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Comparative Photosynthetic Properties of Palisade Tissue Chloroplasts and Spongy Tissue Chloroplasts of *Camellia japonica* L.: Functional Adjustment of the Photosynthetic Apparatus to Light Environment within a Leaf

Ichiro Terashima[1] and Yorinao Inoue[2]

[1] *Department of Botany, Faculty of Science, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan*
[2] *Solar Energy Research Group, RIKEN Institute of Physical and Chemical Research, Hirosawa, Wako-shi, Saitama 351-01, Japan*

The photochemical properties of chloroplasts isolated separately from palisade and spongy tissues of *Camellia* leaves, were compared, and the following results were obtained: (1) The content of the light-harvesting Chl *a*/*b*-protein complex was higher in spongy tissue chloroplasts (S-Chlts) than in palisade tissue chloroplasts (P-Chlts), while the contents of P700 and PS II polypeptides were higher in P-Chlts. (2) Fluorescence induction was slower in P-Chlts, indicating that they had a larger plastoquinone pool than S-Chlts. (3) The quantum yield of PS II electron transport in S-Chlts was appreciably higher, while that of PS I electron transport was higher in P-Chlts. (4) The maximal rates of both PS I and PS II electron transport under saturating light were higher in P-Chlts than in S-Chlts.

From these results, we concluded that the photochemical properties in P-Chlts are adjusted to high light intensity and those of S-Chlts to low intensity enriched in green and far-red; both are adjusted to their respective in situ light environments.

Key words: Chloroplast — Electron transport — Light environment (leaf) — Optical cross section — Palisade tissue (*Camellia japonica*) — Spongy tissue.

Bifacial leaves receive light mostly on their upper surfaces. Because of strong light absorption by chlorophyll, light intensity in the mesophyll steeply declines and simultaneously the spectral composition changes with depth from the irradiated surface (Terashima and Saeki 1983). For example, in a leaf of *Camellia japonica* L., the fluxes of red (680 nm) and green (550 nm) light penetrating through palisade tissue are 2.5% and 22% of the incident light, respectively, so that the spongy tissue is illuminated with low intensity light enriched in green and far-red, while the palisade tissue is illuminated with strong white light (Terashima and Saeki 1983).

Such light conditions of palisade and spongy tissues are qualitatively similar to those of sun and shade plants (or leaves), respectively. Comparative studies have revealed that the properties

Abbreviations: CP I, P700-Chl *a*-protein complex; CPa, PS II reaction center-Chl *a*-protein complex; DCIP, 2,6-dichlorophenol indophenol; LHCP, light-harvesting Chl *a*/*b*-protein complex; MV, methylviologen; PAGE, polyacrylamide gel electrophoresis; P-Chlts (S-Chlts), chloroplasts isolated from palisade (spongy) tissue; PEG, polyethyleneglycol 4,000; Rubisco, ribulose bisphosphate carboxylase/oxygenase; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine.

of chloroplasts in sun and shade plants are different from each other, as they adjust to their respective light environments (Boardman 1977, Björkman 1981). Similar adjustment is known to occur in chloroplasts of the same species of plants grown under different light conditions (Mousseau et al. 1967, Boardman 1977, Wild 1979, Björkman 1981). By analogy, the differences in light conditions between palisade and spongy tissues may cause differentiation in the photosynthetic properties of the chloroplasts in the two tissues. Such indications have been reported by Schreiber et al. (1977) and by Kulandaivelu et al. (1983), who observed that fluorescence induction curves obtained from the upper surfaces of bifacial leaves differ from those from the lower surfaces, as seen between sun and shade leaves. However, the photochemical properties of isolated chloroplast preparations have not been directly compared because of difficulties in isolating chloroplasts separately from the two tissues.

In the present study, we developed a technique to isolate chloroplasts from paradermal sections containing either palisade or spongy tissue of *Camellia japonica* L. leaves. This enabled us to directly compare the photosynthetic properties of palisade tissue chloroplasts (P-Chlts) and spongy tissue chloroplasts (S-Chlts). We report the differences found, such as in the Chl *a*/*b* ratio, polypeptide composition, P700 content, fluorescence spectrum and induction kinetics, and electron transport activity. Our results are discussed in terms of the functional adjustment of chloroplasts to micro light environments within a leaf.

