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The Development of Photosynthesis in a Greening Mutant of *Chlorella* and an Analysis of the Light Saturation Curve[1]

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ABSTRACT

Photosynthetic oxygen evolution considerably precedes the rise in chlorophyll during the greening of a yellow mutant of *Chlorella vulgaris*. Dark-grown cells required 20 times more light to saturate photosynthesis than light-grown or normal cells. The chlorophyll appears to add first to active reaction centers, then to fill in a more general antenna. The carotenoid pigments seem to add more randomly to the reaction centers. The shape of the light saturation curves can be explained with the assumption that an excitation in the antenna can reach several reaction centers. The efficiency of the total unit is constant during the greening process.

The development of photosynthesis and of the structure of the chloroplast in greening plants has been studied extensively because of the unique opportunity to correlate structural development with photosynthetic function. The morphological changes on the scale above 10S of angstroms are well known (14, 18), but the molecular details of this development of photosynthetic activity are less well understood. It is often thought that the development of photosynthesis parallels the biosynthesis of chlorophyll and the formation of grana lamellae. For example, during the greening of dark-grown *Euglena*, both photosynthesis and chlorophyll content are observed to rise together after a lag (2, 20). This rise coincides in time with the formation of grana. Greening of a mutant of *Chlamydomonas* (13, 15) shows a somewhat faster increase of photosynthetic activity than of chlorophyll.

We have studied the greening of a mutant of *Chlorella* (12), paying some attention to the quantitative aspects of measurements of photosynthetic activity. It is assumed that photosynthetic activity of a cell is proportional to the number of active reaction centers present in the cell. The rate of photosynthesis is in turn proportional to the number of active reaction centers under the condition of light saturation. We have found that much more light is required to saturate photosynthetic oxygen evolution in the early stages of greening than in the final fully greened cells. An analysis of these light saturation curves and of the *in vivo* absorption and fluorescence excitation spectra suggests a progressive addition of antenna chlorophyll to rapidly formed active reaction centers.

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MATERIALS AND METHODS

The x-ray-induced mutant 610-y of *Chlorella vulgaris* Beijerinck, Trelease strain, was isolated by Granick (11); the cells had been kept on agar slants and were cloned before these experiments. The cells from individual clones are grown on shakers at room temperature in the dark on a glucose inorganic salt medium, placed in a mineral medium in the dark for 3 days to allow cell division to stop, and then suspended in a 30 mM sodium phosphate buffer, pH 6.4, at a cell density of about 10^7 cells/ml (0.015 ml of packed cell volume per 10 ml of cell suspension). Part of this suspension is exposed to light from a pair of cool-white fluorescence lamps (intensity about 3×10^3 ergs/cm^2·sec) while another part is kept in the dark. Samples are removed from the flasks at intervals during the following 1 to 2 days to measure pigment content or oxygen production. The bright light used for greening under intermittent illumination was an incandescant lamp of intensity about 10^4 ergs/cm^2·sec.

The cell are extracted with 80% acetone containing 10 mM $NaHCO_3$, and absorbance at 663 and 645 nm are recorded to determine chlorophyll content (1). Carotenoids are estimated from the absorbance at 475 nm, $\epsilon = 1.3 \times 10^5$.

In vivo spectra are measured on a layer of cells (about 10^7) deposited on a moist Millipore filter of 13-mm diameter. For absorption spectra the filter is held between two glass cover slips while the measuring beam passes through cells and filter before reaching the photomultiplier; a moist blank Millipore filter is used in the reference beam. Fluorescence excitation spectra are measured using an attachment which allows the cells on the filter to be excited by differing wavelengths of light from the Cary monochromator and their chlorophyll fluorescence ($\lambda > 700$ nm) to be measured by its red sensitive photomultiplier. For fluorescence emission spectra, we remove the usual light source, illuminate the cells with a blue light, and allow the emitted fluorescence to enter the Cary monochromator.

Measurements of O_2 evolution from photosynthesis are made with the oxygen luminometer (3). This instrument is based on the chemiluminescent reaction of O_2 with luminol dianion in dimethyl sulfoxide (21). A stream of argon gas carries the O_2, evolved by the cells, to the luminol solution; the light emitted in the reaction of O_2 and luminol is measured by a photomultiplier. The system is directly calibrated in rate of O_2 evolution by the electrolysis of water, a known current liberating O_2 at a known rate. In these experiments, we have uually measured O_2 production at rates between 5×10^{-11} and 5×10^{-8} moles/min. All measurements were made at 25 C.

