Effect of Light Quality on the Composition, Function, and Structure of Photosynthetic Thylakoid Membranes of Asplenium australasicum (Sm.) Hook

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ABSTRACT

The effect of light quality on the composition, function and structure of the thylakoid membranes, as well as on the photosynthetic rates of intact fronds from Asplenium australasicum, a shade plant, grown in blue, white, or red light of equal intensity (50 microeinsteins per square meter per second) was investigated. When compared with those isolated from plants grown in white and blue light, thylakoids from plants grown in red light have higher chlorophyll a/chlorophyll b ratios and lower amounts of light-harvesting chlorophyll a/b-protein complexes than those grown in blue light. On a chlorophyll basis, there were higher levels of PSI reaction centers, cytochrome f and coupling factor activity in thylakoids from red light-grown ferns, but lower levels of PSI reaction centers and plastocyanin. The red light-grown fronds had a higher PSI/PSII reaction center ratio of 4.1 compared to 2.1 in blue light-grown ferns, and a larger apparent PSI unit size and a lower PSII unit size. The CO2 assimilation rates in fronds from red light-grown ferns were lower on a unit area or fresh weight basis, but higher on a chlorophyll basis, reflecting the higher levels of electron carriers and electron transport in the thylakoids.

The structure of thylakoids isolated from plants grown under the three light treatments was similar, with no significant differences in the number of thylakoids per granal stack or the ratio of appressed membrane length/nonappressed membrane length. The large freeze-fracture particles had the same size in the red-, blue-, and white-grown fronds, but there were some differences in their density. Light quality is an important factor in the regulation of the composition and function of thylakoid membranes, but the effects depend upon the plant species.

The development of etiolated seedlings, in terms of plant growth, chloroplast structure, and function, is greatly influenced by the prevailing light quality (4, 8, 9, 11, 17, 18, 28, 29). It has been reported that both developing and mature seedlings grown under blue light, have higher Chl a/Chl b ratios (4, 11, 17), higher prenylquinone content and lower xanthophyll to carotenoid ratios (11), and higher photosynthetic rates (17), than those grown under red light. These variations were also accompanied by differences in the ultrastructure of the chloroplasts with chloroplasts from plants grown under red light exhibiting greater grana content than those from plants grown under blue light (4, 11, 18). It was proposed (11, 18) that the differences in thylakoid composition, photosynthetic activity, and chloroplast structure of plants adapted to low intensity blue light are similar to 'sun

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The same micrographs were used to construct particle size distribution histograms by measuring particles directly on the micrographs with a ×10 measuring magnifier.

RESULTS

Physiological Differences. After 2 to 3 months in the growth cabinets with blue, white, or red light (Fig. 1), Asplenium developed about ten fully expanded fronds, each about 40 cm long and 12 cm wide. There was, however, a significant difference in the color of these fully mature fronds. Those developed under red light were much paler green than those developed under blue or white light. This difference is partly due to there being significantly less Chl per leaf area or per fresh weight in the red light fronds (Table I). Furthermore, there is less Chl b in fronds developed under red light than those developed under blue or white lights (Table I). Since there is a higher level of fresh weight but much less Chl per leaf area in fronds developed under red light there could be more starch in the fronds. Indeed, fronds developed under red light were thicker and had much more starch content as observed during thylakoid isolation and electron microscopy (see Fig. 3C).

Effect of Light Quality on the Relative Contents of Chlorophyll-Proteins. When the various thylakoids were solubilized in SDS and subjected to discontinuous PAGE under mild conditions, seven green Chl-protein bands were resolved (Fig. 2). These were, in order of increasing mobility: CPla, CP1, LHCP1, LHCP2, CPa, LHCP3, and free Chl (FC). CP1a is an undissociated PSI complex which includes CP1, the Chl a/b-proteins of PSI (LHC1) (13), and colorless polypeptides. CP1 is the β-carotene-P700-Chl a-protein complex, CPa contains the Chl a-proteins of PSI1 complex, and the three Chl a/b-proteins (LHCP1, LHCP2, LHCP3) belong to the main light-harvesting complex of PSI (LHc1-1) (1-3) (Tables II, III, and VIII). Since the relative contents of free pigment were rather low and comparable, direct comparison of Chl content in each Chl-protein complex from ferns grown under blue, white, and red light is possible, although it should be stressed that these comparisons are only relative.

