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Light- and heat-induced denaturation of Photosystem II core-antenna complexes CP43 and CP47

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Abstract

Light- and heat-induced denaturation of the core-antenna complexes of Photosystem II, CP43 and CP47, purified from spinach, has been investigated using absorption, fluorescence and circular dichroism (CD) spectroscopy. Light is found to bring about considerable bleaching of chlorophyll *a* but no apparent change in the protein secondary structure, while heat induces significant unfolding of the protein secondary structure but no apparent destruction of the chlorophyll *a* molecule in the two antenna complexes. Both the destruction of chlorophyll *a* by light and the denaturation of the protein conformation by heat cause the loss of excitonic interaction of chlorophyll *a* in CP43 and CP47, as measured by visible CD activity. Light induces a larger decrease of the chlorophyll *a* fluorescence and the CD activity of CP47 than that of CP43, indicating that the native state of chlorophyll *a* of CP47 is more sensitive to light than that of CP43. The main thermal transitions of protein secondary structure occur at 50°C for CP43 and 63°C for CP47, while the half-loss of chlorophyll *a* excitonic interaction during heating occurs at 45°C for CP43 and 60°C for CP47, suggesting that CP47 is more thermally stable than CP43.

Keywords: Photosystem II; CP43; CP47; Absorption; Fluorescence; Circular dichroism

1. Introduction

Photosystem II (PSII) is a membrane–protein complex located in the thylakoid membrane [1]. It uses solar energy to carry out the primary processes of photosynthesis: charge separation, quinone reduction and water oxidation to molecular oxygen. CP43 and CP47 are two core-antenna complexes in PSII. They accept excitation energy that is harvested by the light-harvesting complex and transfer the energy directly to the PSII reaction center (RC) [2]. In addition to acting as interior antennas, CP43 and CP47 may also perform other structural and functional roles in PSII [3].

The predicted topology of both CP43 and CP47, according to hydropathy plot analysis from the derived amino acid sequences, includes six membrane-spanning α-helixes and five hydrophilic loops connecting these helixes [2]. CP43 and CP47 bind chlorophyll *a* (Chl *a*) and β-carotene, but no chlorophyll *b*. The pigment stoichiometry of CP43 and CP47 is still not clearly known, and the spatial arrangement of the bound pigments has not been determined [4]. Different pigment contents have been reported due to the different methods used for protein purification and pigment and protein quantitation. Akabori et al. calculated that there are 11 Chl *a* molecules in CP43 and 13 in CP47 [5]. Barbato et al. concluded that CP43 and CP47 bind nine to 12 Chl *a* molecules [6]. Alfonso et al. reported 20 Chl *a* molecules in CP43 and 21–22 in CP47 [7]. Zheleva et al. claimed that CP43 and CP47 each contain 12–15 Chl *a* molecules [8]. Several studies showed that the two core-antenna complexes contain different species of Chl *a*; however, the corresponding pigment properties of these Chl *a* species are still not fully understood [4].

Strong illumination results in the photoinhibition of photosynthesis with PSII as the main target. Under high light illumination, the PSII membrane suffers impairment of pigments [9,10], degradation of proteins such as the D1 protein [11,12] and loss of photosynthetic activity. Both CP43 and CP47 are found to be impaired during photoinhibition [13]. CP43 was degraded to three products when PSII membranes

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were treated with Tris and illuminated with white light under aerobic conditions at 25°C [14]. D1 protein, CP43 and CP47 were lost from the PSII membranes and significant cross-linking was induced among them during treatment with alkaline Tris in weak light [15]. Unfortunately, few of these studies investigated the impairment of the Chl *a* molecules and the energy-transfer functions of CP43 and CP47. The specific effects of photoinhibition on these molecules in the PSII membrane cannot be determined due to the presence of other proteins and pigments, as there are more than 25 proteins and about 200–250 antenna chlorophyll molecules for every PSII in higher plants.

