Photosynthetic Properties of ac-31, a Mutant Strain of Chlamydomonas reinhardi Devoid of Chloroplast Membrane Stacking

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Abstract. A pale-green mutant strain of Chlamydomonas reinhardi, ac-31, is characterized by the absence of any stacking of its chloroplast membranes. The capacity for photosynthetic electron transport, phosphorylation, and CO₂ fixation in ac-31 is substantial, and it is concluded that these photosynthetic activities occur within the single membrane. The photosynthetic capacities of wild type and ac-31 as a function of increasing light intensity are compared. Saturation is attained at higher light intensities in ac-31, and the kinetics of the 2 sets of curves are distinctly different. The possibility that energy transfer is enhanced by membrane stacking is suggested by these results. The repeatedly-observed correlation between reduced stacking and disfunctional Photosystem II activities is discussed in view of the observation that ac-31 has no stacking but retains a functional Photosystem II.

In the preceding paper (13) it was demonstrated that the chloroplasts of photosynthetic mutant strains of C. reinhardi exhibit normal disc formation but altered patterns of disc association or "stacking". In 1 class of mutant strains, which includes ac-115, ac-141, and F-34, a preponderance of "unstacked", single discs is found. These strains all lack an active cytochrome 559, a component of the electron transport chain that lies close to Photosystem II (PS II), and none exhibits significant Hill activity with oxidants such as 2,6-dichlorophenolindophenol (DPIP), p-benzoquinone, or potassium ferricyanide. In contrast, the other photosynthetic mutant strains examined are all capable of Hill activity with these oxidants, and none shows a preponderance of single discs.

The observed correlation of defective Hill activity with defective disc association was of interest in light of a recent paper by Homann and Schmid (19), where it is shown that chloroplasts from yellow-green sectors of the variegated tobacco mutant, NC 95, lack both Hill activity and chloroplast membrane stacking. These authors propose that PS II activity is dependent on the closely-packed association of stacked membranes, and suggest that stacking creates a hydrophobic environment where the oxidation of water can occur.

In this paper we describe the ultrastructure and some of the photosynthetic properties of ac-31, a pale-green strain of C. reinhardi. The chloroplast discs of ac-31 exhibit no stacking whatever, and yet PS II activity is very high on a chlorophyll basis and substantial on a cell basis. Other parameters of photosynthetic activity have also been measured, and most of these are judged to be intact. We conclude that stacking is not required for photosynthetic electron transport, photosynthetic CO₂ fixation, or photosynthetic phosphorylation, but that it may possibly play a role in the transfer of excitation energy.

Materials and Methods

Culture of the Organism. Cultures of wild-type, ac-31, and ac-1 strains of C. reinhardi were grown under conditions described in the previous paper (13). Cells grown in minimal salt medium are referred to as minimal-green, and cells grown in an acetate-supplemented minimal medium are referred to as acetate-green. The wild-type strain described in the preceding paper has since been recloned, and the chlorophyll a:b ratio of the clone used in the present experiments is somewhat lower than that of the earlier clone. The photosynthetic and fine-structural properties of the 2 clones are otherwise similar.

Electron Microscopy. Procedures used for specimen preparation are as previously described (13). The plates for Figs. 1-4 were photographed at original magnifications of 42,500×. Measurements of membrane diameters were made on plates of the same magnification, using either a Nikon Shadowgraph (Model C) or a Joyce-Loeb recording microdensitometer.

Chlorophyll Determinations. Total chlorophyll, chlorophylls a and b, and cell number were determined as in the preceding paper (13).

Photosynthetic Capacity. Photosynthetic electron transport reactions were assayed using a model 14 Cary recording spectrophotometer (15,33) with chloroplast fragments prepared from cells disrupted

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by sonication (25). Cyclic and non-cyclic photosynthetic phosphorylation were measured as previously described (11, 14) with chloroplast fragments prepared from cells disrupted by grinding with sand (14).