When compared to ferns grown in white light, those grown in blue light have rather similar relative Chl content in the Chl-protein complexes (Table II). However, those grown in red light have slightly higher Chl contents in CP1a, CP1, CPa, and LHCP3 and lower Chl contents in LHCP1. The effect of light quality during growth on the overall distribution of Chl in PSI (sum of CP1a and CP1) and PSI (sum of CPa, LHCP1, LHCP2, and LHCP3) (Table III) shows clearly that ferns grown in red light have relatively more Chl in PSI and in CPa, but less total Chl in PSI when compared to those grown in white or blue lights. Qualitatively, it appears therefore that the ferns grown in red light have a smaller PSI antenna size with a higher reaction center content. These results agree with those found previously with pea thylakoids (13), where higher Chl a/Chl b ratios in unfractionated thylakoids means less total Chl associated with LHC-II and more with PSI complex.

Effect of Light Quality during Growth of Asplenium on the Electron Transport System. Freshly prepared thylakoids were assayed for partial, as well as coupled and uncoupled, whole chain electron transport rates in PSI and PSII. The PSI and PSII

Table I. Physiological Differences in Asplenium australasicum Grown in Blue, White, or Red Lights of Equal Intensity

<table>
<thead>
<tr>
<th>Light Quality</th>
<th>Total Chl/ Fresh Wt/</th>
<th>Chl a/ Chl b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frond Area</td>
<td>Frond Area</td>
</tr>
<tr>
<td>Blue</td>
<td>20.7</td>
<td>29.8</td>
</tr>
<tr>
<td>White</td>
<td>19.2</td>
<td>26.3</td>
</tr>
<tr>
<td>Red</td>
<td>8.2</td>
<td>34.8</td>
</tr>
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</table>
individual electron transport rates were slightly higher in blue than in red light-grown ferns (Table IV), with slightly higher uncoupled whole chain (PSI + PSII) rates in red light-grown ferns. The higher coupled rate (in the absence of NH$_4$Cl, the uncoupler) in red light-grown Asplenium and the lower stimulation in the presence of uncoupler, indicate that these thylakoids might already be partly uncoupled before the addition of the uncoupler. The observed overall whole chain rates (whether coupled or uncoupled) are much lower than those of most higher plants (16, 17). Such data on partial electron transport activities cannot be used to quantitate the amounts of PSII and PSI complexes in the fern thylakoids, since there may have been some inactivation during their isolation.

A more meaningful approach is provided by determining the concentrations of some electron transport components on a Chl basis (Table IV). The red light-grown fern has a higher concentration of atrazine binding sites (which are an indicator for PSII reaction centers [17]) and Cyt f, but lower concentrations of plastoquinone and P700 than blue light-grown fern. The coupling factor, CF$_1$ AT$	ext{P}$ase activity is almost the same in both blue and red light-grown ferns.

A comparison of the RC II/RC I ratios (measured as the atrazine-binding sites and chemically determined P700, respectively) of Asplenium grown in blue, white, and red light (Table V) shows that these ratios are neither unity nor constant. Indeed, the red light-grown ferns have a much higher RC II/RC I ratio of 4.12 than those of the blue or white light-grown ferns. Melis and Harvey (21) and Melis (22) have also reported that RC II/...
RC I ratios of several obligate shade plants are greater than 1, and greater than the values obtained for sun-adapted plants. With *Atriplex*, we also found a higher RC II/RC I ratio in red light-grown than in blue or white light-grown *Atriplex* (17). Similarly, pea plants grown under far red-enriched light had a higher RC II/RC I ratio than those grown under far red-deficient light (21, 22). Clearly, light quality exerts an effect on the stoichiometry of PSII and PSI reaction centers with higher ratios in red and far red-enriched light than in blue and far red-deficient light.