Heat-inactivation studies, differential scanning calorimetry (DSC) and thermal gel analysis have been used to investigate the thermal denaturation of chloroplast membrane [16–18], PSII membrane [19], PSII core and light-harvesting Chl *a*/*b* protein (LHCP) fractions [20]. The results showed that the PSII membrane exhibited at least four transitions: A_2 (47.5°C), B (54°C), C (59.5°C) and D (66°C) in the 40–70°C temperature range. The four transitions were assigned to denaturation of a component involved in oxygen evolution (A_2); closely associated antenna proteins or Chl *a* binding proteins, including CP43 (B); major components of the core, i.e., D1, D2, cyt-*b*559 and CP47 (C); and LHCP (D) [21]. Fourier transform-infrared (FT-IR) and circular dichroism (CD) spectroscopy were also used to study the thermal stability of the PSII membrane, core complex [22], isolated RC [23] and manganese-stabilization protein (33 kDa protein) [24].

To exclude the effect of other proteins and/or pigments in PSII, isolated CP43 and CP47 are widely used to investigate the spectral properties, the pigment content and organization, the different properties of the Chl *a* species in the proteins, etc. [7,25–28]. In the present paper, the Chl *a* absorbance, fluorescence and CD activity of isolated CP43 and CP47 were measured to investigate and compare the changes of Chl *a* and protein during the light- and heat-denaturation procedures. The relationships between the Chl *a* molecular state, protein secondary structure and Chl *a* excitonic interaction are discussed. The results show that CP47 is more sensitive to light, but more stable thermally than CP43.

2. Materials and methods

2.1. Purification of CP43 and CP47

PSII-enriched membrane was prepared from spinach as previously described [29]. The oxygen-evolving core complex (OECC) was isolated as discussed in Ghanotakis et al. [25]. CP43 and CP47 were then purified according to the method of Alfonso et al. [7] with some modifications. OECC was first treated with 0.8 M Tris buffer (pH 8.0) for 15 min at a final chlorophyll concentration of 0.1 mg ml^{-1} and centrifuged at 40 000*g* for 10 min. The procedure was performed twice to remove completely the 33 kDa extrinsic protein. The pellet was resuspended in 50 mM 2-(*N*-morpholino)-ethanesulfonic acid (MES), 150 mM NaCl and 400 mM sucrose (pH 6.0) at a chlorophyll concentration of 1.5 mg ml^{-1}, and incubated with an equal volume of 20 mM Bis–Tris, 4 M $LiClO_4$ and 15% β-dodecyl maltoside (DM) buffer (pH 6.0) for 20 min with stirring. After that, the sample was dialyzed against 20 mM Bis–Tris (pH 6.0) for 2 h and then loaded onto a DEAE-Fractogel TSK 650S anion-exchange column (1.6 × 12 cm) that had been equilibrated with 20 mM Bis–Tris and 0.05% DM (pH 6.0) at a flow rate of 0.5 ml min^{-1}. The fraction that did not bind to the column and eluted first was CP43. After the elute became colorless, the column was subjected to a 0–200 mM $LiClO_4$, 20 mM Bis–Tris and 0.05% DM (pH 6.0) linear gradient to elute the CP47 and other fractions. To increase the purity of CP43 and CP47, they were then reloaded onto a shorter column (1.6 × 5 cm). The preparations were concentrated using NANOSEP centrifugal concentrators if necessary and stored at 77 K. All the above procedures were performed at 4°C in the dark. CP43 and CP47 were judged to be pure on the basis of SDS–PAGE. The elution profile of the anion-exchange chromatography and the electrophoretic pattern of the fractions were similar to the results of Alfonso et al. [7].

2.2. Light and heat treatment

CP43 and CP47 were suspended in 10 mM Bis–Tris and 0.05% DM (pH 6.0). Sample concentrations were adjusted so that the corresponding absorption maximum in the red region was 0.5. Light treatment was performed by illuminating the samples with weak white light at room temperature. The intensity at the sample cuvette was 500 μE m^{-2} s^{-1}. To study the effect of temperature, samples were heated in steps of 5°C from 25 to 80°C. After each heating step, the samples were incubated for 5 min and the corresponding spectra were then immediately measured.

2.3. Circular dichroism and absorption measurement

Circular dichroism spectra were measured in the far-ultraviolet region (190–250 nm) and the red region (630–720 nm). The 222 nm ellipticity and the overall CD intensity of the doublet signal in the red region were used to determine the changes in the protein secondary structure and the Chl *a* optical activity of CP43 and CP47 during light and heat treatments. CD spectra were recorded with a Jasco J-715 Spectropolarimeter at a scanning speed of 100 nm min^{-1}, a bandwidth of 2 nm, a response time of 1 s and an accumulation of four times in a cell of 1 mm pathlength. The corresponding absorption spectra were obtained from the optical density conversion of the high tension voltage, which was recorded simultaneously with the CD data, using the Standard Analysis program provided by Jasco.