The fixation of carbon dioxide by photosynthesis and photoreduction in whole cells was measured in two ways, either as the incorporation of 14C-labeled NaHCO3, or by a titrimetric method which measures the pH change accompanying the uptake of HCO3- by whole cells in the light.

The rate of 14CO2 fixation by photosynthesis was measured at 40,000 lux and 25° using cells that had been washed once and resuspended in minimal medium. The cell suspension, placed in a small flask, was agitated by a continuous stream of air for 5 min in the light before the addition of NaH14CO3. Samples of 0.1 ml were taken at 1 min intervals for 5 min and plated to stainless steel planchets containing 0.1 ml of a mixture of concentrated HCl and glacial acetic acid (4:1). The samples were dried and counted with the aid of a gas-flow counter. Correction for light-independent 14CO2 fixation was obtained from control experiments run in an identical manner but in the dark.

The fixation of 14CO2 by photoreduction was measured in a similar manner except that the cells were agitated with pre-purified hydrogen that had been passed through a Deoxocartridge. The cell suspension was incubated in the dark for 15 min in order to activate the hydrogenase, whereupon the light was turned on. NaH14CO3 was added after 5 min, and samples were taken and treated as described above for photosynthetic CO2 fixation. The cell suspension contained 10 μl 3,3′,4,4′-dichlorophenyl)1,1-dimethyl urea (DCMU) in order to inhibit oxygen evolution. The rate-versus-intensity curves for the fixation of CO2 by photoreduction were obtained using this method.

Rate-versus-intensity curves for photosynthetic CO2 fixation were obtained by a titrimetric method which gives rates comparable to those obtained by determining the rate of 14CO2 incorporation, and which has the advantage of being both economical and rapid when many measurements are to be made. Cells were washed and resuspended in 2.5 mM NaHCO3. Photosynthetic CO2 fixation was measured by means of a Radiometer titrimeter assembly comprising a Model 28 pH meter, Model 11 titrator, a type SB2RC titrigraph, and a type SBU4 syringe burette. The electrode was an Arthur H. Thomas Company combination pH electrode 4858-L60. Illumination was provided by a 1000 watt tungsten projection lamp, and the temperature was maintained at 25°. The titrimeter was used in the pH stat mode with 0.1 M HCl as the titrant. The pH was maintained at 7.8. and the titrigraph provided a continuous record of the amount of titrant needed to maintain this pH in the reaction vessel. Thus a direct record of the uptake of HCO3- by cells was obtained. A relatively constant, small dark rate was recorded which was the same in the presence or absence of cells, and rates in the light were corrected for this dark rate.

Rate-versus-intensity Curves. The rate-versus-intensity curves for CO2 fixation by photosynthesis and photoreduction were obtained by comparing these reactions at different light intensities from a 1000 watt tungsten projection lamp. Full intensity was 136,500 lux, and lower intensities were obtained with the aid of neutral density filters or neutral density screens. Two ml reaction mixtures were used containing cells equivalent to 12.5 μg chlorophyll per ml. The cells were washed and resuspended in the appropriate medium (minimal medium for photoreduction measurements and 2.5 mM NaHCO3 for measurements of photosynthetic CO2 fixation). The concentration of the cell suspension was adjusted to cells equivalent to 12.5 μg chlorophyll per ml and the suspension (about 100 ml) was placed in the dark where it was agitated with a magnetic stirrer. A different 2 ml sample from this suspension was used for each measurement made, and certain points were checked at the end of the experiment to insure that the cells had not changed in their photosynthetic capacity during the time that they were kept suspended in the dark. At the conclusion of 1 such experiment, the cells remaining in the suspension were fixed and examined with the electron microscope, and they were found to be identical to cells harvested in the light.

Results

Chloroplast Ultrastructure. The fine structure of the chloroplasts of wild-type and certain mutant strains of C. reinhardtii is described in detail in the previous paper (13). Figs. 1 to 4 of the present

Fig. 1. Chloroplast from minimal-grown ac-31, illustrating the maximal degree of disc association observed in the strain. In certain places (e.g. at arrow) an appearance of membrane contact is given but this is in fact a sectioning effect (see text). S, chloroplast stroma containing chloroplast ribosomes; Cy, cytoplasm containing cytoplasmic ribosomes; St, starch. 69,750×.