Materials and Methods

Isolation of chloroplasts—Leaves were collected from current shoots of a field-grown *Camellia japonica* L. tree. The leaf segments (ca. 1 cm^2, 400 μm thick) consisting of 180 μm of palisade tissue and 180 μm of spongy tissue were cut with a freezing microtome into paradermal sections selectively containing either palisade tissue or spongy tissue (Terashima and Saeki 1983). Contamination of venation was carefully avoided. The paradermal sections obtained were immediately frozen by dipping in liquid nitrogen and kept frozen until the last section was obtained.

The sections were ground with a Polytron homogenizer (Kinematica) at maximum speed for 20 s in a medium containing 0.4 M sucrose, 2% (w/v) polyvinylpyrrolidone K-30, 10 mM NaCl, 2 mM $MgCl_2$, and 40 mM HEPES-KOH (pH 7.6). The homogenate was filtered through a layer of 20 μm nylon mesh and the filtrate was centrifuged at 3,000 $\times g$ for 10 min. The pellet was resuspended in the same medium and used for the experiments. By this procedure, about 200 μg of P-Chlts and 100 μg of S-Chlts on a Chl basis were usually obtained from a single leaf. Chl content and Chl *a*/*b* ratio were measured by the method of Arnon (1949).

SDS-PAGE—Chloroplasts were sonicated in a bath-type sonicator (Branson, USA) in a hypotonic medium containing 10 mM NaCl, 4 mM Na-ascorbate, and 20 mM HEPES-KOH (pH 7.8) for 30 s and washed twice with the same medium to remove stromal proteins. The pelleted chloroplast membranes (5 μg Chl) were solubilized in 2% SDS containing 50 mM Na_2CO_3, 50 mM dithiothreitol, 12% (w/v) sucrose, and 0.4% (w/v) bromophenol blue. Electrophoresis was run according to the method of Chua (1980). Gels stained with 0.1% (w/v) Coomassie brilliant blue R-250 were scanned at 560 nm relative to 700 nm with a dual-wavelength TLC scanner (Shimadzu, CS-900).

P700 determination—P700 content was determined by the light-induced absorbance change at 700 nm based on the extinction coefficient of 64 mM^{-1} cm^{-1} reported by Hiyama and Ke (1972). The reaction mixture contained 0.2% (v/v) Triton X-100, 0.4 M sucrose, 10% (w/v) PEG, 10 mM NaCl, 5 mM $MgCl_2$, 100 μM MV, 1 mM Na-ascorbate, 10 μM DCIP, and 20 mM HEPES-KOH (pH 7.0).

Fluorescence spectra—Chlorophyll fluorescence emission spectra were measured at 77K in

a medium containing 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$, and 20 mM HEPES-KOH (pH 7.0) with a spectrofluorometer (Shimadzu, RF-502) with an excitation wavelength of 435 nm (band width 20 nm). Samples were kept in the dark for 10 min before being frozen. No correction of emission spectra was made for the spectral sensitivity of the photomultiplier (Hamamatsu, R-666).

Fluorescence transients—Chlorophyll fluorescence transients were measured at 20°C according to Schreiber and Pfister (1982). Chloroplasts were suspended in the medium for the fluorescence spectrum measurements supplemented with 10% (w/v) PEG and were kept in the dark for 20 min before measurements.

Electron transport activities—PS I electron transport activity was measured by TMPD photooxidation with MV as electron acceptor (Vernon and Cardon 1982) in a medium containing 10% (w/v) PEG, 10 mM NaCl, 2 mM $MgCl_2$, 1 mM KCN, 1 mM TMPD, 1 mM MV, 10 μM DCMU, and 50 mM HEPES-KOH (pH 7.0). PS II electron transport activity was measured by DCIP photoreduction in a medium containing 10% (w/v) PEG, 10 mM NaCl, 2 mM $MgCl_2$, 20 mM HCl-methylamine, 100 μM DCIP, and 50 mM MES-KOH (pH 5.5). Chl concentration was adjusted to 5 μg Chl/ml. The measurements were done with a recording spectrophotometer (Shimadzu UV-300) at 20°C. Actinic light was red light from an incandescent lamp (500 W) passing through a red filter (Toshiba VR-65) and a 10-cm layer of water and then focusing onto the optical cell at a saturating photon flux density of 2,000 μmol quanta m^{-2} s^{-1}. Neutral density filters (Toshiba Electric) were placed between the light source and the red filter when lower photon flux densities were necessary.