In order to observe photosynthesis of cells in phosphate buffer, we add a carbonate-bicarbonate mixture to a final concentration of 10 mM, pH 8. The actinic light from a 500 or 750 watt projector with heat filter is passed through a

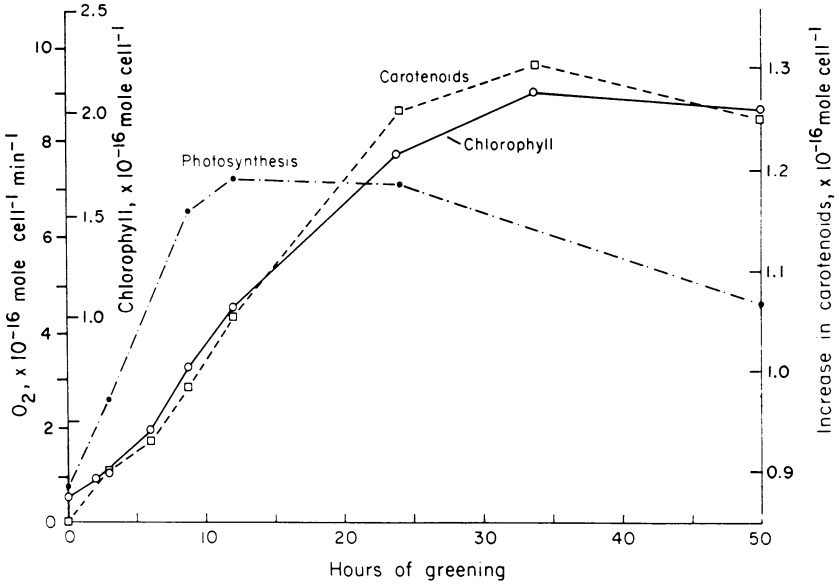

FIG. 1. Increase in photosynthetic ability, chlorophyll, and carotenoids during greening of dark-grown cells of clone 3. The scale for carotenoids is displaced to put the dark-grown level at the origin.

Table I. *Half-Rise Times for Chlorophyll Synthesis and Development of Photosynthetic Ability*

Experiment	Chlorophyll	Photosynthesis	New Carotenoids
		hr	
Clone 3	13	6.75	
Clone 3	14.5	7	
Clone 3	11	6.5	
Clone 3	13	5	13.25
Clone 3	14.5	—	9.25
Clone 3	16	8.5	15
Average	13.67	6.75	12.5
Clone 7	11.5	4	13.5
Clone 8	13.25	5.25	

chromate solution. This yellow light (500–830 nm) is of intensity 3 or 14 $\times$ 10^5 erg/cm^2·sec, respectively, and is decreased with neutral density filters or screens in order to record a light-saturation curve.

The flash lamp used in the estimation of the size of the photosynthetic unit was a General Radio Strobotac, of width about 3 μsec at half intensity. The flash rate was varied from 1 to 10 per second. The background illumination was the yellow light at 2.4 $\times$ 10^3 erg/cm^2·sec for the fully greened cells and 1.8 $\times$ 10^4 erg/cm^2·sec for the low chlorophyll cells. These intensities produced the same rate of O_2 evolution in the two types of cells when the continuous light was applied alone.

RESULTS AND DISCUSSION

Increases in Pigments and Photosynthetic Ability during Greening. Dark-grown mutant cells of *Chlorella* 610-y contain 4 $\pm$ 2% of the chlorophyll found in fully greened cells and evolve about 3 $\pm$ 1% as much O_2 in photosynthesis. In addition to the small amount of chlorophyll, the dark-grown mutant cells also contain one-third to one-half of the carotenoid content of fully greened cells. We will quote photosynthetic activity on a cell basis since this is the stable biological unit. The amount of chlorophyll present is a variable and for this reason the usual unit of rate of O_2 evolution per amount of chlorophyll is inapplicable. The fully greened mutant cells have 1.5 $\pm$ 0.5 $\times$ 10^{-13} g of chlorophyll per cell, about the same as the 2.0 $\pm$ 0.5 $\times$ 10^{-13} g per cell found in our wild-type *Chlorella* grown under the same conditions. In saturating light, the greened mutant cells produce 3 $\pm$ 1 $\times$ 10^{-16} moles of O_2 per min per cell, while the wild-type produce 4.5 $\pm$ 0.5 $\times$ 10^{-16} moles per min per cell.

As in most systems, increase in chlorophyll and photosynthetic ability is not simply triggered by a single short exposure of the dark-grown cells to light. Aliquots of dark-grown cells placed in light for 0.5 or 2 hr, then removed to darkness for periods up to 1.5 days show no further increase in chlorophyll content or in rate of production of oxygen over that found at the time of removal from the light. Thus continuous illumination is required for continued increase in photosynthetic function and for chlorophyll synthesis above the dark-grown level.

Figure 1 shows plots of chlorophyll and carotenoid concentration and rate of photosynthetic O_2 production in saturating light as a function of the time elapsed since the dark-grown cells were placed in the light. Chlorophyll concentration per cell increases steadily and then levels off at its maximum value after more than 24 hr in the light. The carotenoid curve represents only the increase over the dark-grown level and has the same shape as the chlorophyll curve.