Fig. 2. Chloroplast from acetate-grown ac-31, illustrating a region of maximal proximity between discs (arrow) at higher magnification. A gap of ~ 20 Å separates the two membranes. 111,365×.

Fig. 3. Chloroplast from acetate-grown ac-31. Disc membranes tend to be further apart than in minimal-grown (Figs. 1 and 4) cells. 69,750×.

Fig. 4. Chloroplast from minimal-grown ac-31, showing a more representative field than Fig. 1. Discs approach each other in limited regions, but are generally at least 10 Å apart. 69,750×.

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paper depict chloroplasts of ac-31. Most morphological features of the ac-31 chloroplast are comparable to the wild type, but the chloroplast membranes never come together to form a stack. Fig. 3 is a typical field from acetate-grown cells, and the chloroplast discs are seen to be far apart in most places. In a typical field from minimal-grown cells (Fig. 4), the membranes tend to lie closer together than in acetate-grown cells (Fig. 3). Fig. 1 shows a chloroplast from a minimal-grown cell which exhibits the maximum amount of membrane association ever found in ac-31. Here the membranes lie very close together for long distances, but they never touch. In certain places (e.g., Fig. 1, arrow), the membranes appear to make contact, but these regions are produced when 2 adjacent membranes go out of the plane of the section: they have been repeatedly shown by microdensitometer tracings to be wider and less dense than a 2-disc stack.

It should be stressed that a field such as that shown in Fig. 1 was intentionally chosen to indicate the most extreme degree of membrane association in ac-31, and that the discs are not commonly so uniformly close together. Nonetheless, it is evident in all the micrographs that, at least in limited regions, the membranes of adjacent discs often get very close to one another, separated only by a narrow gap. Such a gap is shown at higher magnification in Fig. 2 (arrow). Careful microdensitometer measurements have been made which indicate that the minimum gap distance (i.e. the space between the apposing surfaces of 2 adjacent membranes) is of the order of 17 to 23 Å. Even at this minimum width, the membranes would seem to be too far apart to permit any photosynthetic electron transport between them, and since the gaps are visibly continuous with the aqueous strona, there is no reason to suppose that they constitute a hydrophobic environment.

The 17 to 23 Å gap observed in ac-31 should not be confused with the "A" space which sometimes appears between the stacked membranes of higher plants and which is reportedly 19 Å wide (36). Spaces between stacked membranes are not observed in wild-type C. reinhardtii, nor in any of the other mutant strains we have studied (13): the width of a stacked region (116-122 Å) is consistently twice the width of a single membrane (59-62 Å). Since our fixation procedure for ac-31 is identical to the procedure used for all the other strains, it seems unlikely that we have preserved an "A" space in ac-31 alone.2

Chlorophyll Content. In Table I, the chlorophyll content of ac-31 is compared with wild type. It is evident that minimal-grown cells have a lower chlorophyll content than acetate-grown cells in both strains, and that ac-31 is chlorophyll-deficient compared to wild type under both growth conditions. The chlorophyll deficiency of ac-31 is equivalent to that of ac-1, another pale-green mutant strain of C. reinhardtii having 0.8 to 1.3 µg chlorophyll/10⁵ cells, and yet ac-1 is capable of normal stacking (13); thus, a pigment deficiency does not, per se, produce stacking aberrations. It should be stressed that both ac-1 and ac-31 contain a substantial amount of chlorophyll in comparison with many pigment-deficient strains; yellow strains of C. reinhardtii, for example, have chlorophyll contents of 0.03 to 0.08 µg chlorophyll/10⁵ cells (21, 28), or 10 to 15 fold less than either ac-1 or ac-31.

In addition to being chlorophyll-deficient, ac-31 is also somewhat chlorophyll b-deficient compared to wild type (Table I) or to ac-1. This b-deficiency is, however, modest in comparison with certain b-deficient (18, 30) or b-less (1) mutant strains of higher plants, and yet the higher plants are all capable of substantial membrane stacking (12, 18, 30).