Results

Table 1 and 2 show that the Chl *a*/*b* ratios determined for several samples of P-Chlts and S-Chlts varied considerably with the microhabitat of the leaves in a tree (see Fig. 3). The ratios for P- and S-Chlts of leaves from the sunny part of the tree were higher than those for the respective chloroplasts from the shady part. This agrees with the generally accepted view that the Chl *a*/*b* ratio is higher in sun leaves than in shade leaves (Boardman 1977). In addition to the Chl *a*/*b* ratios depending on the microhabitat, P-Chlts tended to have higher Chl *a*/*b* ratios than S-Chlts; P-Chlts ratios were 0.7 higher when the comparison was made between chloroplasts obtained from the same leaf. The difference may arise because P-Chlts are exposed to more sun.

Fig. 1 shows the SDS-PAGE densitograms of membrane proteins of P- and S-Chlts obtained from a *Camellia* leaf. P-Chlts showed five major bands at approximate molecular masses of 61, 55, 43, 25 and 12 kDa and several minor bands. Comparison of this profile with that of spinach thylakoids, or PS II particles, identified the bands (or group of bands) around 61 kDa, 43 kDa and 25 kDa as apoproteins of CP I, CPa and LHCP, respectively. The large 55 kDa band and the 12 kDa band were also identified as corresponding to large and small subunits of Rubisco, respectively, which could not be removed from the membranes by sonication followed by washing with hypotonic buffer. In the experiment, the amount of sample thylakoids was adjusted with respect to Chl content (5 μg each), so that the contents of CP I, CPa and LHCP could be estimated on a Chl basis from the peak areas on the densitograms. Comparison of the profiles of the two chloroplast preparations revealed that P-Chlts contained relatively larger amounts of CP I and CPa with a smaller amount of LHCP than S-Chlts.

The lower contents of CP I and CPa in S-Chlts suggest that the photosynthetic unit sizes of PS I and PS II are large. This was partly confirmed by the Table 1 data comparing the Chl/P700 ratios of P-Chlts and S-Chlts. The ratio varied considerably with the chloroplast source but S-Chlts clearly showed a higher Chl/P700 ratio when the comparison was made