2.4. Fluorescence measurement

Fluorescence spectra were measured with a Hitachi 850 fluorescence spectrophotometer at room temperature. All the excitation and emission band passes were 5.0 nm, and the scan rate was 120 nm min^{-1} with a time constant of 2 s. The photomultiplier gain was normal.

3. Results

3.1. Light-induced denaturation of CP43 and CP47

The optical absorption spectrum of CP43 measured at room temperature presented a maximum at 671 nm in the red region (640–710 nm), which was similar to that reported earlier [7]. Fig. 1(a) shows that the absorbance maximum continuously decreased during illumination, suggesting that Chl *a* molecules in CP43 were destroyed by light. The fluorescence spectrum for CP43 at room temperature upon excitation at 430 nm displayed a maximum at 683 nm (Fig. 1(b)), which is similar to previous reports [27]. The fluorescence intensity also decreased during illumination accompanied by a blue shift in wavelength. The CD spectrum in the red region (640–710 nm) for CP43 contained a doublet signal with a negative peak at 686 nm and a positive peak at 667 nm, which was mainly caused by the excitonic interaction of Chl *a* in CP43. The result was different from that in the previous report, in which the CD spectrum of CP43 showed a negative band at 670 nm. Both the negative and positive peaks decreased during illumination (Fig. 1(c)). The CD spectrum in the far-ultraviolet region (200–250 nm) for CP43 showed two negative peaks, which changed little during illumination (Fig. 1(d)).

Light-induced changes in the absorption, fluorescence and CD spectra of CP47 are shown in Fig. 2. The absorption spectrum of CP47 at room temperature contained a maximum at 675 nm in the red region. The fluorescence spectrum upon excitation at 430 nm displayed a maximum at 685 nm. The CD spectra of CP47 also contained a doublet signal with negative and positive peaks at 681 and 667 nm in the red region and two negative peaks in the far-ultraviolet region. The CD amplitude in the red region of CP47 was much larger than that of CP43. The Chl *a* absorbance, fluorescence and