Effect of Light Quality on the Relative Photosynthetic Unit Sizes of PSI and PSII. Since the relative amounts of Chl-proteins associated with PSII and PSI are changed as the light quality during growth was altered (Tables II and III), it is important also to examine the light-harvesting antenna unit sizes of the individual photosystems. Since we were unable to measure the light-induced absorbance changes of P680 or P700 spectrophotometrically (as in Ref. 21), we have calculated the relative apparent light-harvesting antenna unit sizes of PSI and PSII by comparing the relative amount of Chl associated with PSI per atrazine binding site, and that of PSI per P700, respectively. This is a qualitative measurement only since the amount of Chl associated with PSII and PSI (Table III) are imprecise measurements. When compared with those of blue light-grown *Asplenium*, ferns grown in red light have a larger apparent PSI antenna size but a lower PSII antenna size. This is partly due to the increase in the concentration of PSII reaction centers combined with a decrease in that of P700 in red light-grown ferns, while the reverse order is found in blue light-grown ferns. Our results suggest that variations in the stoichiometries of PSII and PSI reaction centers may also be accompanied by differences in the antenna unit sizes of PSII and PSI.

Photosynthetic Rates of Intact Fronds. When the net photosynthetic rates of intact *Asplenium* fronds were compared, by measuring the CO₂ fixation rates with an IR gas analyzer at various white light intensities, the fronds all attained maximal photosynthetic rates at 300 to 500 μE m⁻² s⁻¹ (Table VI). Ferns grown in blue and white lights show similar maximal photosynthetic rates whether expressed on unit fresh weight or frond area, while those grown in red light have much lower rates. However, when the rates were expressed on total amount of Chl, ferns grown in red light have significantly higher rates than those grown in blue and white lights. This is due to the fact that there is significantly less Chl in the frond tissue either per fresh weight or per frond area (Table I).

Effect of Light Quality on Chloroplast Structure. Electron micrographs of chloroplasts from ferns grown in blue, white, and red light or extreme shade conditions (~2 μE m⁻² s⁻¹) are shown in Figure 3. No visual differences in the numbers of thylakoids in the grana stacks could be observed on examination of 20 micrographs from each of the red, white, and blue light treatments. This observation was supported by measurement and the calculation of the ratio of stroma lamellae and end grana membrane lengths to appressed membrane lengths. These ratios were comparable for chloroplasts from each of the light treatments. However, it is possible that differences could be obscured by the fact that micrographs of chloroplasts for measurement were randomly selected from frond cells. To reduce this possibility micrographs of chloroplasts confined to the dorsal surface epidermal layer were examined but these also failed to show any differences in numbers of thylakoids in grana stacks between the treatments (data not shown). In contrast, a chloroplast from a frond of a fern grown in extreme shade (Fig. 3D) had greater numbers of thylakoids in the grana stacks.

Chloroplast thylakoid EFs fracture face particle densities are shown in Table VII. The red and blue light treatment particle densities do not differ, but there are fewer particles per μm² from blue light-grown leaves. Particle size distribution histograms of EFs fracture faces showed no difference between the treatments.

**DISCUSSION**

In previous studies on blue or red light effects it has been reported that plants developed under blue light resemble those developed under high intensity of white light, and the reverse for plants developing under red light (11, 18). These results led to the proposal that plants grown in blue light would resemble sun plant species, whereas those grown in red light would resemble shade plant species. However, the present study shows that when *Asplenium*, a shade plant, is grown under blue and red light, it tends to behave more like a sun plant under red light in that it has a higher Chl a/Chl b ratio, less LHC-II, and more PSI Chl. Furthermore, when the sun plant *Atriplex triangularis* was grown under red, white, and blue light of equal intensity, not all of the attributes of red-light *Atriplex* resembled those of shade plants (17). Clearly, the effects of light quality on the composition and function of thylakoid membranes are not the same for all plants.

In the present study, when *Asplenium* is grown in red light it develops fronds with Chl a/Chl b ratios higher than those grown in blue light. This higher Chl a/Chl b ratio correlates with higher and lower relative Chl contents associated with PSII and PSI, respectively (Table III), as is observed in previous studies on the effect of light intensities and qualities on the composition of pea thylakoids (14–16). It has also been reported that the Chl a/Chl b ratios are closely related to the composition and function of pea thylakoids (15, 16), with linear relationships found between Chl a/Chl b ratios and coupling factor CF₁, activity, electron transport rates, and other electron carriers such as plastoquinone, Cyt f, P700, and atrazine binding sites (16). However, in the present study, the increase in Chl a/Chl b ratio in ferns grown in red light is accompanied by higher whole chain electron transport rates, higher levels of atrazine binding sites, and Cyt f (Table IV), but also higher photosynthetic rates in intact fronds (Table VI). It thus seems likely that the higher photosynthetic rates in intact red light-grown ferns are probably facilitated by higher concentrations of atrazine binding sites, Cyt f, and coupling factor CF₁ activity. The present study may also indicate that there may be limits in the transformation of a shade plant to a sun plant, whereby some components of the photosynthetic membrane are favored against the others.

