.

The results of the Western blots indicate that, unlike the membrane-associated electron-transport components, the cellular concentration of Rubisco is independent of growth irradiance. Because of changes in the cellular concentration of electron-transport chains the molar ratio of Rubisco/ETC increases 3.8-fold, from 1.2 to 4.6 between 80 and 1900 μmol quanta per m^2 per s. The change in this ratio corresponds to a change of 4.1-fold in the maximum rate of electron throughput, $1/\tau$, from 68.5 to 277.8 s^{-1} between the lowest and highest growth irradiance levels (Fig. 7). Thus, not only is the relationship between $1/\tau$ and Rubisco/electron-transport chain linear, but the normalized slope between these two parameters is 0.94, which, given experimental error, is not significantly different from unity. The high correlation between these two parameters strongly suggests that the pool of Rubisco relative to the number of electron-transport chains limits the overall rate of photosynthesis at light saturation in this alga.

Let us now consider whether the pool of Rubisco relative to the electron-transport chain is limiting per se, or whether the specific activity of the enzyme is limiting, or some other factor is involved. The results of the in vitro assays of Rubisco activity suggest that the specific activity of the enzyme is constant over the range of growth irradiance levels examined. If Rubisco activity were rate-limiting because of changes in the in vivo specific activity with growth irradiance, which are not reflected in the in vitro assays (i.e., if the ratio between enzyme activity in vivo to that in vitro varied), we would expect that the relationship between Rubisco/electron-transport chain and $1/\tau$ would become nonlinear. This is not the case, and the data suggest that the in vivo specific activity at light saturation is simply proportional to the number of Rubisco molecules present.

The morphometric measurements show that the specific volume of the chloroplast does not change with growth irradiance levels. However, the chloroplast volume available for photosynthetic processes is reduced by 25% in HL cells as compared to LL cells, owing to the increase in the specific volume of the pyranoid and starch globules under HL conditions. Furthermore, the thylakoid membranes' surface density (unit area per unit chloroplast volume) is reduced as the photon flux density increases. Thus, as cells adapted to increased growth irradiance levels, the surface area of thylakoid membranes decreased much more than the stromal volume of the chloroplast. Electron-transport components are distributed over the membrane surface, while enzymes associated with carbon fixation are distributed within the stroma volume. If diffusion of a reductant or energy source from the thylakoids to the stroma were rate limiting, it might be expected that the ratio between $1/\tau$ and the Rubisco/electron-transport chain would change in proportion to changes in the surface area/volume ratio within the chloroplast. This would be reflected in a slope between the two variables which is significantly different from unity. As this was not observed, we infer that within the range of surface area/volume ratios we observed, diffusion of materials from the thylakoids to the stroma is not limiting the rate of carbon fixation in the Calvin cycle.

The data presented here appear to explain why τ varies inversely with growth irradiance level. We recognize that the linear relationship between $1/\tau$ and Rubisco/electron-transport chain may not be the only linear relationship obtainable in the system. Had we measured other enzymes in the Calvin cycle, we might have obtained similar results and it is not our purpose or intention to identify Rubisco, per se, as rate limiting. However, our results clearly imply that carbon fixation and not electron transport between PS II and PS I is rate limiting at light saturation.

Stitt [23] arrived at similar conclusions from different experimental evidence. When steady-state light-saturated photosynthesis in a spinach leaf was interrupted by low light for 10 to 30 s, a temporary enhancement of photosynthesis was observed upon returning to saturating light intensities. Stitt interpreted these results to indicate that there is excess electron-transport capacity over that of carbon fixation capacity available at light-saturated steady-state photosynthesis. After analyzing changes in pool sizes of carbon fixation metabolites, he suggested the capacity for sucrose synthesis ultimately limits the maximal rate of photosynthesis in higher plants. To distinguish between Rubisco activity and ribulose 1,5-bisphosphate regeneration as the rate-limiting step in the Calvin cycle, attention should be focused on metabolite ratios within chloroplasts of cells photosynthesizing at light saturation. Special attention should be paid to the ratio of ribulose 1,5-bisphosphate to PGA or Ru5P. From metabolite ratios, Dietz and Heber [24] concluded that formation of ribulose 1,5-bisphosphate is tightly regulated by light activated enzymes.

Given the surface area of the thylakoid membranes and the cellular concentration of reaction centers, the geometric mean distance between PS II and PS I reaction centers can be calculated, assuming no lateral heterogeneity within the thylakoid membranes. We believe that such an assumption is probably valid in *Dunaliella* because, unlike in higher plant chloroplasts, the chlorophyte shows little evidence of appressed thylakoid membranes, a structural feature which is usually associated with lateral heterogeneity. Our calculations suggest that there are, on average, 1620 nm^2 membrane/electron-transport chain at a growth irradiance of 1750 μmol quanta per m^2

TABLE IV

CALCULATED DISTANCES BETWEEN PHOTOSYNTHETIC ELECTRON-TRANSPORT COMPLEXES AND DIFFUSION TIME OF PQH_2

Cyt, cytochrome.