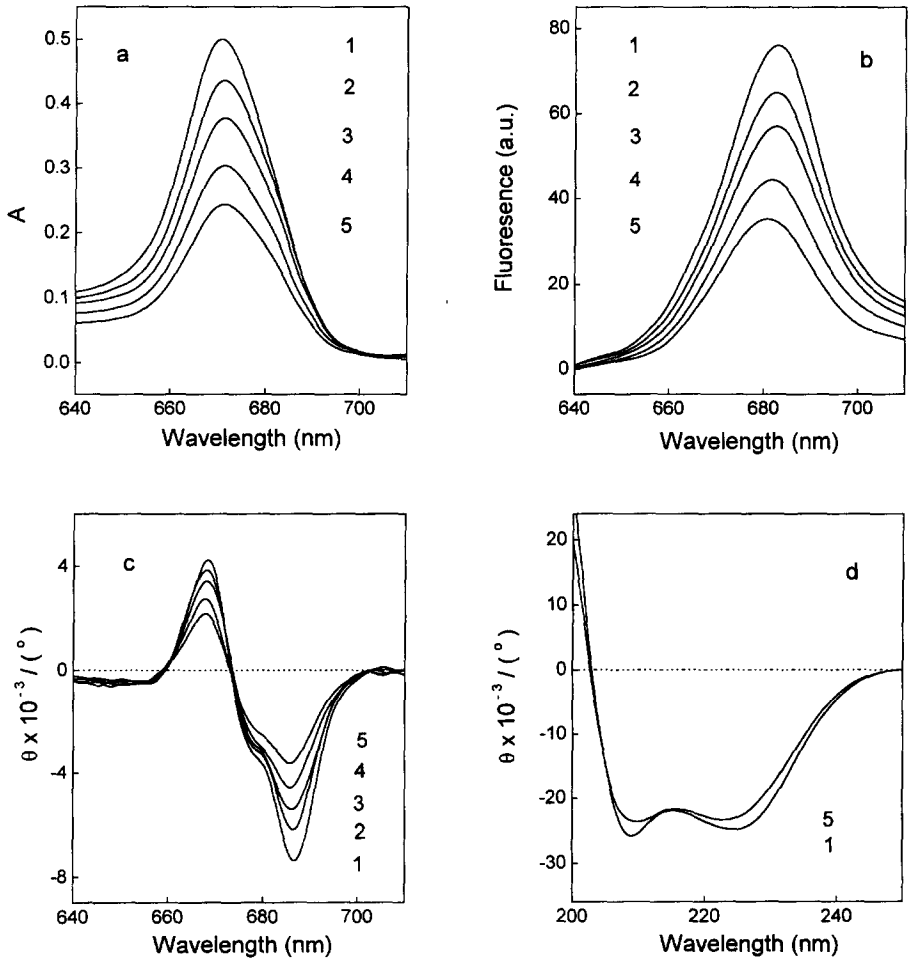

Fig. 1. Effect of white-light illumination on absorption spectra (a), fluorescence emission spectra with excitation at 430 nm (b), CD spectra in the red region (c) and far-ultraviolet region (d) for CP43. Spectra 1–5 were measured at room temperature after 0, 5, 10, 20 and 30 min light treatments, respectively.

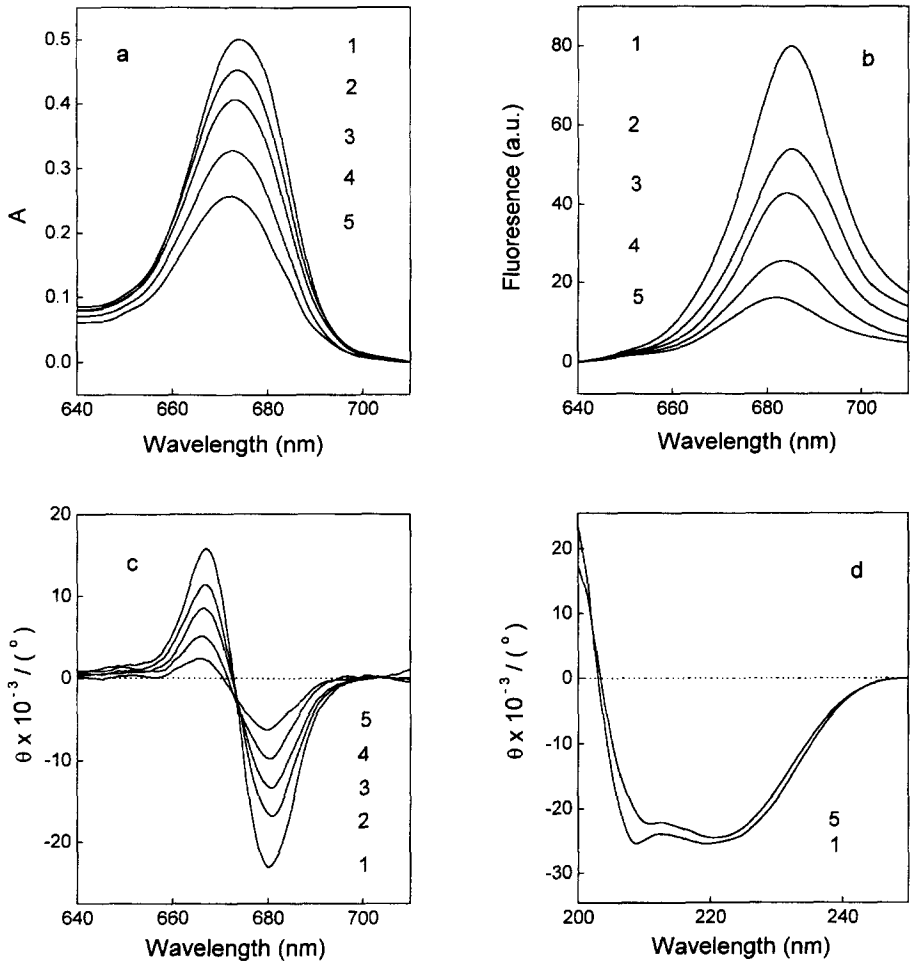

Fig. 2. Effect of white-light illumination on absorption spectra (a), fluorescence emission spectra with excitation at 430 nm (b), CD spectra in the red region (c) and far-ultraviolet region (d) for CP47. Spectra 1–5 were measured at room temperature after 0, 5, 10, 20 and 30 min light treatments, respectively.

