Spectroscopic study of the light-induced changes of the core antenna complex CP43 in photosystem II

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Abstract

In this study, spectroscopic approaches were used to evaluate light-induced changes in chlorophyll protein 43 (CP43). Upon illumination, we observed chlorophyll (Chl) a bleaching with the complete opening of the tetrapyrrrole ring. Electronic transition frequencies to different energy levels of the second singlet electronic state (S2) were changed. In addition, we observed altered rotational and vibrational transitions of Chl a and the CP43 apoprotein as well as variations in the microenvironment surrounding Chl a. CP43 contains three β-carotene (β-Car) and five Chl a molecules, which are involved in the energy transfer from β-carotene to Chl a. We found that these five Chl a molecules bleached more readily than others under constant illumination, with energy transfer from β-Car to Chl a being continuously lost as illumination continued.

Additional key words: absorption; degradation; emission; excitation; fluorescence.

Introduction

Chl protein 43 (CP43) is encoded by PsbC and is composed of six transmembrane α-helical domains as well as five exterior loop domains and stroma exposed N- and C-terminal domains (Bricker and Frankel 2002, Qu et al. 2007). CP43 is located on the surface of PSII core complex, proximal to the D1 of the reaction center (Eijckelhoff et al. 1997, Barber et al. 2000, Hall et al. 2016). This positioning within PSII enables CP43 to readily accept energy absorbed by other membrane proteins such as Chl protein 26 (CP26) and LHCII (Boekema et al. 1999, Yakushevskia et al. 2003), and transfer energy to the PSII reaction center wherein water undergoes oxidation and the photochemical reaction occurs (Hughes et al. 2004, Wang et al. 2018).

CP43 contains 4–5 β-carotene (β-Car) as well as 13 Chl a molecules in CP43 (Casazza et al. 2010, Miś et al. 2015). Chlorophylls are organized into luminal and stromal layers within the thylakoid membrane, with a Chl molecule (Chl 510) being located centrally within this compartment (Fig. 1).

Light is an important environmental factor that influences the photosynthesis process (Montgomery et al. 2008). When PSII membrane is exposed to an alkaline Tris. This loss is thought to result from D1, Chl protein 47 (CP47), and CP43 cross-linking and a concomitant change in D1 degradation efficiency (Mori et al. 1995).

Prior studies have primarily focused on either studying CP43 in the interior of PSII under alkaline conditions or have studied Chl a molecules in an organic solution. Further studies, specifically focused on individual CP43 complexes, are thus needed in order to better understand its functionality in the absence of other PSII components and under normal pH value.

In the present study, we employed spectroscopic approaches to evaluate light-induced changes in CP43, with a specific focus on the impairment of Chl a and changes in energy transfer. Through these approaches, we found that illumination was associated with changes in the frequency of the electronic transition to different energy levels of the second singlet electronic state. This illumination was also associated with altered rotational and vibrational energy transitions for both Chl a and the CP43 apoprotein, with variations in the Chl a microenvironment also being evident. A continuous loss of energy transfer from β-Car to Chl a was also observed in response to illumination, with the five Chl a molecules in CP43 responsible for accepting energy from β-Car being bleached more readily than other molecules.

Materials and methods

CP43 purification: Spinach (Spinacia oleracea L.) PSII particles were isolated using methods previously described...
Core complex extraction was conducted as previously detailed by Ghanotakis et al. (1989). CP43 purification was conducted as outlined by Qu et al. (2006) and Picorel et al. (2011). Samples were diluted to 6 µg(Chl) mL⁻¹ in a 0.05% L-dodecyl maltoside (DM) (Sigma-Aldrich, USA) 20 mmol L⁻¹ Bis-Tris (pH 6.0) solution. All steps were carried out at 4°C in the dark.

**White-light illumination:** A halogen lamp (CDD-1000, Bela Design, China) was used to treat samples with white light at 1,000 µmol(photon) m⁻² s⁻¹. CP43 samples were added to a vessel under constant circulation with water at 4°C, and the sample was continuously stirred during illumination.

**Fluorescence measurements:** Fluorescence spectra were assessed using an F-4600 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) at room temperature with emission and excitation slits both of 5.0 nm in size, and with a time constant of 1 s and a scan rate of 120 nm min⁻¹. The detection wavelength was 683 nm for the fluorescence excitation spectra determination, as it was the peak fluorescence emission spectra position of CP43.