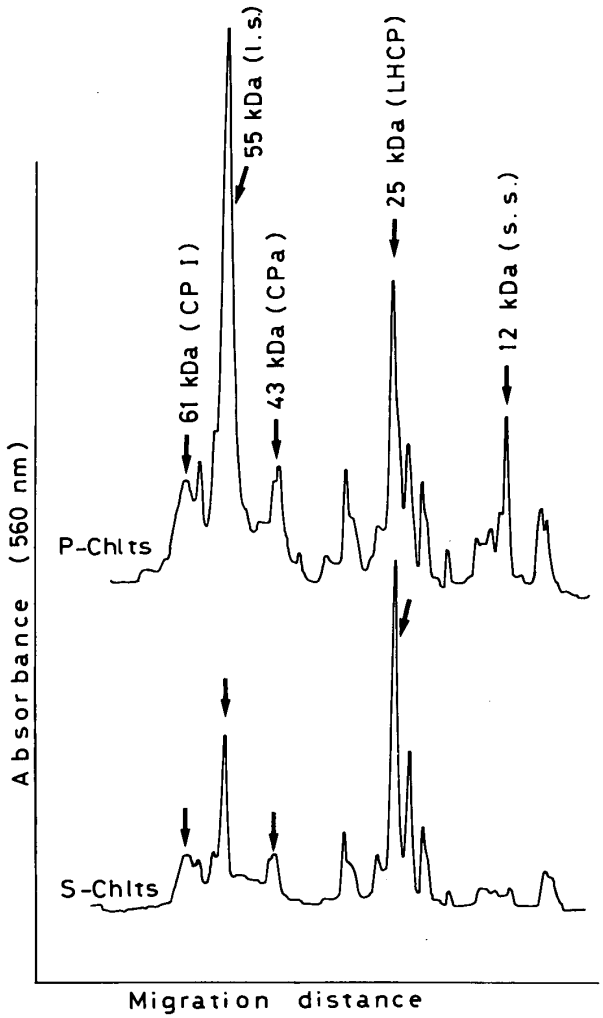

Fig. 1 SDS-PAGE densitograms of thylakoid polypeptides in P- and S-Chlts. The same amount of chloroplasts (5 μg Chl) was loaded. Some bands were assigned: 61 kDa, apoprotein of P700-Chl *a*-protein complex (CP I); 55 kDa, Rubisco large subunit (l.s.); 43 kDa, apoprotein of PS II reaction center-Chl *a*-protein complex (CPa); 25 kDa, apoprotein of light-harvesting Chl *a*/*b*-protein complex (LHCP); 12 kDa, Rubisco small subunit (s.s.). Chl *a*/*b* ratios of P- and S-Chlts were 3.05 and 2.48, respectively.

between P- and S-Chlts obtained from the same leaf. The relatively high content of LHCP in S-Chlts agrees with the low Chl *a*/*b* ratio of S-Chlts, since most of the Chl *b* is known to be associated with LHCP (Thornber 1975, 1979).

Fluorescence spectra at 77 K of P- and S-Chlts showed typical peaks at 684 nm (F684) and 740 nm (F-I) with a shoulder at 695 nm (F695) (Fig. 2). Although the two spectra were similar to each other in shape, the relative height of F684 band to F-I band was greater in S-Chlts. As the three emission bands, F684, F695 and F-I, have been assigned to be emitted from LHCP (Thornber and Highkin 1974), CPa (Satoh and Butler 1978) and CP I (Satoh 1979), re-

Table 1 Chl/P700 ratio of P- and S-Chlts

Chloroplasts	Chl *a*/*b*	Chl/P700
Experiment 1		
P-Chlts	3.44	403
S-Chlts	2.55	562
Experiment 2		
P-Chlts	3.22	563
S-Chlts	2.57	812

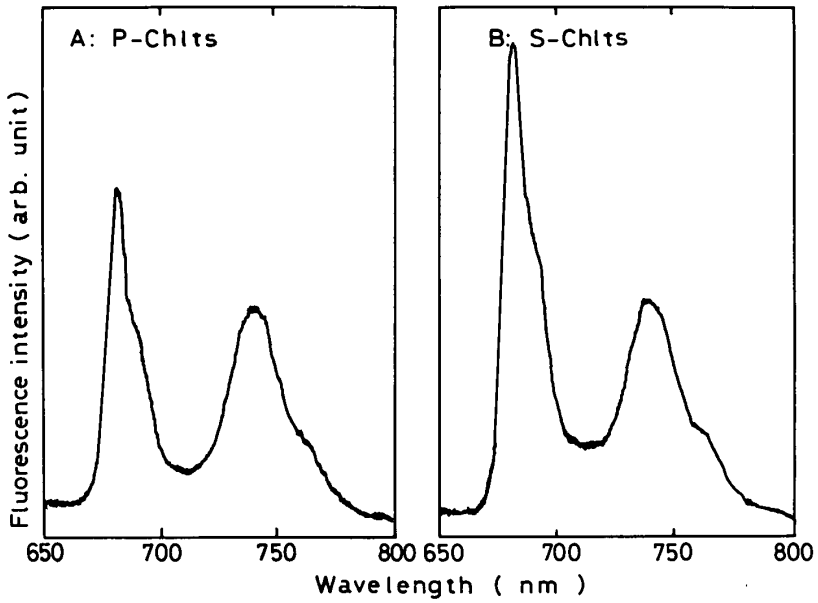

Fig. 2 Low temperature (77 K) fluorescence emission spectra of P-Chlts (A) S-Chlts (B). Chloroplasts were suspended in 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$ and 20 mM HEPES-KOH (pH 7.0). Chl *a*/*b* ratio of P- and S-Chlts were 3.41 and 2.75, respectively.

spectively, the higher F684/F-I ratio in S-Chlts suggests preferential excitation of PS II relative to PS I in S-Chlts. As shown by the densitograms in Fig. 1, more LHCP was present in S-Chlts than in P-Chlts. As most of the LHCP is associated with CPa, the optical cross section of PS II relative to PS I is probably larger in S-Chlts. Thus, the preferential excitation of PS II in S-Chlts may be due to the larger optical cross section of PS II in S-Chlts.