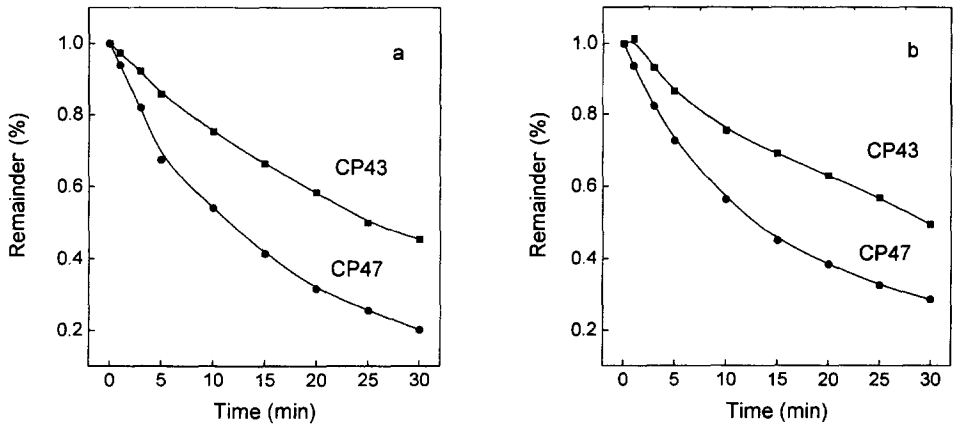

Fig. 3. Dependence of Chl *a* fluorescence intensity (a) and overall CD intensity of the doublet in the red region (b) of CP43 and CP47 on illumination time.

CD activity in the red region all decreased during illumination, while the protein secondary structure, measured by far-ultraviolet CD, was not strongly affected by the light, all of which are similar to the results for CP43.

For both CP43 and CP47, light induced a significant decrease in absorbance, CD activity and fluorescence properties of Chl *a*, but only very small changes in the protein secondary structure as indicated by far-ultraviolet CD. Light-induced losses of Chl *a* absolute absorbance for CP43 and CP47 were similar, with about half of the Chl *a* absorbance lost after 30 min illumination; however, the light-induced losses of Chl *a* fluorescence intensity and CD activity of CP43 and CP47 were significantly different. Fig. 3 shows the changes in the Chl *a* fluorescence and the CD intensity in the

red region as a function of the illumination time. After 30 min illumination, the fluorescence intensity decreased to 45% of the original for CP43 and to 20% for CP47, while the corresponding CD signals decreased to 50% of the original for CP43 and 29% for CP47. The results showed that the fluorescence and CD activity of Chl *a* for CP47 were reduced more rapidly by light than those for CP43.

3.2. Heat-induced denaturation of CP43 and CP47

The absorption spectrum of CP43 decreased little (less than 5%) during heat treatment (Fig. 4(a)), indicating that the molecular structure of Chl *a* was not significantly changed over the experimental temperature range. The CD spectrum of CP43 was obviously changed in both the red and far-ultraviolet regions after high-temperature incubation (Fig. 4(b,c)). In the far-ultraviolet region, for incubation temperatures lower than 45°C, the spectra were not significantly changed compared to the native state. However, for incubation temperatures greater than 50°C, the magnitude of the CD rapidly decreased, which indicated the decrease of the α-helix content. For incubation temperatures higher than 55°C, the spectra did not change much further with increasing temperature, suggesting that CP43 was completely denatured by the heat. The CD activity in the red region was more sensitive to heat than in the far-ultraviolet region. The doublet CD signals in the red region were totally destroyed at 55°C and converted to a small negative band at about 673 nm.

The corresponding spectra for CP47 during heat denaturation are shown in Fig. 5. The heating induced a small decrease of the absolute absorbance, with an obvious blue shift in the wavelength at relatively high temperatures. Both the protein CD signals in the far-ultraviolet region and the Chl *a* excitonic splitting signals in the red region decreased during heating, suggesting the denaturation of both the protein secondary structure and the Chl *a* excitonic interaction.

