Photosynthetic Studies on a Pea-mutant Deficient in Chlorophyll

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Abstract. A chlorophyll-deficient mutant of pea (Pisum sativum) was found as a spontaneous mutation of the variety Greenfeast. Total chlorophyll of the mutant leaves was about one-half that of normal pea leaves per mg dry weight, and the ratio of chl a:chl b ranged from 10 to 18, compared with 3 for normal pea. In each generation the mutant plants gave rise to normal and mutant plants and lethal plants with yellow leaves.

For a normal pea plant, CO₂ uptake was saturated at about 60,000 lux, whereas with mutant leaves, the rate of CO₂ uptake was still increasing at 113,000 lux. At 113,000 lux the mutant and normal leaves showed similar rates of CO₂ fixation per unit area of leaf surface, but on a chlorophyll basis the mutant leaves were twice as active. Hill reaction measurements on isolated chloroplasts also showed that the mutant chloroplasts were saturated at higher intensities than the normal, and that the activity of the mutant was at least double that of the normal on a chlorophyll basis.

It is suggested that the photosynthetic units of the mutant chloroplasts contain about half the number of chlorophyll molecules as compared to the normal photosynthetic units.

Electron microscopy of leaf sections of normal and mutant leaves showed that the mutant chloroplasts contain fewer lamellae per chloroplast and fewer lamellae per granum. The lethal chloroplasts, which were virtually devoid of chlorophyll, were characterized by an absence of grana.

Previous studies with a mutant of barley (Hordeum vulgare L.) devoid of chlorophyll b indicated that chlorophyll b is not essential for photosynthetic activity in a higher plant (6,11,13). Photosynthetic rates obtained with the mutant plants did not differ significantly from those obtained in normal plants (13). Hill reaction measurements on isolated chloroplasts showed that the mutant chloroplasts were more active per mg of total chlorophyll if assayed at saturating light intensities, but less active at low light intensities (6). The mutant chloroplasts were less fluorescent (7), and their molar ratio of total chlorophyll to cytochrome b₅₅ plus cytochrome 559 was only about one-half of the corresponding ratio of normal chloroplasts (4).

It was concluded from these studies that the mutant chloroplasts have less chlorophyll in their light-harvesting assemblies than the normal chloroplasts, but comparable amounts of the components of the photosynthetic electron transport chain (4). It was further suggested that the mutant chloroplasts have a decreased amount of chlorophyll in photosystem 2, relative to photosystem 1 (7). This would result in photosystem 2 absorbing a lower fraction of the incident quanta and would account for the lower photochemical efficiency of the mutant chloroplasts at low light intensities.

Electron microscopy of mutant leaf sections showed fewer grana in the mutant chloroplasts and fewer lamellae per granum, in comparison with the normal chloroplast (8).

In the present studies, we have investigated the photosynthetic properties of a pea mutant deficient in both chlorophyll a and chlorophyll b, and examined the fine structure of its chloroplasts. The level of total chlorophyll is shown to be lower in the pea than in the barley mutant, and a study of its photosynthetic properties enables a useful comparison with the barley mutant, and the chlorophyll-deficient tobacco mutants of Schmid and Gaffron (18,19) and Homann and Schmid (14). A study of the relationship between photosynthetic activity and structure in such chlorophyll-deficient mutants may assist in the elucidation of the molecular organization of the chloroplast. A summary of this work was presented previously (12).

Materials and Methods

Plant Material. The mutant pea was derived from a commercial variety (Pisum sativum, var.

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1 This work was supported in part by a research grant (GB3341) from the National Science Foundation.
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Greenfeast) as a spontaneous mutation. The mutant was observed in a population of plants growing in artificial light at a temperature of 21°/19° and a photoperiod of 16 hr. It was distinguishable from the other plants by the pale-green color of its leaves. The mutant was grown to maturity, and the seed saved and grown for a second generation. Seeds from each of these plants were harvested and grown for a third generation, and this procedure was repeated for a number of generations to date. After the first generation, the plants were grown in a controlled temperature glasshouse of the CSIRO phototron in a 16 hr photoperiod and a temperature of 21°/19°. Normal pea plants were grown under identical conditions.