To compare pigment formation and photosynthetic activity, one must suitably normalize the data. An objective estimate of the rate of biosynthesis is the time it takes to reach one-half of the final yield of pigment or activity. These "half-rise times" are given in Table I for a number of different experiments. The average half-rise time for chlorophyll formation is approximately 14 hr, not very different from the average of 12.5 hr for new carotenoid formation. Definite lags in pigment formation are often observed. The rate of oxygen evolution increases more rapidly with time, reaching half of its maximum value after 6 to 7 hr. No lags in development of photosynthetic activity were observed. The decline in activity after 2 days may be related to the nongrowing conditions of the experiment.

Decreasing the light intensity, under which the dark-grown cells are greening, by a factor of 10 allows them to synthesize only 30% as much chlorophyll and 25% as much carotenoid as control cells greening at the usual intensity. The increase in photosynthetic ability, however, is practically unaffected—oxygen production after 24 hr (at saturating light intensity) is 85% that of the controls. An even lower light intensity for greening essentially stopped chlorophyll production at 1.5% but the oxygen production rate rose to only 12% of normal after 48 hr. Similar effects could be obtained by periodic flashes of bright light. For example, flashes lasting 12 sec delivered every 26 min for 48 hr produced only 9% of normal chlorophyll, but 96% of normal oxygen production. Keeping the total light about constant but having the rate of flashing by using 30 sec flashes every 60 min halved the above figures. This result suggests a finite lifetime for the photoactive intermediates of about 30 min. By such manipulations of the light conditions during greening, we have been able to prepare cells having one tenth as much chlorophyll as fully greened cells but full photosynthetic activity (17), if a sufficiently bright light is used for the photosynthesis measurement.

Light Saturation Curves. The light saturation curve of photosynthesis contains two obvious pieces of information, if we neglect complications (17) at very low light intensities. The linear region reflects the efficiency of the photosynthetic apparatus while the light-independent region at saturation reflects the number of active centers in a cell. The reasoning behind these statements is as follows: for a reaction rate linear in light intensity a quantum yield, or efficiency of the reaction, is directly defined by the slope of this line. This quantum yield need not be simple in a mechanistic sense. In the light-saturated region, the rate is limited by dark reactions, and the slowest such step defines a turn-over time for the system. However, the driving force for these dark relaxation processes is still the light reactions at the reaction centers or traps. In the steady state, the over-all rate will be thus proportional to the number of centers. Again the connection between the linear and the saturated region need not be simple and a particular model is discussed later. We shall discuss these two parameters quantitatively later. At the moment we stress that to study the development of photosynthesis during greening one must consistently compare one or the other of these parameters, keeping constant other variables such as temperature and concentration of carbon dioxide. We find that these light-saturation curves change drastically as the cells green (Fig. 2). Partially greened cells require a more intense light to reach the saturated level of oxygen production than do fully greened cells. The curve for dark-grown cells does not show saturation at all in this experiment because the measuring light is not bright enough. There are indications that a brighter light actually damages the cells. Thus the dark-grown and partially greened (0–4 hr) *Chlorella* never attain their maximum photosynthetic activity. This fact reinforces our conclusion that the oxygen evolving activity precedes the increase in bulk chlorophyll content.

***In Vivo* Spectra of Greening Cells.** An *in vivo* absorption spectrum of dark-grown cells (Fig. 3) reveals strong absorptions at 475 nm, 450 nm, and 420 nm due to carotenoids, along with weaker absorption at 675 nm due to chlorophyll. During greening, the developing chlorophyll peak at 430 nm obscures all but the 475 nm absorption of the carotenoids. These spectra also indicate a lack of chlorophyll *b* (650 nm) in dark-grown cells, while a distinct shoulder representing this peak can be seen after 2 days of greening.

The absence of chlorophyll *b* from the absorption spectra of cells in the early stages of chloroplast formation agrees with its absence from the fluorescence excitation spectra at these times (Fig. 4); here also it appears later during greening. The more striking feature of the excitation spectra is the behavior of the carotenoid peak at 470 to 480 nm. It is very weak in the dark-grown cells, although absorption spectra indicate the presence of carotenoids. During greening this peak increases until it is larger than the blue peak of chlorophyll *a*. Similar observatiosn of the lack of energy transfer from carotenoids in etiolated bean leaves, and the subsequent increase of energy transfer on greening, have been made by Butler (4) and by Goedheer (8).

