Monitoring chiral macroaggregates of LHCII: From isolated chloroplasts to green leaves

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Introduction

Chiral macroaggregates of LHCII in thylakoid membranes might play an important role in the lateral separation of the two photosystems, long-range migration/delocalization of the excitation energy and protection of the photosynthetic apparatus against photoinhibitory damage. In isolated chloroplasts, chiral macroaggregates can be detected by circular dichroism (CD). The chiral macroaggregation was reported to respond to the content of the medium surrounding the chlolroplasts, and to photoinhibitory illumination (Garab, 1996; Gussakovsky et al., 1997).

Chiral macroaggregates can also be detected in isolated chloroplasts by a circularly polarized chlorophyll luminescence (CPL; Gussakovsky et al., 2001). CPL is characterized by an emission anisotropy factor, \( g_{em} \). The CD and CPL methods are, in general, complementary techniques (see Gussakovsky et al., 2001, for detail). CD, which is based on absorption measurements, can readily be applied to isolated chloroplasts in suspensions, but not to green leaf systems. In contrast, CPL, which is based on luminescence measurements, is more applicable to intact leaves. In the present study, we applied the CPL approach to study chiral macroaggregates of LHCII particles in green pea leaves.

Material and methods

Pea plants were grown under continuous cool white light at room temperature. Mature leaves of 14-17 days old plants were used for the measurements. CPL was measured at room temperature by the homemade machine described previously (Gussakovsky et al., 2001), which has been modified for leaf measurements. Chlorophyll luminescence was excited by a laser diode of 2.5 mW, 635 nm. The photochemical quantum efficiency of the leaves was monitored by the modulated chlorophyll fluorescence parameter \( F_v/F_m \) (Schreiber et al., 1994) using a PAM-2000 Portable Chlorophyll Fluorimeter (Heinz Walz, Germany), following 15 min dark adaptation. Photoinhibitory illumination was provided by white light that was heat-filtered through a 10 cm water layer and glass filter resulting in 3-4 mmol m\(^{-2}\) s\(^{-1}\) intensity at the leaf surface.
Results and Discussion

Spectra and intensity of CPL

Figure 1 shows typical spectra of chlorophyll CPL from adaxial and abaxial sides of pea leaves, at the 45/90 variant of the excitation/emission alignment (see below). The spectra closely resemble the CPL spectrum previously reported for isolated pea chloroplasts having a maximum at 690 nm (Gussakovsky et al., 2001) and a negative band at about 670 nm. For intact pea leaves with photochemical efficiency \( F_v/F_m \) of 0.84-0.77, the \( g_{em} \)-factor at 690 nm ranged from \( 9 \times 10^{-4} \) to \( 74 \times 10^{-4} \) in different leaf samples. In isolated pea chloroplasts, the \( g_{em} \)-factor was in the same order of magnitude (Gussakovsky et al., 2001). The similarity of both the spectrum and magnitude for isolated chloroplasts and intact leaves (in both adaxial and abaxial sides), suggests that they reflect the same CPL phenomenon, namely chiral macroaggregation.

![Figure 1. Spectra of circular polarization of chlorophyll luminescence (CPL) of intact pea leaves from the adaxial (1) and abaxial (2) sides. The measurements were done at the 45/90 optical alignment (see Table 1), using excitation light of 635 nm and spectral resolution of 7.4 nm.](image)

Effect of beam alignment and leaf sidedness on the measured CPL

Maximal CPL signals were detected from the adaxial side of pea leaves when luminescence was excited at 45°, and the emission collected at 90° to the plane of the leaf (Table 1). The circularly polarized emission detected at 45°, was significantly reduced when the excitation angle was set to either 45° or 90°. However, the difference in the \( g_{em} \)-factor for the 45/45 and 90/45 variants was not statistically significant. We chose the 45/90 optical alignment for all further studies of CPL from adaxial side. Using neutral glass filter to attenuate the excitation intensity in the range of 110-350 \( \mu \)mol m\(^{-2}\)s\(^{-1}\), we found no effect on CPL at all different optical alignments (data not shown). Hence, the variation of CPL probably reflects the optical properties of the leaf.

Epidermal cells were shown to serve as a lens for perpendicular incident light beams penetrating into the palisade or spongy layers (Poulson and Vodelmann, 1990). The circularly polarized emission from adaxial side might indeed relate to this effect, with the CPL being maximal under focusing, while smaller under non-focusing conditions. It is also possible that the lower CPL signals measured in intact leaves, compared with isolated pea chloroplasts, result from this lens effect of the epiderm. The independence of the \( g_{em} \)-factor on the excitation angle (for emission collection at 45° to the adaxial plane) might suggest that CPL does not depend on the focusing effect for the non-polarized excitation light. For the abaxial side, the different optical alignments did not affect the CPL signal (Table 1). The palisade (at the adaxial) consists of highly organized cells, while the spongy tissue cells of the abaxial side are randomly oriented (Villani and DeMason, 1997). Hence, the dependence of the adaxial CPL signal on the optical alignment might relate to the spatial organization of cells.
Table 1