Preparation of Chloroplasts. Leaves (3-5 g fresh wt) either from mutant or normal plants were ground in a mortar, chilled in ice, with 15 ml of 0.05 M phosphate buffer (pH 7.2), containing 0.3 M sucrose and 0.01 M KCl. The resulting brei was filtered through 2 layers of Miracloth, and the chloroplasts sedimented by centrifugation at 1000g for 10 min. The chloroplasts were resuspended in the sucrose-phosphate buffer (30 ml) and again centrifuged at 1000g for 10 min. Chloroplasts obtained from 3 g of mutant leaves were resuspended in 3 ml of sucrose-phosphate buffer and those from 3 g of normal leaves were resuspended in 6 ml.

Chlorophyll Determinations on Chloroplasts or Leaves. Total chlorophyll was determined in 80% acetone with a Cary recording spectrophotometer, using the equations of Arnon (1). For determinations with whole leaves 1.0 g fresh wt samples were ground in 80% acetone in a Servall Omnimixer. The residue obtained after centrifugation was extracted with 80% acetone in the Omnimixer, and the procedure repeated. The 3 supernatants were combined and made up to 250 ml. Dry weights were determined after heating samples at 80° until a constant weight was achieved, usually 24 hr. Leaf areas were obtained by outlining leaves on mm paper.

Chl a:chl b ratios of mutant leaves or mutant chloroplasts were determined in ethyl alcohol by a sensitive spectrofluorimetric method, full details of which will be published elsewhere. Briefly, the ethanol extracts were diluted to an absorbancy of 0.2 at 436 nm, and cooled to the temperature of liquid nitrogen. Fluorescence emission spectra were recorded on a fluorescence spectrometer incorporating automatic correction for photomultiplier and monochromator responses, and variation in energy output of the light source (7). The extract was excited at 474 nm, the wavelength maximum for chlorophyll b, and the chl a:chl b ratio determined from the relative amplitudes of the fluorescence emission at 680 nm (Chl a) and 656 nm (Chl b). The method was calibrated with mixtures of purified chlorophyll a and chlorophyll b.

Absorption spectra of intact leaves at 77°K were recorded on a Cary Model 14R spectrophotometer, equipped with a scattered transmission accessory (6).

Carbon Dioxide Fixation. Carbon dioxide uptake by leaves of mutant and normal plants was determined with an infra-red gas analyzer (Grubb Parsons). A leaf attached to the plant was enclosed in a perspex airtight container, and an air sample containing 300 ppm of CO₂ was pumped across the leaf at a rate of 2000 cc/min. The leaf was illuminated with 4 Mazda FBRU 1000 watt mercury vapor high pressure lamps, and the light intensity was varied with neutral grey "Sarlon" shade cloth. Light intensities were measured with a undirectional Kipp thermopile and also with a selenium light meter (El Instruments). The temperature of the leaves was determined with a thermocouple placed under an identical set of leaves in the same cabinet as the experimental leaves. The temperature of the cabinet was 17.5°.

Photochemical Activities of Chloroplasts. Hill reaction measurements were made as described previously (1), except that ferricyanide reduction was measured directly in a Cary spectrophotometer by the absorbance decrease at 418 nm.

White-light intensities in the range of 1400 to 200,000 lux were obtained from a 250 watt photoflood lamp operated at 200 volts. The cuvette containing the reaction mixture was positioned at varying distances from the lamp. Light intensities were measured with a Weston meter, Model 603, calibrated in ft-c.