Growth Rates. Growth curves for wild type, ac-1 and ac-31 on minimal medium are given in Fig. 5. The ac-31 growth rate is slow, even compared with the comparably chlorophyll-deficient ac-1. Growth rates on acetate-supplemented media are, of course, much higher for all 3 strains (not shown).

Photosynthetic Capacity. The fact that ac-31 can grow on minimal medium indicates in itself that the strain is capable of photosynthesis. An analysis

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1 Weier et al. (36) have proposed that the "A" space contains chlorophyll and other hydrophobic components, and that these are normally extracted during ethanol dehydration such that the space is no longer observable with the electron microscope. Our rapid dehydration procedures (total time in ethanol ≤10 min) extract little chlorophyll, the tissue is still very green when it is sectioned, and no "A" space is observed. We thus cannot agree that the "A" space corresponds to the presence of chlorophyll.

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<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth conditions</th>
<th>Total chl</th>
<th>Chl a</th>
<th>Chl b</th>
<th>Chl b</th>
<th>Chl a:chl b</th>
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<tr>
<td></td>
<td>µg/10⁶ cells</td>
<td>µg/10⁶ cells</td>
<td>mg/10⁶ cells</td>
<td>%</td>
<td>ratio</td>
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<td>Wild type</td>
<td>Acetate</td>
<td>4.2 ± 0.51</td>
<td>2.6 ± 0.3</td>
<td>1.7 ± 0.09</td>
<td>40</td>
<td>1.7 ± 0.08</td>
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<tr>
<td>Wild type</td>
<td>Minimal</td>
<td>2.5 ± 0.3</td>
<td>1.7 ± 0.3</td>
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<td>34</td>
<td>1.9 ± 0.2</td>
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<td>ac-31</td>
<td>Acetate</td>
<td>1.1 ± 0.07</td>
<td>0.84 ± 0.3</td>
<td>0.23 ± 0.09</td>
<td>23</td>
<td>3.4 ± 0.4</td>
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<td>ac-31</td>
<td>Minimal</td>
<td>0.70 ± 0.08</td>
<td>0.49 ± 0.05</td>
<td>0.16 ± 0.03</td>
<td>23</td>
<td>3.0 ± 0.4</td>
</tr>
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</table>

1 Mean ± standard deviation for 3 independent experiments.
Table II. Carbon Dioxide Fixation by Photosynthesis and Photoreduction by Wild-type and ac-31 C. reinhardi

Photosynthetic CO₂ fixation was measured at 25° in 2 ml reaction mixtures of minimal medium containing cells equivalent to 12.5 µg chlorophyll/ml. After equilibration for 5 min in the light, 0.2 ml of NaH¹⁴CO₃ (0.5 µc per mole per ml) was added. Samples were taken at 1 min intervals.

The fixation of CO₂ by photoreduction was measured at 25° in 2 ml reaction mixtures of minimal medium containing cells equivalent to 12.5 µg chlorophyll/ml and 10 µM DCMU. Following equilibration in the dark with hydrogen gas, the light was turned on, and 0.2 ml of NaH¹⁴CO₃ (0.5 µc per mole per ml) was added after 5 min. Samples were taken at 1 min intervals.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Photosynthesis</th>
<th>Photoreduction</th>
</tr>
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<tr>
<td>Wild type</td>
<td>115¹</td>
<td>0.336²</td>
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<tr>
<td>ac-31</td>
<td>184</td>
<td>0.202</td>
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</table>

¹ µmoles CO₂ fixed per hr per mg chlorophyll.
² µmoles CO₂ fixed per hr per 10⁶ cells.

Table III. Electron Transport Reactions by Chloroplast Fragments of Wild-type and ac-31 C. reinhardi

For the Hill reaction with DPIP, the cuvette in the same compartment of the spectrophotometer contained chloroplast fragments (10 µg chlorophyll) prepared by the sonication of cells, and the following in µmoles: potassium phosphate, pH 7.0, 20; KCl, 40; MgCl₂, 5.0; and DPIP, 0.1. The final volume was 2.0 ml. The DPIP was omitted from the control cuvette in the reference compartment.