F684/F-I ratios of P- and S-Chlts were plotted against the Chl *a*/*b* ratio for five different leaves collected from various parts of a tree (Fig. 3). They increased as the Chl *a*/*b* ratios decreased, indicating a strong correlation between the two ratios. As already mentioned, the lower Chl *a*/*b* ratio in chloroplasts denotes a higher LHCP content. As LHCP is mainly associated with CPa, its high content probably means a larger optical cross section of PS II. Thus, the strong correlation between the F684/F-I ratio and the Chl *a*/*b* ratio confirms the above view that the preferential excitation of PS II in S-Chlts results from a larger optical cross section of PS II due to a higher content of LHCP.

Fluorescence induction curves were measured for P- and S-Chlts (Fig. 4). The transient curve of P-Chlts was characterized by a slow induction accompanied by high Fo but low Fm levels with small variable fluorescence (Fm—Fo). Slow fluorescence induction is generally considered to result from a large plastoquinone pool size on the acceptor side of PS II and/or a small optical cross section of PS II. The plastoquinone pool sizes estimated from the integrated areas above the transient curves relative to those in the presence of DCMU (10 μM) (Zankel and Kok 1972, Öquist and Martin 1980) were 10 and 5 for P- and S-Chlts, respectively. If we take into account that the optical cross section of PS II of P-Chlts is smaller, as shown by the experiments of Fig. 2 and 3, both the large plastoquinone pool size and the small optical cross section of PS II are additively responsible for the slow induction observed.

Light saturation curves of PS II electron transport activity in P- and S-Chlts are shown in Fig. 5-A. At light intensities higher than about 150 μmol quanta m^{-2} s^{-1}, P-Chlts activity was higher than that of S-Chlts, while at intensities lower than 70 μmol quanta m^{-2} s^{-1}, S-Chlts activity was higher. The two intensity curves crossed each other at about 100 μmol quanta m^{-2} s^{-1}. Since the activity is light-limited at low intensities, the higher activity of S-Chlts

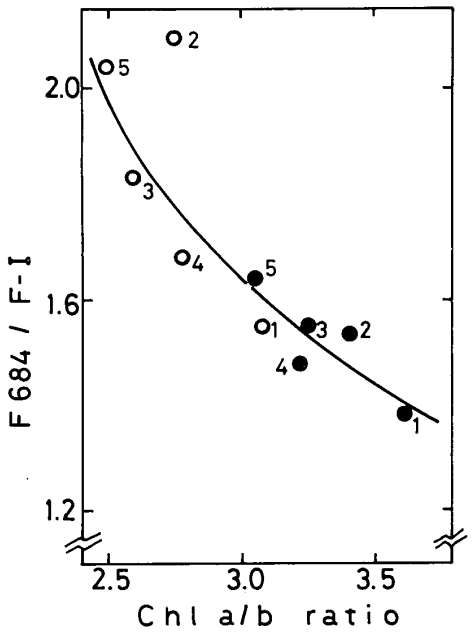

Fig. 3

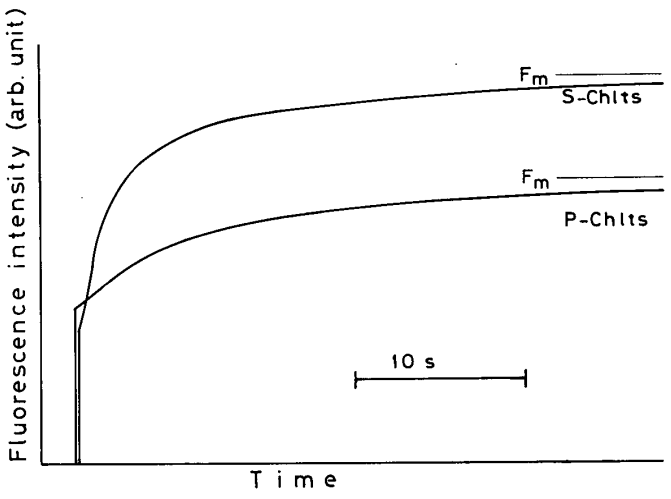

Fig. 4

Fig. 3 F684/F-I ratio plotted as a function of Chl *a*/*b* ratio for P-Chlts (●) and S-Chlts (○). Values beside the circles stand for the leaf segment samples. Open and solid circles with the same number give data for P- and S-Chlts isolated from the same leaf segment.