It was also noted that, compared to the spectrum of dark-grown cells, the fluorescence emission spectrum of the fully

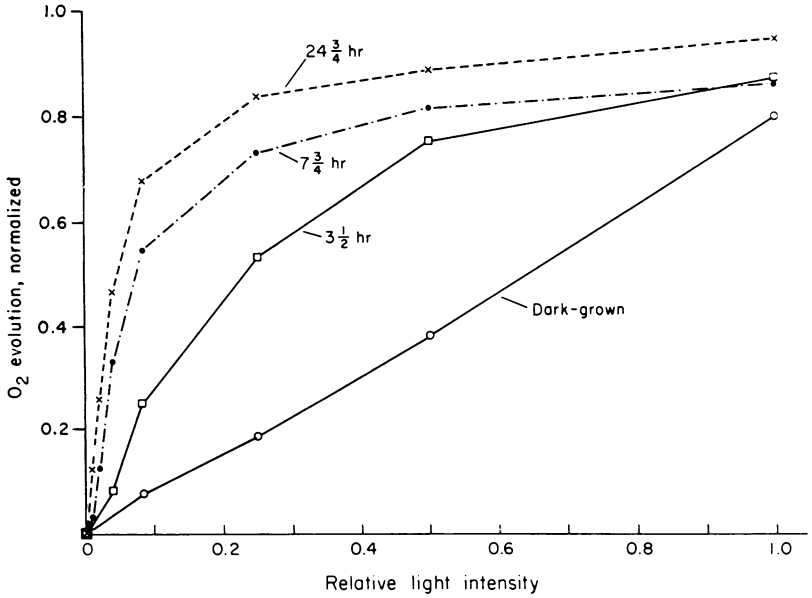

FIG. 2. Light saturation curves for photosynthesis in clone 7 of the mutant *Chlorella*. Curves are for dark-grown cells and for cells greened for 3.5, 7.75, and 24.75 hr. The ordinate represents oxygen evolution per cell and its scale is different for each curve: full scale is 0.23, 4.5, 6.8 and 9.1 $\times$ 10^{-16} moles O_2 min^{-1} $cell^{-1}$ respectively. Full light intensity is 1.4 $\times$ 10^6 ergs/cm^2·sec.

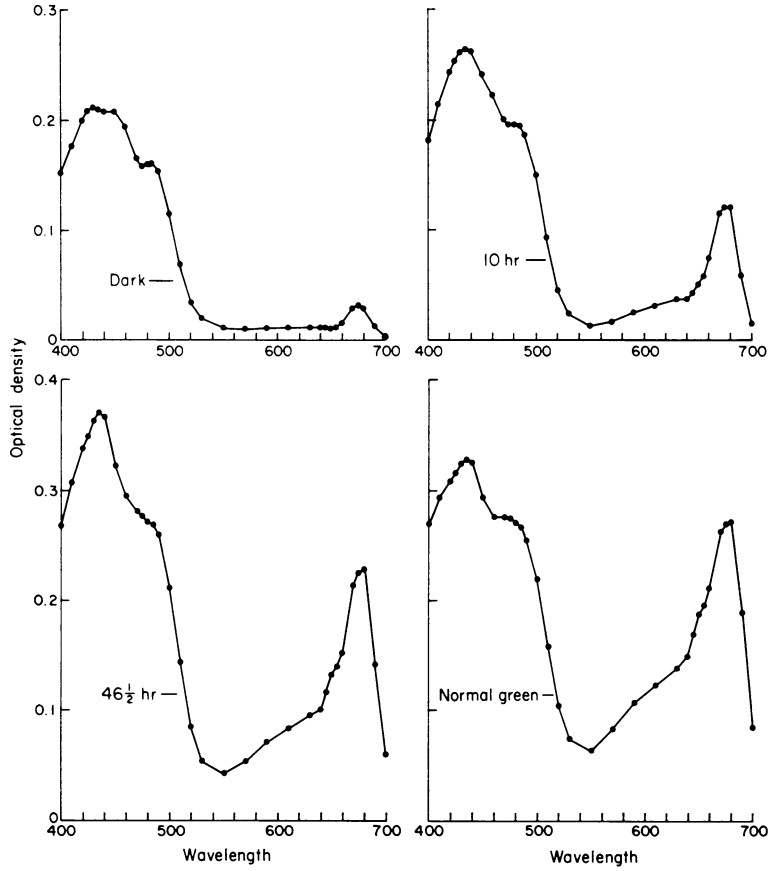

FIG. 3. *In vivo* absorption spectra of *Chlorella* cells. Mutant clone 3 cells were greened for 0 hr, 10 hr, and 46.5 hr. The spectra were recorded continuously; the points are for plotting after baseline corrections.

greened cells is skewed in the direction of longer wavelengths (>700 nm). Preliminary experiments indicate that the fluorescence quantum yield is higher and the fluorescence lifetime is longer for the dark-grown cells.