Heat-induced modification of the protein and pigment of CP43 and CP47 can be clearly observed when the relative changes of the CD activities in the far-ultraviolet and red regions are plotted against the incubation temperature. Fig. 6 shows these graphical representations as sigmoidal curves for temperatures from 25 to 80°C. The inflection points of the 222 nm ellipticity-change curves were 50°C for CP43 and 63°C for CP47, while the inflection points of the total doublet

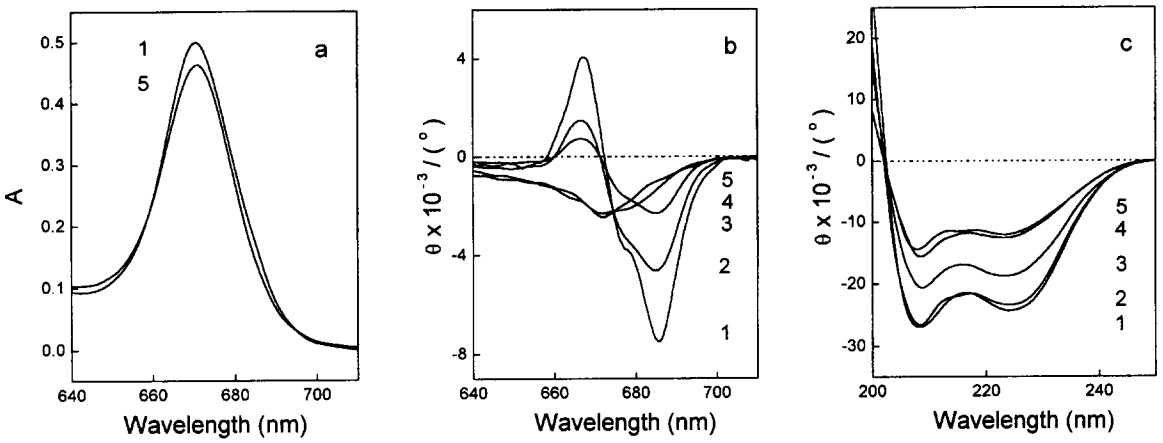

Fig. 4. Effect of heat incubation on absorption spectra (a), CD spectra in the red region (b) and far-ultraviolet region (c) for CP43. Spectra 1–5 were measured after 5 min heat treatment at 25, 45, 50, 55 and 70°C, respectively.

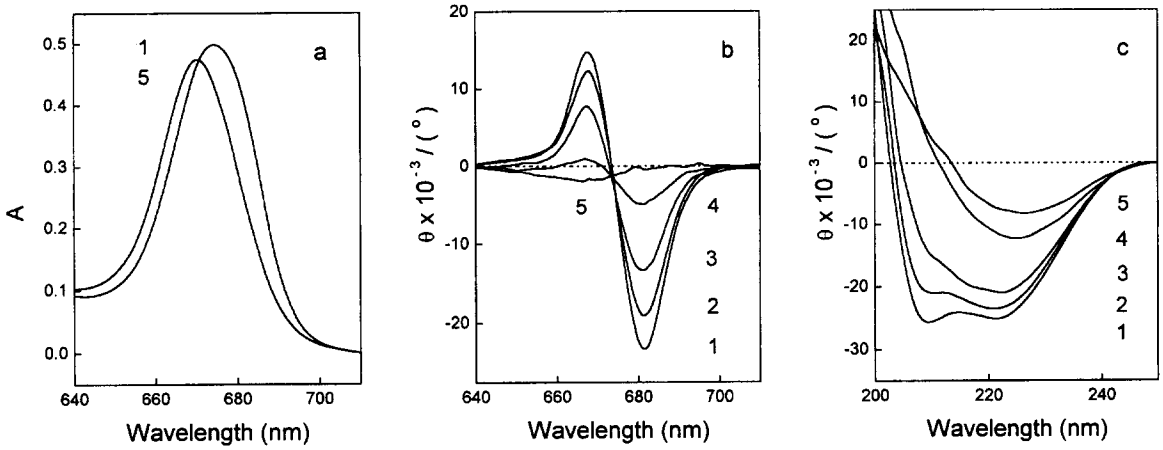

Fig. 5. Effect of heat incubation on absorption spectra (a), CD spectra in the red region (b) and far-ultraviolet region (c) for CP47. Spectra 1–5 were measured after 5 min heat treatment at 25, 55, 60, 65 and 70°C, respectively.