Fig. 4 Fluorescence induction curves of P-Chlts and S-Chlts. Chloroplasts were suspended in 0.4 M sucrose, 10% (w/v) PEG, 10 mM NaCl, 5 mM $MgCl_2$ and 20 mM HEPES-KOH (pH 7.0). Chl concentration was adjusted to 5 μg Chl ml^{-1}. Chl *a*/*b* ratios of P- and S-Chlts were 3.42 and 2.55, respectively.

observed at low intensities implies more efficient excitation of PS II in S-Chlts under light-limited conditions. This result is consistent with those mentioned earlier (Fig. 2 and 3) that the optical cross section of PS II is larger in S-Chlts. Detailed measurements of the initial slope of

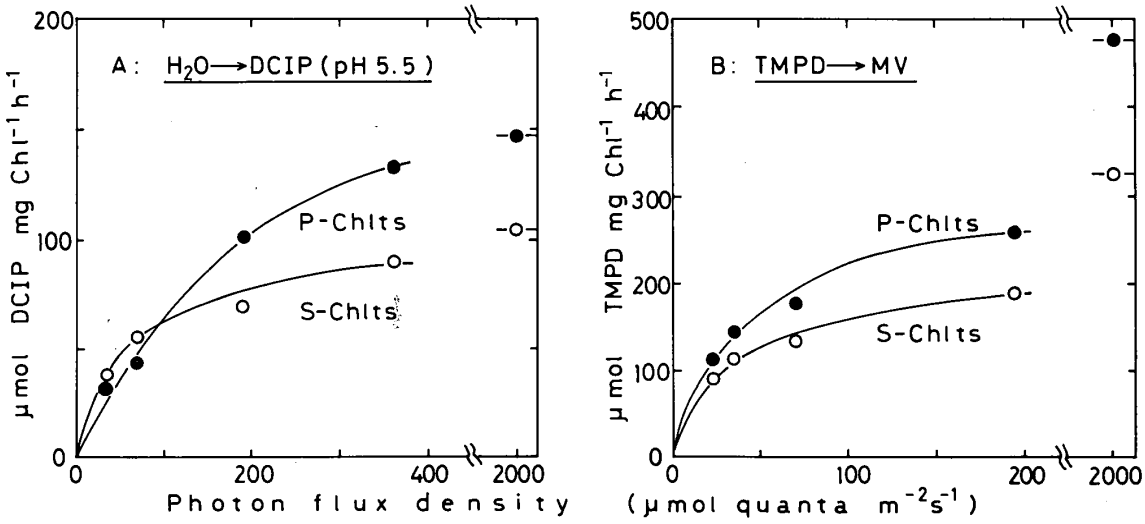

Fig. 5 Light saturation curves of the rate of PS II electron transport (A), and PS I electron transport (B). Activities were measured spectrophotometrically by DCIP photoreduction at pH 5.5 for PS II and by TMPD photooxidation for PS I. Chl concentration was adjusted to 5 μg Chl ml^{-1} in all measurements to make light absorption by each sample equal. For details, see **Materials and Methods**. Chl *a*/*b* ratios of P- and S-Chlts were 3.23 and 2.67, respectively.

Table 2 Photosynthetic electron transport activity in P- and S-Chlts at saturating light intensity

Chloroplasts	Chl *a*/*b*	PS I activity (TMPD → MV)	PS II activity (H_2O → DCIP)
		(μmol mg Chl^{-1} h^{-1})	
Experiment 1			
P-Chlts	3.40	601	189
S-Chlts	2.62	358	151
Experiment 2			
P-Chlts	3.34	636	192
S-Chlts	2.68	335	119

the light intensity curves revealed that the quantum yield (in relative units) of PS II photoreaction in S-Chlts was reproducibly higher than that in P-Chlts by a factor of 1.5–1.9 depending on the chloroplast source.

Fig. 5-B shows the intensity dependence of PS I electron transport in P- and S-Chlts. At saturating intensities, the P-Chlts activity was considerably higher. It was also higher at low intensities and detailed measurements of the initial slopes of the intensity curves revealed that the quantum yield of PS I photoreaction was higher in P-Chlts. Thus, for S-Chlts, the quantum yield of PS I is low but that of PS II is high while for P-Chlts, the PS I yield is high but the PS II yield is low. If we assume that most of the light energy absorbed by chlorophyll is transferred to either the PS I or the PS II reaction center, this complementary relationship can be reasonably understood.