	Area for electron-transport chain (nm^2/chain)	PS II → PS I distance (nm)	PS II → Cyt b_6f distance (nm)	PQH_2 [a] diffusion time (ms)
HL	1620	29.5	15	2.4
LL	790	20.6	10.3	1.1

[a] Estimated from Einstein equation, $d^2 = Dt$, where d is the distance between RC II and Cyt b_6f and D is the diffusion coefficient -10^{-9} $cm^2 \cdot s^{-1}$ [25].

per s (HL) and 790 nm^2 membrane/electron-transport chain at a growth irradiance of 100 μmol quanta per m^2 per s (LL). These calculations suggest that the weighted mean distance between PS II reaction centers is 45.8 nm and 32.0 nm in HL and LL cells, respectively. Taking into account the average stoichiometric ratio between PS II and PS I for both cell types, we estimate that the average distance between PS II and PS I is 29.5 nm in HL cells and 20.6 nm in LL cells.

If we assume that the cytochrome b_6/f complex is localized halfway between PS II and PS I, and the diffusion coefficient for PQH_2 within the thylakoid membrane is 10^{-9} $cm^{-2} \cdot s^{-1}$ [25], it is possible to calculate the time interval for electron transport from PS II to cytochrome to b_6/f. Our calculated values are 2.4 and 1.1 ms for HL and LL cells, respectively (Table IV), and are in good agreement with PQH_2 turnover times measured directly [26]. It should be pointed out that these calculations suggest that, if the diffusion and oxidation of PQH_2 were rate limiting at light saturation, we would expect that τ would be larger for HL cells than for LL cells, which is just the opposite of what is observed. In LL cells the calculated turnover time for PQH_2 is 8-times lower than τ. This calculation strongly suggests that the diffusion (and oxidation) of PQH_2 is not rate limiting in LL cells. In HL cells, τ is only 50% higher than the calculated diffusion time of PQH_2, implying that in spite of the morphological and stoichiometric alterations in the photosynthetic apparatus which occurred in response to high growth irradiance levels, the diffusion of PQH_2 is not likely to be rate limiting.

The results of these calculations may be used to predict a growth irradiance level where light-saturated photosynthesis will become limited by the oxidation of PQH_2. Extrapolating from the linear relationship between Chl a/cell and τ we can predict the minimal turnover time possibly achievable in *D. tertiolecta* at 18°C is 2.3 ms, a value which is similar to the time interval estimated for the reoxidation of PQH_2. To achieve this turnover time cells would be growing at about 3000 μmol quanta per m^2 per s (i.e., 1.5-times full sun intensity at local noon in temperate latitudes) and have a Rubisco/electron-transport chain ratio of 7.8. Under such conditions, electron transport, rather than carbon fixation, would become rate limiting.

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References

1 Myers, J. and Graham, J. (1971) Plant Physiol. 48, 282–286
2 Herron, H.A. and Mauzerall, D. (1972) Plant Physiol. 50, 141–148

3 Falkowski, P.G. and Owens, T.G. (1980) Plant Physiol. 66, 592–595
4 R.W. Eppley. (1972) Fish. Bull. 70, 1063–1085
5 Healey, F.P. (1973) CRC Rev. Microbial. 69–113
6 Falkowski, P.G. (1980) in Primary Productivity in the Sea (P.G. Falkowski, ed.), pp. 99–119, Plenum Press New York
7 Richardson, K. Beardall, J. and Raven, J.A. (1983) New Phytol. 93, 157–181
8 Gallager, J.C. and Alberte, R.S. (1985) J. Exp. Mar. Biol. Ecol. 94, 233–250
9 Falkowski, P.G., Owens, T.G., Ley, A.C. and Mauzerall, D.G. (1981) Plant Physiol. 68, 969–973
10 Dubinsky, Z., Falkowski, P.G. and Wyman, K. (1986) Plant Cell Physiol.27, 1335–1349
11 Jeffrey, S.W. and Humphrey, G.F. (1973) Biochem. Physiol. Pflanz 167, 191–194
12 Raps, S., Wyman, K., Siegelman, H.W. and Falkowski, P.G. (1983) Plant Physiol. 72, 829″2
13 Marsho, T.V. and Kok, B. (1971) Methods Enzymol. 23, 515–522
14 Barr, R. and Crane, F.L. (1971) Methods Enzymol. 23A, 372–408
15 Bendall, D.X., Davenport, H.E. and Hill, R. (1971) Methods Enzymol. 23A, 327–344
16 Laemmli, U.K. (1970) Nature (London) 277, 680–685
17 Bennett, J., Jenkins, G.I. and Hartley, M.R. (1984) J. Cell Biochem. 25, 1–13
18 Badour, S.S. (1978) in Handbook of Physiological methods (Hellebust, J.A. and Craigie, J.S., eds.), pp. 209–217, Cambridge University Press, Cambridge
19 Weibel, E.R., Kistler, G.S. and Scherle, W.R. (1966) J. Cell. Biol. 30, 23–51
20 Myers, J. (1980) Primary Productivity in the Sea (Falkowski, P.G., ed.), pp. 1–16, Plenum Press, New York
21 Falkowski, P.G., Wyman, K., Ley, A.C. and Mauzerall, D.C. (1986) Biochim. Biophys. Acta 849, 183–192
22 Haehnel, W. (1986) Annu. Rev. Plant Physiol. 35, 659–693
23 Stitt, M. (1986) Plant Physiol. 81, 1115–1122
24 Dietz, K.J. and Heber, U. (1986) Biochim. Biophys. Acta 848, 392–401
25 Wang, Z, Berry, E.A. and Crofts, A.R. (1986) (1987) in abstracts of the Proceedings of the VIIth International Conference on Photosynthesis (August 10–15, 1986; Brown University, Providence, RI) (Biggins, J., ed.), Martinus Nijhoff, Dordrecht, in the press
26 Haehnel, W. (1976) Biochim. Biophys. Acta 440, 506–521