Electron Microscopy. For electron microscopy several leaf pieces, approximately 1 mm², were cut from the second leaf of normal, mutant or lethal pea plants and fixed in 3% glutaraldehyde (purified by vacuum distillation) in 0.025 M phosphate buffer pH 7.2 for 1.5 hr at 20°. After washing in phosphate buffer for 1 hr the leaf pieces were post-fixed in 2% osmium tetroxide in 0.025 M phosphate buffer pH 7.2 for 2 hr at 20°, washed in buffer, dehydrated in ethanol, and embedded in an araldite epon mixture (17). The resin was vacuum infiltrated at 85° and polymerized at the same temperature for 24 hr before sectioning with an LKB ultra-microtome. Sections were stained with saturated uranyl acetate in 50% ethanol for 1 hr followed by Fiske's lead stain (9) before examination in a Philips EM200 electron microscope.

Electron micrographs of the chloroplasts were examined for distinctness and resolution of lamellae and 10 each of the mutant and normal were selected. The numbers of single lamellae, groups of lamellae (grana) per chloroplast, and number of lamellae per group were recorded for each chloroplast and the results analyzed statistically.

Results

Chlorophyll Content of Leaves. The pale-green leaves of the original mutant plant gave an absorp-
Chlorophyll contents of leaves from the second generation of plants are shown in Table I. Leaves from young mutant plants contained about one-half of the total chlorophyll of the normal plants of the same age. The plants were sampled again after further growth for 3 weeks. The chlorophyll contents were higher by about 25%, but the relative amounts in the normal and mutant remained about the same. The chl a:chl b ratio of mutant leaves declined from 15 at 3 to 4 weeks to 10.3 at 8 to 9 weeks.

The chlorophyll contents reported in Table II were made on the individual leaves used for the CO₂-uptake studies. The mutant leaves contained slightly less than half the chlorophyll of the normal leaves on a fresh weight basis. Per unit area, the mutant leaves have 40% of the chlorophyll of the normal leaves.

Chlorophyll was virtually absent from the leaves of the lethal plants, being barely detectable in ethanol extracts by the sensitive spectrofluorimetric method.

**Carbon Dioxide Fixation.** Measurements of CO₂ uptake by leaves of intact plants are shown in Table III. For the normal plant, CO₂ uptake was saturated at about 60,000 lux, whereas with mutant leaves, the rate of CO₂ uptake was still increasing at 120,000 lux. Measurements of CO₂ uptake at intensities >120,000 lux were unreliable because of heating. The phytotron cabinet was maintained at 17.5°C, but the temperature of the underside of a leaf increased from 23°C at 3000 lux to 33°C at 120,000 lux. At 120,000 lux, the mutant leaves showed a slightly

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**Table I. Chlorophyll Contents of Leaves From Normal and Mutant Plants**

The mutant plants were of the second generation grown in a 16 hr photoperiod at a temperature of 21°C:19°C in a glasshouse and supplemented with artificial light. Age refers to time after planting the seed. The weights of the mutant and normal leaves expressed as a percentage of the fresh weight were 15.8 and 16.0, respectively. The chl a:chl b ratio of the mutant was determined by spectrofluorimetry of ethanol extracts, and total chlorophyll by spectrophotometry of acetone extracts.

<table>
<thead>
<tr>
<th>Age of plant</th>
<th>Mutant 3-4 weeks</th>
<th>Normal</th>
<th>Mutant 6-7 weeks</th>
<th>Normal</th>
<th>Mutant 8-9 weeks</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a + chl b (mg/g dry wt)</td>
<td>6.05</td>
<td>12.0</td>
<td>7.33</td>
<td>15.2</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>chl a/chl b</td>
<td>15</td>
<td>2.9</td>
<td>...</td>
<td>...</td>
<td>10.3</td>
<td>2.9</td>
</tr>
</tbody>
</table>

**Table II. Leaf Areas and Chlorophyll Contents of Single Leaves**

The individual leaves were used for CO₂ uptake measurement before detachment from the plants. The mutant plants were from the third generation grown in a 16 hr photoperiod at a temperature of 21°C:19°C in a glasshouse supplemented with artificial light.