Energy Transfer from Carotenoids. Fluorescence excitation spectra measure how effective different wavelengths of light are in exciting chlorophyll fluorescence. The presence of a peak at wavelength where another pigment absorbs indicates that the other pigment is transferring energy to chlorophyll. This is clearly the case for the carotenoid peak in the spectrum of the fully greened cells (Fig. 4). Therefore, the total absence of energy transfer from carotenoids to chlorophyll *a* in dark-grown cells is unexpected in view of the presence of substantial amounts of carotenoids, as shown by the absorption spectrum of these cells. In fact the carotenoids in these cells actually screen the blue or Soret band of chlorophyll. The fully greened cells have a ratio of blue (440 nm) to red (680 nm) fluorescence excitation bands of 0.6, *versus* 0.3 for the dark-grown cells. We estimate that the carotenoids in the dark-grown cells absorb about one-half the light, an estimate in agreement with that of Emerson and Lewis on *Chlorella* (10).

As new carotenoids are synthesized during greening, energy transfer increases, and the question arises whether only the *new* carotenoids can transfer energy or if gradually all of them become able to do so. The latter might be the case, for instance, if the carotenoids in the dark-grown cells are free to migrate or are not close enough to other molecules, initially, for efficient energy transfer. As new carotenoids and chlorophylls are synthesized, they might fill in between the original molecules, reducing the average distance enough to allow *all* the carotenoids to transfer energy to chlorophyll. Alternatively, the carotenoids in the dark-grown cells might be permanently in the wrong places, so that only the newly synthesized molecules are capable of energy transfer.

An experiment using isonicotinic acid hydrazide (1 or 3 mg/ml) to block carotenoid synthesis (>95%) shows an increase in energy transfer from carotenoids as the cells synthesize chlorophyll (Table II), implying that the pre-existing carotenoids are able to function as accessory pigments. Thus, some of the new chlorophyll molecules must be placed close to the carotenoids, or else the carotenoids are able to migrate into the antennae sites. We note that the efficiency of energy transfer correlates quite well with the relative amount of chlorophyll in the cell (Table II). This suggests that the chlorophyll placement is the critical factor, and not carotene migration. The latter should be a function of time, not chlorophyll content. The breadth of the absorption peak in this region of the spectrum make the exact wavelength chosen to measure carotenoids immaterial. Thus chlorophyll *b* cannot account for this increase, and in fact our measure of it at 650 nm indicates it is very small in the early time range.

In the absence of an inhibitor, the ratio of the 475 nm carotenoid peak to the 680 nm chlorophyll peak in the excitation spectra increases with a half-rise time of 10 hr, close to the 13 hr half-rise time of the new carotenoid and chlorophyll synthesis. Since chlorophyll fluorescence is roughly proportional to the amount of chlorophyll in the cell and since chlorophyll is increasing at about the same rate as the carotenoids, the ratio of the carotenoid to chlorophyll peaks would be a constant if every new carotenoid molecule was added to the right spot for efficient energy transfer to chlorophyll. This would be the case if a photosynthetic unit grew the way most

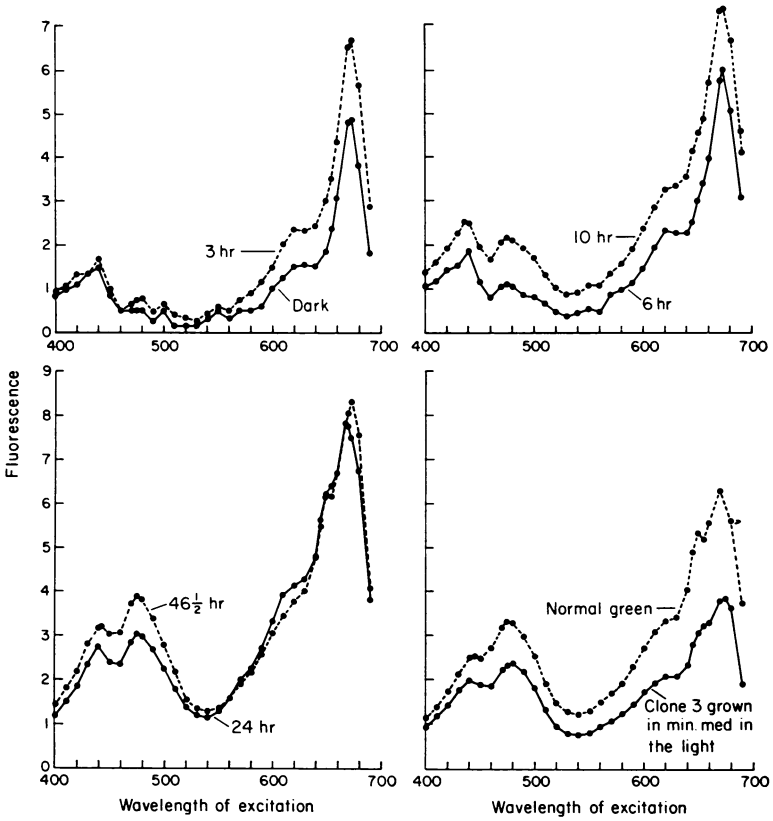

FIG. 4. *In vivo* fluorescence excitation spectra of *Chlorella* cells. Dark-grown mutant clone 3 cells were greened for 0, 6, 10, 24, and 46.5 hr About 10^7 cells were used for all spectra except that of 46.5 hr, for which we used 7×10^6 cells. Clone 3 and normal green cells were grown on mineral medium in the light. Correction for the quantum output of the tungsten source and monochromator efficiency would raise the intensity at 430 nm by a factor of two relative to that at 660 nm. This would make the excitation spectra of the fully greened and normal cells resemble the absorption spectra in Figure 3. The dots are for plotting after baseline corrections.