For the Hill reaction with NADP, the cuvette contained chloroplast fragments (10-15 µg chlorophyll) prepared by the sonication of cells, and the following in µmoles: potassium phosphate, pH 7.0, 20; KCl, 40; MgCl₂, 5.0; NADP, 0.5; and ferredoxin prepared from wild-type C. reinhardi, 0.05. Half a unit of ferredoxin-NADP reductase, prepared from wild-type C. reinhardi, was also added. The final volume was 20 ml. Ferredoxin, ferredoxin-NADP reductase, and NADP were omitted from the control cuvette.

For the photoreduction of NADP from the DPIP-ascorbate couple, the reaction mixture contained, in addition to the components for the NADP Hill reaction, the following in µmoles: DPIP, 0.1; sodium ascorbate, pH 7.0, 10; and DCMU, 0.02. The control cuvette contained everything but ferredoxin, ferredoxin-NADP reductase, and NADP.

All reactions were run at a light intensity of 20,000 lux and 25°.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Hill reaction</th>
<th>NADP reduction with DPIP-ascorbate couple</th>
</tr>
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<tr>
<td></td>
<td>DPIP</td>
<td>NADP</td>
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<tr>
<td>Wild type</td>
<td>84¹</td>
<td>0.398²</td>
</tr>
<tr>
<td>ac-31</td>
<td>265</td>
<td>0.169</td>
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</table>

¹ µmoles photoreduced per hr per µg chlorophyll.
² µmoles photoreduced per hr per 10⁶ cells.
Table IV. Cyclic and Non-cyclic Photosynthetic Phosphorylation by Wild-type and ac-31 C. reinhardtii

The reactions were run at 25°C in 25 ml Erlenmeyer flasks. The reaction mixture (2 ml) contained chloroplast fragments (40–80 μg chlorophyll) prepared from cells disrupted by grinding with sand, and the following in μmoles: glycylglycine buffer, pH 8.0, 40; MgCl₂, 10; ADP, pH 7.5, 5; AMP, pH 7.5 in NaOH, 5; and potassium phosphate buffer, pH 8.0, 10, containing 0.5 to 1.0 μcurie³²P₁. For cyclic photosynthetic phosphorylation, the mixtures contained 0.067 μmole of phenazine methosulfate and 0.02 μmole of DCMU. For non-cyclic photosynthetic phosphorylation, 2 μmoles of potassium ferricyanide were added. All reaction flasks were flushed continuously with nitrogen throughout the experiment. Reactions were terminated after 3 min of illumination (30,000 lux) by turning off the lights and by adding 0.2 ml of 20% trichloroacetic acid to each flask. Dark controls were run in flasks covered with black tape.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cyclic</th>
<th>ATP formed</th>
<th>Non-cyclic</th>
<th>Ferricyanide reduced</th>
<th>P/2 e⁻</th>
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<tr>
<td>Wild type</td>
<td>154¹</td>
<td>0.730²</td>
<td>102¹</td>
<td>0.306²</td>
<td>488¹</td>
</tr>
<tr>
<td>ac-31</td>
<td>1270</td>
<td>1.73</td>
<td>491</td>
<td>0.669</td>
<td>970</td>
</tr>
</tbody>
</table>

¹ μmoles per hr per mg chlorophyll.
² μmoles per hr per 10⁶ cells.

Fig. 6. Rate-versus-intensity curves for wild type (□ · · · · · · · · □), ac-31 (○ — — — — — — □), and ac-1 (△ · · · · · · · △). The rate of photosynthetic CO₂ fixation was measured against light intensity (% of 136,000 lux). The titrimetric method was used. Cells were washed and resuspended at a chlorophyll concentration of 12.5 μg/ml in 2.5 mM sodium bicarbonate. Two ml were placed in the thermostated vessel of the titrimer, and they were agitated with a magnetic stirrer. The temperature was maintained at 25°C. Measurements were made in the dark and in the light for at least 5 min. The values plotted in the figure have been corrected for the dark uptake of HCO₃⁻. Light was provided by a 100 watt tungsten lamp.