**Absorption spectra measurements:** A UH5300 spectrophotometer (Hitachi, Tokyo, Japan) was used to measure room temperature absorption spectra, with a 0.5-nm data interval and a 2 nm s⁻¹ scan rate.

**Statistical analysis:** All experiments were repeated at least three times. The analyses were done with SPSS 20.0 (IBM, Chicago, USA).

**Results**

**CP43 absorption spectra:** The room temperature absorption spectrum of CP43 exhibited two main peaks at 436 and 671 nm (in the blue and the red region, respectively), with several other small peaks also being evident (Fig. 2). This analysis did not indicate the presence of a pheophytin Qx (1-0) peak at 542 nm, suggesting that the tetrapyrrole ring of Chl a was completely degraded, and did not only loose the Mg atom. Under illumination, both of these main peaks decreased in size over time, indicating that the Chl a within CP43 was destroyed over time in an illumination-dependent manner.

No shift in absorption maximum was detected following the sample illumination (Fig. 2, Table 1). The full width at half maximum (FWHM) of the peak at 671 nm did gradually increase over the first 15 min of illumination, before rapidly increasing from 15–30 min and then not changing further after 30 min. The ratio of absorbance at 436 to 419 nm also decreased in time in response to illumination.

**CP43 fluorescence emission spectrum:** The fluorescence spectrum is highly sensitive to changes of chromophores in a given protein. We determined that upon excitation at 436 nm at room temperature, CP43 exhibited a fluorescent emission peak at approximately 683 nm (Fig. 3). In response to illumination, this fluorescence emission spectrum decreased gradually; with the emission peak

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Fig. 1. β-Car and Chl a organization in CP43 (view along the membrane). (A) Relative positions of Chl a tetrapyrrole rings in CP43 and of the main apoprotein chain of CP43. (B) β-Car and Chl a organization in CP43. Chls 501–504 are located proximal to the lumenal side of the membrane, Chls 505–513 are to the stromal side. Chl 510 is located within the middle of the membrane. Data were derived from 3JCU in RCSB PDB, and from an article by Wei et al. (2016) pertaining to the spinach (Spinacia oleracea L.) PSII crystal structure. PROTEINVIEW was used to prepare this figure.
shifting from 683 nm to 682.6, 682.0, 680.8, 680.2, 679.4, 678.6, and 678.0 nm when illuminated for 2, 5, 10, 15, 20, 30, and 60 min, respectively.

Upon excitation at 480 nm at room temperature, CP43 also exhibited a fluorescent emission peak at approximately 683 nm (Fig. 4). Following 2, 5, 10, 15, 20, 30, and 60 min of illumination, these peak values declined by 18.1, 35.2, 51.5, 61.2, 69.0, 75.8, and 86.4%, respectively, while the peak position shifted from 683 nm to 682.4, 682.0, 681.4, 680.2, 679.8, 678.8, and 677.8 nm, respectively.

**β-Car to Chl a energy transfer rate:** In order to calculate the energy transfer rate from β-Car to Chl a, we used the formula \( F/A \times 100\% \) according to the methods of Shan et al. (2000) and de Weerd et al. (2003), where \( A \) is corresponding to the absorption intensity at 488 nm and \( F \) is corresponding to the fluorescence excitation spectrum intensity at 488 nm. We calculated that the energy transfer rate between β-Car and Chl a in native CP43 was 34.1%, which is in line with the previous reports mentioned above. In addition, we found that this energy transfer rate decreased in response to prolonged illumination (Table 2).

The β-Car and Chl a pairs with energy transfer were presented in Table 3. Data were derived from the spinach (Spinacia oleracea L.) PSII crystal structure (3JCU in RCSB PDB, Wei et al. 2016).

| Table 1. Spectrum intensity, FWHM of the peak at 671 nm and \( A_{436}/A_{419} \) ratio of CP43 over the course of 1,000 μmol(photon) m\(^{-2}\) s\(^{-1}\) illumination. Percentage decreased were calculated as \( (A_0 - A)/A_0 \times 100\% \), where \( A \) is the absorbance of CP43 at 671 nm under illumination, and \( A_0 \) is the absorbance of CP43 at 671 nm when without illumination. FWHM – full width at half maximum.