crystals grow. The fact that we find this ratio to increase means the efficiency of transfer is proportional to the amounts of both the carotenoids and the chlorophyll. This indicates a random process is occurring during the filling of a photosynthetic unit. As new chlorophyll and carotenoid molecules are synthesized the average distance between pigment molecules is reduced and transfer of energy is facilitated. A more quantitative statement is difficult because of the presence of the pool of old carotenoids. However, the fact that the half-rise time of the fluorescence excitation at 475 nm to that at 680 nm is some 30% less than the time for chlorophyll and new carotenoid synthesis is in agreement with the result of the experiment with isonicotinic hydrazide that the old carotenoids progressively contribute to the energy transfer to chlorophyll.

Development of Photosynthetic Units. A complete photosynthetic unit includes both reaction centers with enzymes and the bulk (or "antenna") chlorophyll which absorbs light and transfers its energy to the reaction centers. Chloroplast development might proceed by the production of one complete unit after another, or by the rapid formation of functional reaction centers followed by a slow filling in of bulk chlorophyll around the centers. In the first case, we expect chlorophyll content and photosynthetic ability to rise in parallel and we expect the cell to show the same light-saturation curve at all times. In the second case, photosynthetic ability should rise relatively more rapidly than chlorophyll content and in the early stages of greening the traps should have less chlorophyll close by and so be less effective in collecting light energy. As chlorophyll is filled in, the light-saturation curves should change and approach the effectiveness of fully greened cells.

Table II. *Increase in Energy Transfer from Carotenoids to Chlorophyll during Greening With and Without a Net Increase in Carotenoids*

Greening Time	Control		Isonicotinic Hydrazide[1]			
	R[2]	Chl[2]	R	Chl	R	Chl
hr			*1 mg/ml*		*3 mg/ml*	
0	0.4	0.02	0.3	0.02	0.3	0.02
2	0.5	.03	0.3	.02	0.3	.02
4	1.1	.08	0.6	.03	0.5	.02
8	1.0	.24	0.7	.09	0.4	.03
24	2.0	.97	1.0	.40	0.8	.15
49	2.0	1.00	1.5	.61	1.2	.27

[1] The carotenoid content of the control cells increased 53% during the greening period while that in the low and high concentration of inhibitor changed by +5% and −5% respectively. Similarly, the light-saturated rate of oxygen production reached a level of 73% and 50% of the level of the control cells.

[2] R: the ratio of the fluorescence excitation intensity at 470 nm to that at 675 nm. Corrections for variation in the base line produce an error of about 0.1 in this ratio.

[3] Chl: the amount of chlorophyll in the cells normalized to that in the fully greened mutant.

This latter model agrees well with the data presented. The experiment on greening in dim light shows that the smaller amount of chlorophyll synthesized is used mainly for the reac-

tion centers and only the final accumulation of bulk or antenna chlorophyll is diminished by the dim light.

The size of the photosynthetic unit is defined by the classical repetitive flash technique of Emerson and Arnold (9). In applying this technique with the luminometer as the oxygen detector, we found that if the cells were in darkness except for the brief saturating repeated flashes, the size of the photosynthetic unit was very large; that is, the oxygen emitted per flash was very low. If, however, a constant background light was added to the flashes, then about 10 times as much oxygen was evolved by the flashes. The background intensity was chosen to be in the lower part of the linear region of the light saturation curve for each kind of cell. Thus the background light had to be 10 times brighter for the low chlorophyll cells greened in dim light than for the fully greened cells. The effect of the constant background light could *not* be duplicated by supplying the cells with oxygen in the gas stream at the same rate as they would have evolved photosynthetically in the presence of the background light. Therefore, the increase in yield of oxygen is a photo effect. This problem has been shown (6) to be caused by loss of the photochemical intermediates at low partial pressure of oxygen. With these complications in hand, the partially greened cell, as expected, had a much smaller photosynthetic unit size than the fully greened ones: roughly 900 *versus* 2400 chlorophyll per molecule of oxygen. If the ratio of total chlorophyll in the greened cells to that in the partially greened cells, 7.6, is divided by the ratio of the sizes of their photosynthetic units, 2.7, we obtain the ratio of total (flash) units in these cells, namely 2.8. This is twice the ratio of their light saturated rates of oxygen production, 1.3, which we have assumed proportional to the number of (continuous light) units. This difference does not affect the conclusions of this paper, and a discussion of these different estimations of the photosynthetic units is forthcoming (6). A smaller size of the photosynthetic unit in a mutant of *Chlorella pyrenoidosa* (22) has also been inferred from the light saturation curves.