Even on a cell basis, the photosynthetic rates of ac-31 are at least half the rates of wild type. If chloroplast membrane stacking were required for any of the reactions tested, the rates would be expected to be nil.

An interesting feature of the photosynthesis of ac-31 is its high coupling ratio (P/2e⁻) for non-cyclic photosynthetic phosphorylation and its high rates of cyclic phosphorylation compared to wild type. A possible explanation for this phenomenon is that the fused discs of wild-type chloroplasts are somehow more subjected or sensitive to damage during the process of cell disruption than are the free discs of ac-31, and that this damage somehow uncouples both cyclic and non-cyclic phosphorylation from photosynthetic electron transport. A more interesting possibility is that stacking may somehow serve to regulate rates of photosynthetic phosphorylation.

Photosynthetic Efficiency. Fig. 6 gives plots of photosynthetic CO₂ fixation against increasing light intensity (rate-versus-intensity curves) for ac-31, ac-1, and wild type, and Fig. 7 shows the initial
Fig. 7. Rate-versus-intensity curves for wild type (■——△) and ac-31 (●——○——●). Each set of points represents a separate experiment. The procedure was the same as described in the text and for Fig. 6. A, B, and C indicate the three different phases of the rate-versus-intensity curve for ac-31.
portions of such curves for 5 different experiments, 2 with wild type and 3 with ac-3I. Two features of these plots are of interest. One is that the rates of CO₂ fixation for ac-3I tend to saturate at somewhat higher light intensities than either ac-I or wild type. This difference is most readily explained by the somewhat lower chlorophyll a:b ratios of ac-3I compared to the other 2 strains (table I), since b-deficient strains of higher plants are known to saturate at high light intensities (1, 18). In other words, ac-3I, by virtue of its b-deficiency, would be expected to trap photons less effectively than either ac-I or wild type.

If a chlorophyll b-deficiency were the only factor limiting the efficiency of photosynthesis by ac-3I, then one would expect its rate-versus-intensity curve to resemble the curves obtained for ac-I and wild type except that the initial linear slope seen in ac-I and wild type would be longer and less steep in ac-3I. In fact, a second difference is noted, for it is evident in Fig. 6 that the initial slope for ac-3I is not linear, but has instead 2 distinct components. These components are explicitly delineated in Fig. 7: a rapid initial phase (Fig 7, A) is followed by a less rapid second phase (Fig. 7, B) until saturation is approached (Fig. 7, C). Such biphasic kinetics are observed in repeated experiments. As Fig. 7 indicates, and they are not evident in the rate-versus-intensity curves obtained for the b-less mutant of barley (1), indicating that they are not the consequence of a b-deficiency alone. It would seem, then, that perhaps 2 factors are limiting the photosynthetic efficiency of ac-3I. At low light intensities, 1 rate-limiting step appears to dictate the amount of photosynthesis the cell can carry out, whereas at high intensities, a second step becomes limiting until a final state of saturation is attained.

It was stated earlier that the distance separating adjacent disc membranes in ac-3I is usually a large one, but that the membranes sometimes approach each other and may come as close together as 17 to 23 Å. Distances of this magnitude, although probably too great to permit electron transport, are not too great to allow substantial transfer of excitation energy by a mechanism of inductive resonance. For example, according to Forster theory (7), transfer efficiency is still very high when the distance separating donor and acceptor molecules is in this range. We are led to propose, then, that 1 of the 2 steps that limits the photosynthetic efficiency of ac-3I is possibly a less efficient transfer of excitation energy from existing sites of photon trapping to reaction centers. We have in mind a model in which at least some transfer of excitation energy normally occurs between donor and acceptor molecules that are located on or within 2 different membranes; that when these membranes are adjacent, i.e. stacked, energy transfer between such molecules is very efficient, but that when they are separated, as in ac-3I, this transfer becomes less efficient, and this somehow limits the rate of photosynthesis. Clearly this model has only circumstantial support at present, but we feel it has sufficient interest to be put forth in this paper.