The ratio of atrazine binding sites to P700 (Table V) varies considerably in red light- and blue light-grown ferns, indicating that the stoichiometry of the PSII and PSI reaction centers is not necessarily constant, but may vary. Significant differences in RC II/RC I ratios between obligate shade and sun plants were reported earlier (21, 22). Furthermore, as the concentration of atrazine binding sites in ferns grown in red light increases, there is a concomitant decrease in P700, and in ferns grown in blue light, the decrease in the concentration of atrazine binding sites is contrasted with the increase in P700. Similarly, in *Atriplex* grown under blue and red light, the RC II/RC I ratio was higher in red light (17). This differential effect of light quality on the ratio of PSII centers/PSI centers has also been observed in peas.

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**Table VI. Maximal Photosynthetic Rates of Intact Fronds of Asplenium Grown Under Blue, White, and Red Lights of Equal Intensities**

<table>
<thead>
<tr>
<th>Light Quality</th>
<th>Maximal Photosynthetic Rates of Intact Fronds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg CO₂ h⁻¹ g⁻¹</td>
</tr>
<tr>
<td>Blue</td>
<td>1.21</td>
</tr>
<tr>
<td>White</td>
<td>1.24</td>
</tr>
<tr>
<td>Red</td>
<td>0.57</td>
</tr>
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</table>

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FIG. 3. Cross-section electron micrographs of the ultrastructure of Asplenium chloroplasts from fronds grown in (A) blue, (B) white, and (C) red light of equal intensity, or in (D) extreme shade in a glasshouse for 6 months (magnification, × 16,000).
under far red-enriched and -deficient light conditions (19) with a higher ratio being found with far red-enriched light. In all cases, it appears that the ratio of PSII/PSI reaction centers is greater in plants grown under far red-enriched light compared to plants grown in blue- or far red-depleted light. Our studies (15) and those of Melis (21, 22) which demonstrate that light quality exerts a marked influence on the stoichiometries of PSII and PSI reaction centers are in sharp contrast to those of Whitham and Orth (26, 27) who found that several plant species including summer or winter grown spinach had a constant stoichiometry of PSI reaction center:Cyt f/PSI ratio of 2:1. The great variations found in the ratios of Chl a/Chl b, Chl/P680, Chl/Cyt f, and Chl/P700 and in the relative amounts of Chl in the Chl-proteins in a wide variety of plants grown under different light intensities and qualities (e.g. 3, 8, 9, 11, 14-18, 20-22, 28, 29), we believe, argue strongly for the idea that plants adapt to natural light conditions by incorporating suitable and well-balanced changes in the composition and function of their thylakoid membranes to provide efficient and optimal photosynthesis (17). 

Light quality also exerted an effect on the apparent lightharvesting antenna sizes of PSII and PSI (Table V). Red light-grown ferns have a larger apparent PSI unit compared to blue light-grown ferns; conversely, the PSI unit size is apparently larger when the ferns are grown under blue light. Our results (16, 17) appear to indicate that variations in the stoichiometries of PSII and PSI reaction centers may be accompanied by variations in the PSI and PSI antenna unit sizes. In contrast, Melis and Harvey (21, 22) found no changes in the photosynthetic unit sizes of PSI and PSII in peas grown under far red-enriched and far red-depleted illumination.

The small difference in Chl a/Chl b ratios in ferns grown in blue and red lights (2.37 versus 2.62, Table I) seems to be out of proportion with the larger differences observed in the relative amount of Chl associated with LHCP complexes as well as other Chl-protein complexes (Tables II and III). When the absorption spectra from 400 to 750 nm of each green gel band after SDS-PAGE were compared, it becomes clear that the 650-nm peak varied slightly within Chl-protein complexes with respect to the light quality during growth, indicating that the Chl a/Chl b ratios could be different (data not shown). Indeed, when the Chl-proteins were eluted from these green bands (10) and the Chl a/Chl b ratios determined, they were found to be slightly higher in CP1a, CP1, and CPa complexes (i.e. Chl-proteins which include reaction centers), but lower in LHCP3, LHCP2, and LHCP1 in ferns grown in red light than those grown in blue light (Table VIII). Thus, the slight decrease in Chl b in the light-harvesting Chl-protein complexes of blue light-grown ferns is accompanied by an increase of Chl b in CP1a, CP1, and CPa, resulting in only slight differences in the Chl a/Chl b ratios of thylakoids. Thus it appears that the pigment composition of PSI and PSII (amounts of Chl a/b-proteins relative to Chl a-proteins of each photosystem) are also changed by light quality.