Electron photomicrographs by Dr. S. Schor show that the chloroplasts of the fully greened mutant resemble those of normal green *Chlorella*. Those of the dark-grown cells are reduced to outer membranes with some vesicles. The mutant greened in dim light with low chlorophyll content but good photosynthetic ability also has a largely undeveloped chloroplast, with few if any stacked thylakoids, only many vesicles. Yet this mutant carries out oxygen evolution based on fixation of carbon dioxide just as well as the fully greened mutant, with respect both to quantum yield and to rate in saturating light. It would appear that chloroplasts highly organized on a scale of 1000 Å are not required for an effective photosynthetic apparatus. This conclusion has been previously reached by many workers based on observations of blue-green and red algae.

Analysis of the Light Saturation Curves. We have shown (17) that there is a region of dependence of oxygen evolution on the square of the light intensity at low intensities. Neglecting the "toe" in the light saturation curve, we here analyze the saturation region alone. The shape of this curve has been the subject of much experiment and theorizing (19). The trouble lies in the fact that the experimental curves break more sharply than the usual hyperbolic saturation curves used to explain this type of behavior in enzyme kinetics, fluorescence quenching, and linear photochemical cycles. Let us first note that the exact shape of the light saturation curve may be a function of wavelength. The available data on distribution of excitation in the two photosystems (for discussion see (16)) suggests that these wavelength effects will be small outside of the red drop region. They were minimized in the present experiments by the simple expedient of broad-band illumination.

The simplest model to fit these "sharp" light-saturation curves is that of allowing the excitation in the antenna molecules to visit more than one trap or reaction center. We propose, essentially, a highly simplified version of a model used by Clayton (5) to relate fluorescence to photosynthesis. A unit is all antenna chlorophyll and all traps available to a single excitation. The probability that all traps in a unit are filled is X^n, where X is the fraction of total traps filled and n is the number of traps in a unit. There are thus N/n units, where N is the total number of traps in a preparation. The probability that a unit contains at least one empty trap is $(1 - X^n)$. We assume that an excitation continues to move (or is sufficiently delocalized) until it finds an empty trap in the unit. The rate of excitation will then be $N\sigma I(1 - X^n)$, where σ is the cross section of a trap with its share of antenna chlorophyll to quanta of light, and I is the light intensity. We have also taken the liberty of including the average effectiveness of energy transfer in the cross-section. This allows for some loss in transferring energy to the trap. All indications are that the effectiveness is very close to one. This implies that the rate of transfer is far greater than the rate of loss. The rate of flow through the photosynthetic system is $NX\alpha$ where α is the effective rate constant for the bottle neck or rate-determining step in the photosynthetic pathway. In the steady state the rate of excitation trapping equals the rate of throughput and thus

$$\sigma I(1 - X^n) = X\alpha$$

For $n = 1$, this equation predicts the usual hyperbolic saturation curve:

$$\text{Rate} = (N\alpha\sigma I)/(\alpha + \sigma I)$$

For $n = N$ (a large number) it predicts a straight line from the origin, of slope $N\sigma$, with a sharp break at $X = 1$ (rate $= N\alpha$). A simple way to characterize a curve intermediate to these two extremes is by the light intensity necessary to reach a given fraction, f, of the full rate:

$$I_f = (f\alpha)/(\sigma(1 - f^n))$$

This follows from our simple assumption that the rate is proportional to the fraction of traps filled. With two other characteristics of the curve, the light saturated rate $R_s = N\alpha$ and the slope in the linear region, $S = N\sigma$, the dependence of the curve on n is given by

$$(R_s)/(SI_f) = (1 - f^n)/(f) = r$$

For the convenient choice of $f = \frac{1}{2}$, the midpoint of the curve, r varies from 1 to 2 as n varies from 1 to N (*i.e.*, ∞). Table III gives some data relevant to this problem. The average value of r for the 17 entries without qualification marks is 1.92 ± 0.15. The average value for all but one extreme (4.4) is 1.95 ± 0.25 (rms errors). This would be fit by a value of $n > 3$. A larger range of r could be obtained by choosing f closer to unity, but the error in our data, specifically in the evaluation of R_s, precludes a more precise determination of n at the moment. A sampling of the light saturation curves given by Rabinowitch (19) leads to the same result. The conclusion that more than one trap is available to each excitation is strongly indicated.