In contrast to the differences observed for wild type and ac-3I with respect to their rate-versus-intensity curves for CO₂ fixation by photosynthesis, the curves obtained for photorespiration were found to be identical (not shown). It seems reasonable to assume, therefore, that the factors affecting photosynthetic efficiency in ac-3I do not alter a process that depends on the operation of PS I alone.

Photorespiration. The apparent rate of photosynthesis can be affected by photorespiration (reviewed in ref. 38). Since differences between wild type and ac-3I photosynthetic rates might account for some of the differences seen in rate-versus-intensity curves (39), it became important to test for this possibility. Photorespiration rates are dependent both on CO₂ tension and light intensity (38). We therefore measured growth rates of ac-3I and wild type at high (5% in air) and low (atmospheric) CO₂ concentrations in the expectation that, if photorespiration rates differed in the 2 strains, the ratio of the growth rate on high CO₂ to that on low CO₂ for wild type would be different from the ratio obtained for ac-3I. In fact, the ratios were the same. In a similar experiment, CO₂ fixation by photosynthesis was measured for the 2 strains both at high and low CO₂ concentrations and at high (136,500 lux) and low (12,000 lux) light intensities. Again, the ratios obtained for wild type and for ac-3I did not differ. Thus, photorespiration differences are not thought to contribute to the differences in the rate-versus-intensity curves seen in Figs. 6 and 7.

Discussion

The ac-3I Mutation. The close association of biological membranes is a rare phenomenon, found only in the chloroplast, in the specialized “tight” junctions between certain cells (6), and perhaps in the myelin sheath surrounding nerve cells. It is not established whether chloroplast membranes actually fuse together or only become very closely apposed; in either case, the association is apparently a strong one, for it is retained in sonicated and sand-ground chloroplast fragments of C. reinhardtii (unpublished observations).

The ability of chloroplast membranes to form a stack can thus be considered as one of their distinctive characteristics, and this ability can evidently be lost as a consequence of the ac-3I mutation. The ability to stack may be a property of the membrane proteins themselves. In this case, the ac-3I mutation might result in an alteration of the charge properties, or perhaps the conformation, of these proteins such that they can no longer interact with one another. Alternatively, stacking may be mediated by some component, a stacking “factor,” which
acts as a kind of “glue” to hold adjacent membranes together.

Three allelic xantha mutant strains of barley have been described that also appear unable to form closely-aggregated grana (37), and possibly these strains suffer from a defect similar to that affecting ac-21. Mutations at the xantha and ac-21 loci exhibit Mendelian segregation, and ac-21 has been mapped to linkage group V of the C. reinhardii genome (4). Thus a property that is particularly associated with chloroplast membranes is apparently under at least partial nuclear control.

Stacking and Photosynthesis. Stacking is found in the chloroplasts of most algae and higher plants (reviewed in ref. 24). Important exceptions are the blue-green, red, and brown algae (2, 3, 5, 10, 16, 27, 29). The major light-harvesting pigments in these algal groups are the phycobilins and fucoxanthins, and it is possible that their presence effects modifications of the membrane architecture (8, 9, 17). In any case, the existence of chloroplasts that do not require stacking for photosynthesis does not negate the hypothesis that stacking is important for the photosynthesis of chloroplasts in which stacking occurs.

As mentioned earlier, Homann and Schmid (19) have suggested that PS II activity is dependent on stacking in higher plants. Two published observations do not support this suggestion. Obad et al. (28) claim that in regreening chloroplasts of a C. reinhardii yellow mutant, the resumption of Hill activity slightly precedes the onset of stacking, and Izawa and Good (22) find that spinach chloroplasts can be so disrupted by exposure to Tricine and low salt that all membrane fusion disappears, and that such preparations will reduce Hill oxidants at very high rates. The former authors suggest that the fusion of discs may be important in “quantum conversion efficiency,” whereas the latter suggest it may somehow be involved in CO₂ fixation.