<table>
<thead>
<tr>
<th>Fresh weight of leaf (g)</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
<th>Expt. 1</th>
<th>Normal</th>
<th>Expt. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf area (cm²)</td>
<td>13.0</td>
<td>22.5</td>
<td>13.3</td>
<td>25.1</td>
<td></td>
</tr>
<tr>
<td>Chl a + Chl b (µg/g fresh wt)</td>
<td>1040</td>
<td>1005</td>
<td>2090</td>
<td>2260</td>
<td></td>
</tr>
<tr>
<td>Chl a + Chl b (µg/cm²)</td>
<td>17.5</td>
<td>17.3</td>
<td>49.0</td>
<td>41.2</td>
<td></td>
</tr>
</tbody>
</table>
Table III. Carbon Dioxide Uptake by Normal and Mutant Plants

The mutant plants were from the third generation. Plants were illuminated with 4 "Mazda" FBRU 1000 watt high pressure mercury vapor lamps and the light intensity was varied with neutral grey "Sarlon" shade cloth. Light intensities were measured with a selenium light meter, and with a Kipp thermopile. The equilibrium leaf temperature was determined with a thermopile placed beneath an identical set of leaves. CO₂ uptake was measured with an infra-red gas analyzer. Total chlorophyll was determined on acetone extracts of leaves.

<table>
<thead>
<tr>
<th>Light Intensity (lux × 10⁻³)</th>
<th>Equilibrium leaf temperature (°C)</th>
<th>Mutant</th>
<th>Normal</th>
<th>Mutant</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Expt. 1</td>
<td>Expt. 2</td>
<td>Expt. 1</td>
<td>Expt. 2</td>
</tr>
<tr>
<td>11.3</td>
<td>33</td>
<td>18.4</td>
<td>20.2</td>
<td>18.9</td>
<td>23.3</td>
</tr>
<tr>
<td>7.49</td>
<td>30</td>
<td>16.4</td>
<td>19.4</td>
<td>18.9</td>
<td>23.3</td>
</tr>
<tr>
<td>4.25</td>
<td>27.5</td>
<td>14.6</td>
<td>17.3</td>
<td>17.9</td>
<td>21.8</td>
</tr>
<tr>
<td>3.35</td>
<td>25.8</td>
<td>12.4</td>
<td>13.7</td>
<td>16.3</td>
<td>19.0</td>
</tr>
<tr>
<td>0.93</td>
<td>24</td>
<td>6.4</td>
<td>7.4</td>
<td>9.7</td>
<td>10.0</td>
</tr>
<tr>
<td>0.25</td>
<td>23</td>
<td>3.6</td>
<td>4.1</td>
<td>5.3</td>
<td>5.3</td>
</tr>
</tbody>
</table>

Fig. 1. Light saturation curves for the reduction of NADP⁺ by isolated chloroplasts. The reaction mixture contained (in 3 ml), chloroplasts containing 25 μg chlorophyll (normal) or 12 μg (mutant), and (in μmoles): tris-HCl buffer (pH 8.0), 40; NaCl, 70; MgCl₂, 10; NADP⁺, 0.6 and a saturating amount of spinach ferredoxin. The rate of NADP⁺ reduction was calculated from the increase in absorbance at 340 nm, after an illumination time of 2 min.

Fig. 2. Light saturation curves for the reduction of trichlorophenol indophenol by isolated chloroplasts. The reaction mixture contained (in 3 ml), chloroplasts containing 8 μg chlorophyll (normal) or 2 to 4 μg (mutant), and (in μmoles): tris-HCl buffer (pH 7.8), 40; NaCl, 70; and TCIP, 0.06. The reaction rates were measured by the decrease in absorbance at 620 nm after illumination for 45 sec or 2 min (for low light intensities).