We can obtain information on the relative efficiency of the chlorophyll in the photosynthetic units during greening by further analysis of the light saturation curve. Dividing the initial slope of these curves, $S = N\sigma$, by the saturated rate, $R_s = N\alpha$, and by the chlorophyll content c, gives us

Table III. *Parameters of Light-saturation Curves*

Experiment	Greening Time	Chlorophyll	Initial Slope (S)[1]	Half-saturating Intensity ($I_{1/2}$)[1]	Maximum Rate (R_s)[2]	Ratio (r)[3]	Efficiency (e)[4]
	hr	*μg/2 × 10⁷ cell*		%			
Clone 8	0	0.154	0.63	22	25	1.8	0.17
	1.5	0.217	3.4	14	≥90	≥1.9	≥.17
	3	0.332	8.2	12.5	195	1.9	.13
	8.5	0.930	48	3.5	390	2.3	.13
	23.5	2.2	180	1.2	460	2.0	.18
Clone 7	0	0.061	0.12	>52	>12	~2.0	<.15
	1.75	0.187	2.0	>30	>120	~2.0	<.09
	3.5	0.304	9.2	≥19	≥260	~1.5	≤.12
	5.75	0.56	20	≥13	≥410	~1.6	≤.09
	7.75	0.94	36	6	≥385	≥1.8	≤.10
	10.5	1.49	45	5.5	440	1.8	.07
	24.75	3.01	75	4	560	1.8	.04
	47	2.98	95	2	375	2.0	.09
Clone 3	0	0.084	0.17	>45	>15	~2.0	<.13
Control[5]	4	0.215	3.6	≥25	>170	~1.9	<.10
	9.5	0.98	29	6	320	1.8	.09
	24	3.34	100	3	580	1.9	.05
Dim[5]	4	0.165	2.3	≥36	>170	~2.0	<.08
	9.5	0.308	5.6	25	≥270	≥1.9	≤.07
	24	0.85	39	7	490	1.8	.09
Clone 3	0	0.14	0.69	≥25	49	≤2.8	.10
	3	0.27	3.7	25	170	1.8	.08
	12	1.2	39	6	480	2.0	.07
	24	2.06	72	4	470	1.6	.07
	50	2.3	75	2	300	2.0	.11
Clone 3	0	0.12	0.042	>50	>5.2	~2.4	<.06
	2	0.27	0.34	>50	>74	~4.4	<.02
	4	0.40	1.5	50	>170	>2.3	<.02
	5	0.53	3.5	35	240	2.0	.03
	8.5	1.07	15	15	425	1.9	.03
	23.25	2.49	44	7	650	2.1	.03

[1] The initial slope S and the half-saturating intensity, $I_{1/2}$, are read from the light-saturation curves of photosynthetic rate *versus* intensity.

[2] The light-saturated rate of photosynthesis is R_s.

[3] Ratio r of R_s to $SI_{1/2}$.

[4] Dividing the initial slope S by the saturated rate R_s and the chlorophyll content c gives the efficiency e.

[5] Control and dim refer to cells greened under the usual intensity and cells greened by a light one-tenth as bright.

$e = (\sigma)/(c\alpha)$, the relative efficiency per chlorophyll of a photosynthetic trap. As full greening is approached, this division by the chlorophyll under-estimates the efficiency because the contribution of each chlorophyll molecule to the cross section decreases due to heterogeneous light absorption (7). This reaches a factor of ½ in *Chlorella* at the maximum absorption in the red, but is even less when averaged over broad band illumination, relevant to the present discussion. The results of this calculation of e, shown in the last column of Table III, indicate that the relative efficiency is remarkably constant. In any particular experiment e varies by a factor of 2, and often less, while the chlorophyll content varies by a factor of 20. The optical cross section per chlorophyll is a constant determined only by molecular properties and will be only slightly affected by heterogeneous light absorption, and electronic interactions between neighboring pigments for the broad wavelength range of light used in the present experiments. However, our definition of the total cross section per trap, σ, included any losses in the energy transfer processes. The constancy of e strongly argues that both the effectiveness of the energy transfer and the limiting rate constant of the photosynthetic apparatus, α are constant during the greening process. This means that the photosynthetic unit is assembled as a very tight system. At no time during greening is there any appreciable amount of free chlorophyll. Only in the dark-grown cells do we have evidence for the presence of nonphotosynthetically active chlorophyll.

CONCLUSION

A study of the greening process in this mutant of *Chlorella* has shown that the active photosynthetic unit of reaction center, electron transfer agents, and enzymes is assembled first, followed by addition of more antenna chlorophyll. Fully active cells containing only one-tenth the usual chlorophyll can be prepared. The carotenoid component seems to be added somewhat randomly. Analysis of the light-saturation curves, which strongly depend on greening, show that the excitation energy is able to visit several traps and that the efficiency of these traps is relatively constant. These results are most easily explained by a model wherein the largest part of the chlorophyll (and carotenoids) in fully greened or wild-type cells is an extensive two-dimensional mat. Funnels of most of the remaining chlorophyll (as a protein complex?) connect this large antenna with the reaction centers or traps. This model ex-

plains many details of the photoconversion process and is useful in planning new experiments.

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