Fig. 5 also shows that at saturating light intensity, both PS I and PS II activities are higher in P-Chlts than in S-Chlts. The rate-limiting factor(s) of electron transport under light saturation is generally considered to be the abundance and/or the turnover rate of the components of the electron transport chain. The difference in PS II electron transport activity (H_2O→DCIP at pH 5.5) observed between P- and S-Chlts seems to have resulted from the difference in the amount of the components; P-Chlts were estimated to contain more PS II reaction center, based on the peak area of 43 kDa proteins on the densitogram in Fig. 1, and also a larger plastoquinone pool size based on the fluorescence transients. As for the components involved in PS I electron transport (TMPD→MV), about 1.4-fold more P700 on a chlorophyll basis was present in P-Chlts (Table 1). This may be related to the P-Chlts having higher PS I activity at saturating intensity. Although decisive characterization with respect to the abundance of electron transport components in P- and S-Chlts must await more detailed quantitative studies, it is noteworthy that for chloroplasts preparations from the same leaf, both PS I and PS II activities under light saturation were higher in P-Chlts than in S-Chlts (Table 2).

Discussion

The in situ light environment of spongy tissue is characterized by low intensity illumination enriched in green and far-red. This favors excitation of PS I, which preferentially absorbs far-red light. Under such conditions, effective excitation of PS II by green light will be required to maintain a balanced electron flow through the non-cyclic electron transport chain. The present study suggested that in S-Chlts, preferential excitation of PS II occurs due to a large optical cross section of PS II arising from an abundance of LHCP.

Our hypothesis did not agree with those proposed for leaves grown under far-red light or for the leaves of shade plants. Melis and Harvey (1981) measured the contents of PS II

quencher, Q, and P700 in leaves grown under far-red illumination, and concluded that the energy imbalance under far-red light is offset by increased abundance of PS II reaction centers relative to PS I reaction centers (Q/P700) in far-red-grown leaves. A similar view of the changes in the PS II population has been reported for shade leaves by Björkman (1981), who proposed an increase in PS II reaction center in shade leaves, assuming a stoichiometric relationship between LHCP and CPa. However, we found no indication of an increase in PS II reaction center in S-Chlts of *Camellia* leaves. The CPa/CP I ratio estimated from the areas on densitograms in Fig. 1 showed similar values both for S- and P-Chlts, although the contents (on Chl basis) of both reaction centers were considerably lower in S-Chlts. As far as the S- and P-Chlts of *Camellia* leaves are concerned, effective excitation of PS II in S-Chlts seems to result from the larger optical cross section of PS II, as shown by the fluorescence spectra (Fig. 2), and the Chl *a*/*b* ratio (Table 1 and 2), and not from an increase in the population of PS II reaction centers. Support for this comes from a recent report by Leong and Anderson (1983) that the stoichiometric relationship between LHCP and CPa is not always very rigid in pea plants grown under various light conditions.

The present study also suggested that P-Chlts have smaller unit sizes of both PS I and PS II but with larger plastoquinone pools than S-Chlts, which leads to a higher maximum rate of electron transport in P-Chlts under light saturation (Fig. 5, Table 2). These results are analogous to those reported for sun and shade leaves (Mousseau et al. 1967, Boardman 1977, Wild 1979, Björkman 1981), and consistent with the widely accepted view that light intensity during growth determines the maximal rate of photosynthetic activities, namely P- and S-Chlts are adjusted to high-intensity and low-intensity environments, respectively, although both are located in a single leaf.

Adjustments of photosynthetic properties appear to suit the energy economy in a leaf. Photosynthetic production in a whole leaf is most efficient when P-Chlts have a small light-harvesting system but a large pool of electron carriers and S-Chlts have a large pigment system but a small pool. From this point of view, the existence of two different types of chloroplasts, "sun chloroplasts" and "shade chloroplasts" within a leaf, may be a general phenomenon in bifacial leaves. We have found some such distinctions in *Nicotiana*, *Phytolacca* and *Spinacia* leaves. The sun and shade chloroplast system within a leaf should be helpful for optimizing the utility of light flux penetrating the leaf as well as effectively allocating light energy within a leaf based on the different optical properties of palisade and spongy tissues, as previously reported by Terashima and Saeki (1983).

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