Fig. 3. Light saturation curves for the reduction of ferricyanide by isolated chloroplasts. The reaction mixture contained (in 3 ml) chloroplasts containing 25 μg chlorophyll (normal) or 12 μg (mutant), and (in μmoles): tris-HCl buffer (pH 8.0), 40; NaCl, 70; MgCl₂, 10; ferricyanide, 1.0. The reaction rates were measured by the decrease in absorbance at 418 nm after illumination for 2 min.
lower rate of CO₂ fixation than the normal leaves per unit area of leaf surface, but on a chlorophyll basis the mutant leaves with a CO₂ uptake of 252 μmoles/mg chl hr were twice as active as the normal leaves.

It was considered preferable to present the CO₂ uptake measurements without correction for dark respiration, as it is likely that photorepiration was responsible for a larger part of the CO₂ evolved by the illuminated leaves. No attempt was made to measure photorepiration.

Photochemical Activities of Isolated Chloroplasts. Figs. 1, 2, and 3 show the influence of light intensity on the Hill reaction activities of chloroplasts isolated from normal and mutant leaves. With normal chloroplasts, the rate of reduction of NADP⁺ was saturated at about 10,000 lux (Fig. 1), and this agrees reasonably well with the saturating intensities observed previously with spinach and normal barley chloroplasts (6). In contrast, the mutant chloroplasts required about 40,000 lux for saturation of NADP⁺ reduction (Fig. 1). At saturating intensities, the mutant chloroplasts reduced NADP⁺ at the rate of 185 μmoles/mg chl × hr, compared with 80 μmoles/mg chl × hr for normal chloroplasts. This 2.2 fold difference in activity for NADP⁺ reduction on a chlorophyll basis closely duplicates the CO₂ uptake results.

When trichlorophenolindophenol (TCIP) was used as an electron acceptor however, the mutant chloroplasts were almost 5 times as active in dye reduction as the normal chloroplasts, if assayed at the very high light intensity of 200,000 lux (Fig. 2). The other feature of TCIP reduction by the mutant chloroplasts is that it was not possible to saturate the reaction even at an intensity of 200,000 lux. Normal chloroplasts were saturated at about 40,000 lux.

Ferriyanide reduction by the mutant chloroplasts was saturated at about 60,000 lux, compared with about 20,000 lux for normal chloroplasts (Fig. 3). The saturating activities were 850 and 280 μmoles ferriyanide reduced per mg chl per hr.

Electron Microscopy. Electron micrographs of chloroplasts from normal and mutant leaves are shown in Figs. 4 and 5. The grana are more distinct in the normal chloroplast and it appears that there are more lamellae per grana in the normal than in the mutant chloroplast. Table IV shows the results of the statistical analysis made on the lamellar counts from the 2 types of chloroplast. The number of grana per chloroplast and the number of single lamellae do not differ significantly, but the total number of lamellae per chloroplast and the number of lamellae per grana are highly significantly lower in the mutant. The number of large grana (with 8 or more lamellae) are also significantly lower in the mutant than in the normal.

Fig. 6 is an electron micrograph of a chloroplast from the yellow lethal mutant. It is characterized by an absence of lamellae pairing. Ribosomes are abundant within the chloroplast, from which it is concluded that the failure of the lethal mutant to synthesize normal grana or to form paired lamellae is not due to an absence of ribosomes.

Discussion

It is interesting to compare our chlorophyll-deficient pea mutant with the barley mutant lacking chlorophyll b, described previously (6,11,13), and the chlorophyll-deficient tobacco mutant (SU/Su) studied by Schmid and Gafron (18,19) and Homann and Schmid (14). The mutant barley leaves have about 70% of the total chlorophyll of the leaves of normal barley plants (6) while the mutant tobacco has about 20 to 30% of the chlorophyll of the normal tobacco (14). Our pea mutant leaves with 50% of the chlorophyll of normal pea leaves are intermediate between the mutant barley and tobacco leaves in their

Table IV. Statistical Analysis of Numbers of Lamellae and Grana in Normal and Mutant Chloroplasts

Approximately 50 electron micrographs each of normal and mutant chloroplasts were taken at random and 10 each of normal and mutant chloroplasts were selected for distinctness and resolution of lamellae. The number of single lamellae, number of grana and number of lamellae per grana were counted and the results subjected to an analysis of variance.