When the ultrastructure of Asplenum chloroplasts from fronds grown in blue, white, and red lights were compared (Fig. 3, A, B, and C), more starch was detected in the red light-grown fern. Furthermore, chloroplasts from fronds grown in red light contain many more plastoglobuli than those from fronds grown in white and blue light (Fig. 3, A, B, and C). Meier and Lichtenthaler (20) have reported that chloroplasts from radish seedlings grown under high light intensity contain more and larger plastoglobuli and in this respect, Asplenum grown under red light appears to resemble radish seedlings grown under high light intensity. However, under the experimental light conditions used, little differences in relative lengths of grana and stroma thylakoids could be measured although there were differences in Chl a/Chl b ratios (Table I) and photosynthetic rates (Table VI). Usually, these biochemical differences are associated with structural differences in chloroplasts of plants grown under various intensities of white light or sup-shade conditions (8, 9).

The thylakoid EFs fracture face particle size distribution in chloroplasts from fronds grown under blue, white, and red light were not significantly different. However, the distances between the particles in chloroplasts from fronds grown under blue and red light are significantly different. This result may indicate some differences in the polypeptides of the thylakoid membranes of fronds grown under blue and red light, and SDS-PAGE under denaturing conditions showed that there were quantitative differences (data not shown).

In conclusion, the marked modulations in the composition, function, and structure of Asplenum australisacum thylakoids grown in red, white, and blue light of equal intensity, do not support the generalization (11, 18) that plants grown under blue light resemble sun plants and under red light resemble shade plants. Both this study and that with Atriplex trianinarius (17) lead us to conclude that the plant species is very important in the complex response to the effects of light quality and quantity. In 1959, Van der Veen and Meijer (25) demonstrated that the stem elongation of different plants responded differently to red and blue light. At a particular light intensity, the ‘Mirabilis’ type showed greater inhibition of stem elongation by blue light than by red light, whereas with the ‘Gherkin’ type, red light was more inhibitory than blue light. However, these effects were dependent on light intensity. If the light intensity were low enough, blue light was always less effective than red light, but at high enough light intensity, the effect was reversed. The critical intensity (that intensity at which the effects of red and blue light were similar) depended on the plant species, and was much lower for the Mirabilis than the Gherkin-type plants (25). These stem elongation studies clearly show the complex interaction between light quality and intensity, and how the responses will vary with different plant species. The future challenge is to unravel the coordinated effects of light quality and quantity on the photo-regulation of thylakoid membranes and the molecular mechanisms responsible for photoregulation.

Acknowledgments—We thank all the Phytotron staff for maintaining the plants and Mrs. C. Miller for expert technical assistance with the electron microscopy.

REFERENCES


Table VII. Chloroplast Thylakoid EFs Fracture Face Particle Density

<table>
<thead>
<tr>
<th>Light Quality</th>
<th>No. of Particles Counted</th>
<th>No. of Particles per μm² ± SD</th>
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</thead>
<tbody>
<tr>
<td>Red</td>
<td>1282</td>
<td>1350 ± 184</td>
</tr>
<tr>
<td>White</td>
<td>2545</td>
<td>1407 ± 148</td>
</tr>
<tr>
<td>Blue</td>
<td>1645</td>
<td>1028 ± 118</td>
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</table>

Table VIII. Variation in the Chl a/Chl b Ratios of the Individual Chl-Protein Complexes Eluted from Green Gel Segments Immediately after Mild SDS-PAGE

<table>
<thead>
<tr>
<th>Light Quality</th>
<th>Chl a/Chl b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CP1a</td>
</tr>
<tr>
<td>Blue</td>
<td>5.23</td>
</tr>
<tr>
<td>White</td>
<td>5.32</td>
</tr>
<tr>
<td>Red</td>
<td>5.83</td>
</tr>
</tbody>
</table>
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