<table>
<thead>
<tr>
<th>Variant</th>
<th>Angle between leaf plane and light beam</th>
<th>$g_{em} \times 10^{-4}$ (average of 14 leaves ± standard deviation)</th>
<th>Excitation</th>
<th>Emission</th>
<th>Adaxial side</th>
<th>Abaxial side</th>
</tr>
</thead>
<tbody>
<tr>
<td>45/90</td>
<td>45</td>
<td>45.4 ± 4.6</td>
<td>90</td>
<td>90</td>
<td>48.7 ± 7.6</td>
<td>44.5 ± 6.3</td>
</tr>
<tr>
<td>45/45</td>
<td>45</td>
<td>32.4 ± 5.5</td>
<td>45</td>
<td>45</td>
<td>44.5 ± 6.3</td>
<td>42.6 ± 4.8</td>
</tr>
<tr>
<td>90/45</td>
<td>90</td>
<td>35.5 ± 5.8</td>
<td>45</td>
<td>45</td>
<td>44.5 ± 6.3</td>
<td>42.6 ± 4.8</td>
</tr>
</tbody>
</table>

**Kinetics**

The intensity of the excitation laser beam was sufficient to drive photosynthetic electron transport. Indeed, the time course of the total luminescence intensity has showed a curve typical for chlorophyll luminescence changes in intact leaves, namely a fast rise followed by a slower decrease, reaching a steady state within about 70 s (Fig. 2). This behavior reflects the photochemical processes occurring in the photosynthetic apparatus after the onset of actinic illumination. In contrast, the $g_{em}$-factor remained essentially unchanged throughout the measuring time, indicating no changes in the chiral macroaggregates status during the photochemical equilibration.

![Fig.2. Time course of the total luminescence (1) and the $g_{em}$-factor at 690 nm (2) for the adaxial side of intact pea leaves. The measurements were done at the 45/90 optical alignment (see Table 1), using excitation and emission wavelengths of 635 and 690 nm, respectively. For more details see Materials and Methods.](image)

**Effect of photoinhibitory illumination**

Attached pea leaves were exposed to photoinhibitory illumination and assayed for CPL as well as the chlorophyll fluorescence parameter $F_v/F_m$. The latter measures the reduction in the photochemical quantum capacity of PS II reaction centers, occurring during photoinhibition (Schreiber, 1994). Figure 3 shows the $g_{em}$-factor at 690 nm as plotted versus $F_v/F_m$. In this series of experiments, the initial $F_v/F_m$ ranged between 0.72-0.81 and $g_{em}$-factor gave the averaged value of $53 \times 10^{-4}$ (n=24) depending on sample. No correlation was found between the CPL and the initial $F_v/F_m$ values.

After photoinhibitory illumination for durations varying between 12-20 min (which did not lead to a visual reduction of the leaf turgor), the $F_v/F_m$ ratio was reduced to values ranging between 0.68-0.002. Again, the CPL signal did not correlate with $F_v/F_m$. The average $g_{em}$-factor after the photoinhibitory illumination was only 26% lower than in untreated leaves (Fig.3). Nevertheless, significant reduction of CPL was observed for leaves, which developed visual symptoms of leaf wilting at the end of the illumination (triangles in Fig.3). For these leaves, the $g_{em}$-factor ranged between $-1.4 \times 10^{-4}$ to $17.2 \times 10^{-4}$ with a mean value of $g_{em}/g_{em,0} = 0.17 \pm 0.18$, and the $F_v/F_m$ values were also close to zero (0.003-0.076). Therefore, we further studied the effect of leaf dehydration (drought stress) in more details (see below).
The results indicate that the photoinhibitory illumination by itself causes only minor reduction in the chiral macroaggregate content of attached pea leaves. This change does not correlate with the reduction in the photochemical quantum efficiency of the PS II complexes. We therefore hypothesize that chiral macroaggregation of LHCII particles might serve as a structural support for the proper functioning of the photosynthetic apparatus, while it is not directly involved in determining the efficiency of PSII centers.

**Drought stress**

To reveal the effect of leaf dehydration on the chiral macroaggregate level, drought stress was applied to detached pea leaves by three modes. (i) Slow dehydration at room temperature for 61 hours under room light (15-20 µmol m\(^{-2}\) s\(^{-1}\)) cause gradual decrease of the g\(_{em}\)-factor down to zero (g\(_{em}/g_{em,o} = 0.03±0.08\)) at RLH < 40% (Fig.4). Under these conditions, full disruption of chiral macroaggregates occurred at F\(_v/F_m < 0.4\). (ii) Fast drought stress, which was induced under similar conditions, but with supplemented mild heating during 3.5 hours, and which reduced RLH down to 70%, resulted in a strong cooperative decrease down to zero of both the g\(_{em}\)-factor and F\(_v/F_m\). At F\(_v/F_m > 0.1\), the g\(_{em}\)-factor remained almost unchanged, while at F\(_v/F_m < 0.04\), chiral macroaggregates were essentially abolished (data not shown). (iii) Dehydration that developed during 20 min photoinhibitory illumination closely resembled the fast drought response: a sharp drop of g\(_{em}/g_{em,o}\) occurred within a narrow range of RLH (70-80%). However, photoinhibitory illumination led to negative CPL (g\(_{em}/g_{em,o} = -0.21±0.14\)).

**Conclusions**

CPL is the only technique that enables to monitor chiral macroaggregates of LHCII in intact green leaves. It thus provides a non-destructive tool for structural studies of antennae macrostructures inside chloroplasts of attached leaves under varying environmental conditions (diseases, agro-technical treatments, climatic stresses etc.). The present study, in which CPL was tested in intact leaves for the first time, revealed that the chiral macroaggregation of
LHCI is highly responsive to the leaf hydration status, while it is relatively insensitive to photoinhibitory illumination.

References


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