<table>
<thead>
<tr>
<th>No. of grana/chloroplast</th>
<th>No. of lamellae/chloroplast</th>
<th>Lamellae/grana</th>
<th>No. of single lamellae/chloroplast</th>
<th>No. of grana/chloroplast with 8 or more lamellae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>40.3</td>
<td>270.5</td>
<td>6.52</td>
<td>8.8</td>
</tr>
<tr>
<td>Mutant</td>
<td>38.6</td>
<td>179.1</td>
<td>179</td>
<td>12.0</td>
</tr>
<tr>
<td>Difference</td>
<td>1.7 N.S.1</td>
<td>91.4⁺</td>
<td>-3.2 N.S.</td>
<td>10.4⁺</td>
</tr>
</tbody>
</table>

1  N.S. Not significant.
2  Highly significant, P<0.001.
Fig. 4. Normal barley chloroplast showing well developed grana (G).

Fig. 5. Mutant barley chloroplast showing fewer lamellae per granum (G) than the normal.
Fig. 6. Plastid from the lethal barley having no grana and a few single lamella (L).
chlorophyll deficiency. Rates of CO₂ fixation by
the various mutant plants at saturating light inten-
sities, when expressed on a chlorophyll basis bear an
inverse relationship to the chlorophyll content of
the leaves. Thus, the normal barley plants gave a
saturation rate of CO₂ fixation which was 70% of
the rate obtained with the mutant barley plants (13).
The normal pea plant gave 46% of the rate obtained
with the mutant pea, and the normal tobacco about
24% of the rate of the mutant tobacco.

Hill reaction activities at saturating intensities
on isolated chloroplasts from barley and pea plants,
with NADP⁺ as oxidant, parallel the CO₂ uptake
rates of intact leaves. The normal barley chloro-
plasts exhibited 75% of the NADP⁺ reducing ac-
tivity of the mutant barley chloroplasts on a chloro-
phyll basis, (6), and the normal pea chloroplasts
43% of the activity of the mutant pea. Normal
barley chloroplasts reduced ferricyanide at 72% of
the rate observed with the mutant barley, while
normal pea chloroplasts had 33% of the ferricyanide-
reducing activity of the mutant pea. The inverse
relationship between chlorophyll content of the leaves
and activity of isolated chloroplasts was obeyed for
TCIP reduction by mutant and normal barley (6),
but it did not hold so exactly for the pea chloroplasts,
where the mutant showed considerably higher rates
of TCIP reduction.

The results reported in this paper suggest that
the photosynthetic units of the mutant pea chloro-
plasts contain about half the number of chlorophyll
molecules of the photosynthetic units of normal
chloroplasts. For the purposes of this discussion,
we choose to define the photosynthetic unit as the
minimum assembly of pigment molecules and asso-
ciated electron carriers required for the transport of
an electron from OH⁻ to NADP⁺ (3), rather than
as the number of chlorophyll molecules involved in
the fixation of 1 molecule of CO₂ (10).

In a normal spinach (5) or pea chloroplast (un-
published observations) there is 1 molecule of cyto-
chrome f per 400 chlorophyll molecules, from which
it is concluded that the photosynthetic unit in a
normal pea chloroplast contains about 400 chlorophyll
molecules. The amount of P-700 supports this con-
clusion (15). The 400 chlorophyll molecules are
thought to be divided about equally between the 2
pigment assemblies of photosystem 1 and 2 (3) lead-
ing to the concept of 2 units each containing
approximately 200 light-harvesting chlorophyll mole-
cules.

It is postulated from our present studies that the
photosynthetic units (photosystems 1 + 2) of the
mutant pea chloroplast contain about 200 chlorophyll
molecules. Our recent finding, to be reported in a
later publication, that the mutant pea chloroplasts
contain twice the amount of cytochrome f, cyto-
chromes b₅ and 559, of the normal pea chloroplasts,
when expressed on a chlorophyll basis supports this
conclusion.