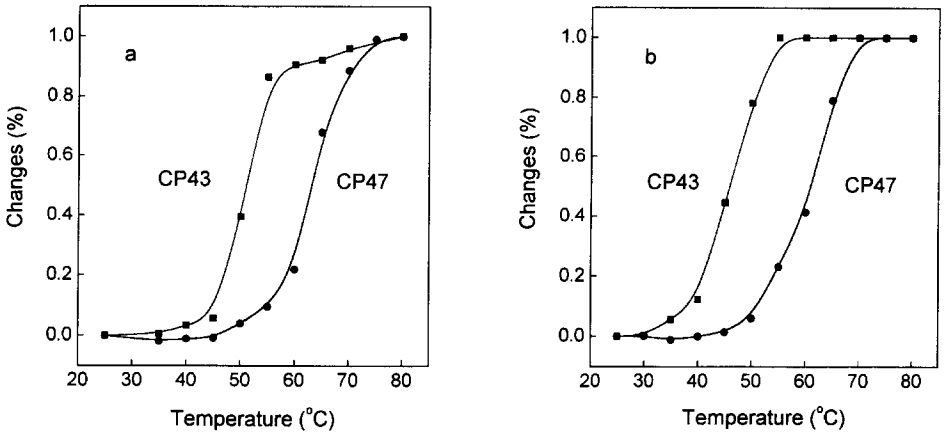

Fig. 6. Relative changes of CD 222 nm ellipticity in the far-ultraviolet region (a) and of the overall CD intensity of the doublet in the red region (b) for CP43 and CP47 as a function of incubation temperature.

CD intensity-change curves were 45°C for CP43 and 60°C for CP47. The results indicate that CP47 is more thermally stable than CP43.

4. Discussion

The absorption band in the red region for both CP43 and CP47 is due to the Chl *a* electronic $Q_y(0–0)$ transition, which reflects the electronic state of Chl *a*. The decrease of the absorption band in the red region during illumination indicated that the Chl *a* structure was destroyed. CD is a powerful technique for the structural analysis of biological systems, which can provide insight into the molecular architecture of the photosynthetic antenna system [30]. The far-ultraviolet CD spectrum is widely used to assess quantitatively the overall secondary structure content of the protein [31,32], and the ellipticity at 222 nm can be used to estimate the α-helix content [33]. The decreased CD activity in the far-ultraviolet region during heat treatment reflected the decreased α-helix content in the protein secondary structure due to the heating. The CD spectrum in the red region is very sensitive to the microenvironment of the chromophore, including the position, orientation and distance between the chromophores. The doublet CD signals in the red region of CP43 and CP47 were mainly ascribed to the excitonic interaction between the Chl *a* molecules, which is one of the energy-transfer modes in the antenna complexes [34,35]. The decrease of the doublet in the red region indicated the modification of the native microenvironment of the pigments and the destruction of the Chl *a* excitonic interaction and also implied the loss of energy-transfer function.

The Chl *a* molecules were destroyed by light, which caused the chlorin ring to open and further decay to a product without significant absorption in the red region. The previous fluorescence and excitonic interactions of these Chl *a* molecules were lost. These resulted in the decreased absorbance, fluorescence intensity and CD activity in the red region during illumination. Although the Chl *a* molecular structure was not apparently changed during heat incubation, the distance and orientation of the pigments were affected as a consequence of protein structure modification. The doublet CD signals in the red region for CP43 and CP47 were mainly from the excitonic interaction of Chl *a* molecules and the intrinsic CD signal of monomeric Chl *a* was very weak with only a small negative peak in the red region [36]. The thermally induced decrease of the doublet CD signals and conversion to a small negative band reflected the loss of the excitonic interaction and thermal transition to an unordered state of the Chl *a* molecules in CP43 and CP47. Both light and heat treatment destroyed the excitonic interaction of Chl *a* molecules in the two antenna complexes and thus the energy-transfer function, but the mechanisms were different. The light-induced loss of Chl *a* excitonic interaction was mainly caused by the destruction of the Chl *a* molecule during illumination, while the protein secondary structure was not apparently changed. The thermally induced inactivation was due to conformational change of the protein, while the Chl *a* structure was not changed. The results showed that both the Chl *a* structure and the protein native conformation were necessary for the Chl *a* excitonic interaction and the energy-transfer function of the antenna complexes.