<table>
<thead>
<tr>
<th>Illumination time [min]</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance intensity</td>
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<td>0.496</td>
<td>0.452</td>
<td>0.395</td>
<td>0.353</td>
<td>0.319</td>
<td>0.267</td>
<td>0.198</td>
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<tr>
<td>Percentage decreased [%]</td>
<td>-</td>
<td>8.0</td>
<td>16.1</td>
<td>26.7</td>
<td>34.5</td>
<td>40.8</td>
<td>50.5</td>
<td>63.3</td>
</tr>
<tr>
<td>FWHM [nm]</td>
<td>23.8</td>
<td>24.2</td>
<td>24.3</td>
<td>25.3</td>
<td>24.8</td>
<td>26.5</td>
<td>32.0</td>
<td>32.0</td>
</tr>
<tr>
<td>( A_{436}/A_{419} )</td>
<td>1.18</td>
<td>1.17</td>
<td>1.15</td>
<td>1.15</td>
<td>1.11</td>
<td>1.10</td>
<td>1.08</td>
<td>1.05</td>
</tr>
</tbody>
</table>

Fig. 2. Changes in the CP43 absorption spectra at room temperature in response to 1,000 μmol(photon) m\(^{-2}\) s\(^{-1}\) illumination. CP43 was suspended in a 0.05% DM, 20 mmol L\(^{-1}\) Bis-Tris, pH 6.0 buffer at a sample density of 6 μg mL\(^{-1}\) of Chl a.

Fig. 3. CP43 fluorescence emission spectra at room temperature following excitation at 436 nm upon 1,000 μmol(photon) m\(^{-2}\) s\(^{-1}\) illumination. CP43 was suspended in a 0.05% DM, 20 mmol L\(^{-1}\) Bis-Tris, pH 6.0 buffer at a sample density of 6 μg mL\(^{-1}\) of Chl a.

Fig. 4. CP43 fluorescence emission spectra at room temperature following excitation at 480 nm upon 1,000 μmol(photon) m\(^{-2}\) s\(^{-1}\) illumination. CP43 was suspended in a 0.05% DM, 20 mmol L\(^{-1}\) Bis-Tris, pH 6.0 buffer at a sample density of 6 μg mL\(^{-1}\) of Chl a.
Table 3. Distances between β-Car and Chl a pairs in CP43. Only β-Car and Chl a pairs with an inter-chromophore distance of < 5 Å are shown. Distances were measured edge-to-edge between the porphyrin of Chl and the β-Car aryl ring. Data are derived from the spinach (Spinacia oleracea L.) PSII crystal structure (3JCU in RCSB PDB, Wei et al. 2016).

<table>
<thead>
<tr>
<th>Chl</th>
<th>β-Car 514</th>
<th>β-Car 515</th>
<th>β-Car 517</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl 501</td>
<td>-</td>
<td>3.99 Å</td>
<td>-</td>
</tr>
<tr>
<td>Chl 505</td>
<td>-</td>
<td>4.7 Å</td>
<td>-</td>
</tr>
<tr>
<td>Chl 511</td>
<td>-</td>
<td>-</td>
<td>3.85 Å</td>
</tr>
<tr>
<td>Chl 512</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chl 513</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

Discussion

Electronic transition: The absorption spectra of Chls primarily arise as a consequence of electronic transitions to S1 and S2 singlet states from the ground state within the tetrapyrrole ring. Ring V of the tetrapyrrole ring destroys the degeneracy coming from the ring geometry and then splits the two singlet states. Consequently, the resultant absorption spectra of Chl a exhibit two primary bands (B- and Q-bands), with the 419 and 436 nm peaks being assigned to the B-band (Houssier and Sauer 1970). Microenvironmental factors can alter the electronic transition frequency between different energy levels for a given chromophore, thus altering the associated absorption intensity (Zhao and Zhou 2000). The observed illumination-dependent reduction in the A436/A419 ratio (Fig. 2, Table 1) indicates a change in the frequency of electronic transition to different energy levels of the second singlet electronic state (S2); the A436/A419 ratio would not change if only bleaching of Chl a happened. This also suggested that there were changes in the microenvironment surrounding Chl a in this experimental context.