The present studies do not allow a conclusion
about the distribution of chlorophyll molecules be-
tween the photosystems of the mutant pea chloro-
plasts. Our earlier work (6,7) with the barley
mutant chloroplasts suggested that photosystem 2 of
the mutant chloroplasts may contain less pigment
molecules compared with photosystem 1. The mutant
barley chloroplasts showed a lower photochemical
efficiency at low light intensities (6) and lower
quantum yields of fluorescence (7) than the normal
barley chloroplasts. The Hill reaction curves (Figs.
1, 2, and 3) in the present paper do not show the
cross-over points shown by the barley chloroplasts
at 10 to 20 × 10³ lux (6) but the ratio of the Hill
activity of mutant and normal chloroplasts decreases
as the light intensity is lowered. At the lower in-
tensities the ratio is less than 2, which suggests that
the quantum efficiency of the Hill reaction in the
mutant chloroplasts is lower than that for the normal
chloroplasts. Experiments are in progress to deter-
mine the quantum efficiencies of the mutant and
normal pea chloroplasts in monochromatic light of
low intensity. The higher saturation intensities
observed with the mutant pea are considered to be
due mainly to the lower chlorophyll content of the
mutant's photosynthetic units, although a dispropor-
tion of chlorophyll between the photosystems would
also be expected to increase the saturation intensities.

The structural differences between the normal
and mutant pea chloroplasts particularly in respect to
the number of grana per chloroplast are less than for
the normal and mutant barley. The pea is more
deficient in total chlorophyll than the barley, but the
barley completely lacks chlorophyll b. The absence
of chlorophyll b may impair grana formation or
membrane pairing although the presence of grana in
the barley mutant shows that chlorophyll b is not
essential for grana formation.

The location of chlorophyll within the higher
plant chloroplast has not been resolved. Fluores-
cence microscopy indicates that the red fluores-
cence of chlorophyll arises mainly from the grana (20),
but this observation does not exclude chlorophyll
from the intergrana lamellae, since the concentration
of membranes in the grana is so much higher than
in the intergrana regions. Some evidence is avail-
able which suggests that chlorophyll is uniformly
distributed throughout the internal membrane system
of the chloroplast (16). In their model of the
chloroplast membrane Weier and Benson (21) postu-
late that chlorophyll is confined to the zones of
membrane pairing (partitions).

Measurement of the total length of partitions on
3 mutant and 3 normal chloroplasts selected at ran-
dom indicated that the mutant chloroplasts contained
approximately 50% of the partition length of the
normal chloroplasts. This finding could be taken as
evidence to support the view that chlorophyll is
confined to the partitions, assuming a correlation
between length and area of partition. Location of
chlorophyll in the partitions would suggest further that the area of partition occupied by a photosynthetic unit is determined by the number of chlorophylls in the unit and not by the carriers of the photosynthetic electron transport chain.

When measurements were made of the total length of partitions in electron micrographs of 3 mutant and 3 normal barley chloroplasts obtained previously (8) the total length of the partition in the mutant was only 35% that of the normal barley chloroplast. Therefore, we are unable to conclude that there is a direct relationship between chlorophyll content and length of partition.

Grana or membrane pairings appear to be completely absent from the lethal chloroplast. Chlorophyll is barely detectable in the leaves of the lethal plant and it seems likely that chlorophyll is essential for the formation of grana. The pea lethal chloroplasts resemble the chloroplasts in the yellow areas of the variegated tobacco materials studied by Homan and Schmid (14). These areas, however, did contain measurable amounts of chlorophyll a and chlorophyll b, and the chloroplasts, although low in Hill-reaction activity, were able to photoreduce NADP⁺ if provided with an artificial electron donor (14).

Acknowledgments

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Literature Cited