As the core-antenna complexes CP43 and CP47 were impaired during photoinhibition, the light-induced destruction of Chl *a* in purified CP43 and CP47, which we observed in vitro, may also occur in vivo when the PSII suffers photoinhibition under strong illumination. Our present results for isolated CP43 and CP47, namely that the CD activity and fluorescence intensity of Chl *a* for CP47 decreased more rapidly during illumination than those for CP43, suggest that the native microenvironment of Chl *a* in CP47 is more sensitive to light than that of CP43. Since the native architecture of Chl *a* is the basis for the energy-transfer function of the antenna complex, the results imply that the energy-transfer function of CP47 may be more easily impaired than that of CP43 when they were in intact PSII membrane during photoinhibition.

Since the CD activity in the far-ultraviolet and red regions reflects the protein secondary structure and the Chl *a* excitonic interaction, the inflection points in Fig. 6 could be considered to be the thermal transition temperatures in the protein secondary structure (50°C for CP43 and 63°C for CP47) and in Chl *a* excitonic interaction (45°C for CP43 and 60°C for CP47). The results showed that the Chl *a* excitonic interaction was more thermally sensitive than the protein secondary structure, which agrees with previous reports of other chlorophyll-binding proteins [37,38]. The thermal transition temperatures for both the protein secondary structure and the Chl *a* excitonic interactions of CP47 were higher than for CP43. The present study investigated the thermal stability of isolated CP43 and CP47 and excluded the effect of other PSII components; therefore, the results reflect their different protein conformations and Chl *a* interactions.

The DSC and thermal gel analysis study using the PSII membrane and core complex showed that the 50% solubility decrease temperature ($T_{1/2}$) was 54°C for CP43 and 58°C for CP47 in the PSII membrane. In the PSII core complex, $T_{1/2}$ was 43°C for CP43, 52°C for CP47, and about 51–52°C for the RC, since the core complex does not contain the light-harvesting proteins as does the PSII membrane [20]. The FT-IR study on the purified RC showed a much lower thermal transition at 42°C [23]. The results indicated that the interaction among the proteins in PSII had obviously affected the thermal stability of the proteins. For instance, the thermal transition temperature of the isolated RC is much lower than it was in the PSII core complex, whose main components are the RC, the two core-antenna complexes and the extrinsic 33 kDa protein. Previous studies have shown that the extrinsic 33 kDa protein was released from the PSII core complexes at about 40°C during heat treatment [18]. Since the thermal stability of isolated CP47 was much higher than that of the isolated RC and the $T_{1/2}$ values of CP47 and RC were similar when they were located in the PSII core complex, the higher thermal stability of the RC in the PSII core complex than in the isolated state suggests that the close association between the RC and CP47 increased the thermal stability of the RC. The relatively lower $T_{1/2}$ of CP43 in the isolated state and in the PSII core complex reflected its inherent lower thermal stability and that it is more loosely bound to the CP47 and RC. These results are in good agreement with previous reports showing that CP47 is bound tightly to the RC and may perform a more significant role for a stable and functional assembly of PSII than CP43 [39,40].

Each batch of CP43 and CP47 in our preparation was tested by SDS–PAGE, absorption, fluorescence and CD spectroscopy, and the results were similar for each successful preparation. In addition, the results of light and heat treatment were similar for different batches of CP43 and CP47 (data not shown). The good reproducibility suggested that CP43 and CP47 in our preparation were in a relative stable state. The different effects of light and heat treatments on CP43 and CP47 reflected their different protein structures and pigment arrangements. The two chlorophyll-binding proteins CP43 and CP47, acting as core antennas in PSII, are structurally similar and thought to have evolved from a common ancestor [41]; however, several differences were found, including the spectroscopic properties [7], structural and functional roles in PSII [2], etc. More detailed studies of the structure and function of CP43 and CP47 by other techniques are needed to understand better their protein structures and pigment architectures and to elucidate and compare the specific light- and heat-induced changes of the proteins and pigments of the two core-antenna complexes.

Acknowledgements

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