Vibrational and rotational energy levels: Electronic, rotational, and vibrational transitions are related to the overall absorption spectra. The electronic transition is associated with the spectrum peak, while the remainder of the spectrum is comprised of a series of vibrational and rotational transitions. As such, the overall appearance of a given absorption spectrum is closely related to the vibrational states of a given chromophore (Albani 2007). The observed illumination-induced increases in FWHM (Fig. 2, Table 1) are consistent with significant changes in the vibrational and rotational transitions of Chl a. The electronic states of chromophores are not linked to those of a given protein with which they interact, while the vibrational states of chromophores are influenced by those of the given protein (Renger 1992). As such, changes in Chl a vibrational states are also related to changes in those of the overall CP43 apoprotein.

Microenvironmental polarity surrounding Chl a: Microenvironmental polarity can influence the maximum position of the emission profile of a given chromophore. When the polarity decreases, fluorescence maximum blue shifts (Zhao and Zhou 2000, Xu and Wang 2006). In response to illumination, CP43 exhibited a blue-shifted maximal fluorescence emission profile in response to excitation at 436 nm (Fig. 3). This suggests a reduction in the microenvironmental polarity surrounding Chl a. Previous works (Groot et al. 1999, Di Donato et al. 2007) suggested a narrow absorption band for CP43 at 5 K, which peaks at 682.5 nm. This band is correlated to a single isolated Chl a molecule, with which other Chl a molecules are weakly coupled. This Chl a is located in a polar environment, and its exact assignment is not clear now. The absorption of this Chl a originates from specific pigment–protein interactions, and is involved in the polar environment. Namely, the origin of this absorption is environmental rather than excitonic. In addition, this Chl a is lost more easily and it dominates the emission spectrum. Therefore, it contributes more to the blue shift than other Chls.

Energy transfer from β-Car to Chl a: Carotenoids are important pigments participating in photosynthetic antenna reactions, wherein they serve as accessory pigments enabling rapid singlet–singlet energy transfer to antenna Chl and further to the reaction center, thus extending the overall spectral absorption range (Hashimoto et al. 2016, Collini 2019). The shoulder observed at approximately 480 nm in the CP43 absorption spectrum is attributable
to β-Car (Fig. 2), which primarily functions in CP43 via transferring its excitation energy to Chl a. Since β-Car is essentially nonfluorescent, the observed fluorescence in response to excitation at 480 nm, therefore, reflects such energy transfer from β-Car to Chl a. Following illumination, fluorescent emission in response to 480 nm excitation was reduced (Fig. 4). This suggests a sustained loss of energy transfer from β-Car to Chl a, as supported by Table 2.

Both the fluorescence peak and the overall fluorescence emission spectra of native CP43 upon excitation at 480 nm was nearly identical to that observed in response to 436 nm excitation following normalization (Figs. 3, 4). This further supports the model wherein energy transfer between β-Car and Chl a occurs, supporting the functional and structural integrity of the CP43 samples used in the present analysis.

The energy transfer from β-Car to Chl a occurs through what is known as the ‘Dexter mechanism’. In this mechanism context, the small distance (< 5 Å) between two given chromophores is a key determinant of energy transfer (Shan et al. 2000, de Weerd et al. 2002, Qu et al. 2007). There are a total of five Chl a molecules in CP43 that are sufficiently close to β-Car to allow this mechanism to function (Fig. 1, Table 3). As such, energy transfer from β-Car to Chl a within CP43 can readily occur between the pigment pairs detailed in Table 3.

Beside structure stabilization and light harvesting via singlet state energy transfer, carotenoids are very effective quenchers of Chl triplet states, preventing the sensitized formation of singlet state oxygen. In isolated pigment–protein complexes, the rate of intersystem crossing is a key determinant of energy transfer (Collini E.: Carotenoids in photosynthesis: The revenge of the “accessory” pigments. – Chemistry 5: 494-495, 2019)


The details of the microenvironmental changes of CP43 upon illumination remain to be studied further.

The decreases in calculated energy transfer (Table 2) and in fluorescence emission intensity following 480 nm excitation (Fig. 4) were greater than the observed decreases in absorption intensity (Fig. 2). This suggests that the five Chl a molecules in CP43 capable of accepting energy from β-Car bleached more readily than did other Chl a molecules.

References

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