The experiments with ac-21 reported in this paper demonstrate that chloroplast membrane stacking in C. reinhardii is not required for CO₂ fixation by photosynthesis or by photoreduction, cyclic and non-cyclic photosynthetic phosphorylation, the light-induced reduction of Hill oxidants from water, or for the light-induced reduction of NADP from water or from the dye-ascorbate couple. Unless C. reinhardii proves to be very different from a higher plant, then, we cannot agree with Homann and Schmid’s suggestion that PS II activity requires the hydrophobic environment provided by stacked membranes, nor with the concept (35) that electron transport and photophosphorylation occur within chloroplast “partitions” or stacks.

Reduced Stacking and Defective PS II. In contrast to ac-21, chloroplasts have been described in which stacking is markedly reduced, but in which substantial stacking still occurs. These include the chlorophyll b-less mutant of barley (12), Mn-deficient C. reinhardii (34), chloramphenicol-treated C. reinhardii (20), and the mutant strains ac-21 (23, 26) and ac-115, ac-141, and F-34 (13) of C. reinhardii. In every case, the cells are further characterized by the absence or inactivity of components closely associated with PS II. In several cases, moreover, the cells can be cured of their PS II deficiency (Mn can be added back to Mn-deficient cells, for instance), and normal patterns of stacking are recovered at the same time that PS II activity is restored (13, 20, 23, 24).

An apparent paradox thus arises: defective PS II and a reduction in stacking frequently occur together, whereas an active PS II is found in ac-21 which has no stacking at all. These observations can be reconciled by proposing that a disorganized PS II has the effect of curtailing normal membrane stacking. This suggestion carries the implication that components of PS II are localized on or near the membrane surface which normally makes contact with adjacent membranes, as opposed to the surface facing the intra-disc space. Thus, a normal PS II organization would allow, or even facilitate, an effective stacking process, whereas an abnormal organization would impede it. Indeed, the disorganization of PS II might become so severe that stacking becomes physically impossible, a situation that perhaps arises in the yellow-green chloroplasts of the NC 95 tobacco mutant (19).

Stacking and Chlorophyll. It has been suggested that chlorophyll is packed within a granum to prevent its being inactivated by photooxidation (31). It has also been predicted that if chlorophyll is present in a green alga or higher plant, grana will also be present (35, 36). Since chloroplasts of ac-21 contain substantial quantities of chlorophyll and no grana, and since this chlorophyll is clearly very “active,” these proposals are not supported by our results.

The fact remains that ac-21 is deficient in chlorophyll. We do not believe that the missing chlorophyll corresponds to the missing inter-disc “glue,” since ac-1 has similarly low chlorophyll levels and normal stacking (13). However, it is possible that the absence of stacking may secondarily produce a pigment deficiency, and that stacking is, at least in part, “a device to pack more chlorophyll into the chloroplast” (32).

If this were the only “function” of chloroplast membrane associations, one would expect that the growth rates of ac-21 and ac-1 on a minimal medium would be comparable, that is, that both strains would be limited in their photosynthetic capacity to a similar extent by their similar chlorophyll deficiencies. In fact, ac-21 grows much more poorly than ac-1 on minimal medium (Fig. 5). We are thus drawn to the conclusion that stacking does participate in photosynthesis, but that its role is more subtle than those that have previously been proposed. Other aspects of the photosynthesis of ac-21, such as the light-induced pH change, the 520 nm absorbance
changes, control of photosynthetic phosphorylation, fluorescence phenomena, and particularly phenomena relating to the transfer of excitation energy, are currently being studied in this laboratory in an attempt to define this role more explicitly.

Meanwhile, we wish to stress that, at least in C. reinhardtii, the complete apparatus for photosynthetic electron transport and for photosynthetic phosphorylation, and approximately one third of the cell's chlorophyll, appear to be built into the structure of the single chloroplast membrane.

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Literature Cited


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