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Light and Heat Induced Denaturation of Photosystem Ⅱ Core Antenna Complex CP47*

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Abstract Light and heat induced denaturation of CP47, the core antenna complex of photosystem Ⅱ purified from spinach, were investigated using absorption and circular dichroism spectra. Light caused the destruction of chlorophyll a and excitonic interaction of chlorophyll a in CP47, while the protein secondary structure was not apparently changed. Heat induced the destruction of protein secondary structure and excitonic interaction of chlorophyll a, but the chlorophyll a molecule was not damaged. The results suggest that both the chlorophyll a molecular structure and the protein native conformation are necessary for excitonic interaction of chlorophyll a and the energy transfer function of the chlorophyll a binding protein.

Key words photosystem Ⅱ; core antenna; CP47; circular dichroism

Introduction

Photosystem Ⅱ (PS Ⅱ) of higher plants is a membrane protein complex located in the thylakoid membrane, which consists of more than 20 proteins[1]. It uses solar energy to carry out the primary processes of photosynthesis: charge separation, quinone reduction, and water oxidation to molecular oxygen. CP47 is a core antenna complex of PS Ⅱ. It accepts excitation energy which is harvested by the light-harvesting complex and transfers the energy directly to the PS Ⅱ reaction center (RC)[2]. In addition to acting as an interior antenna, CP47 may also perform structural and functional roles in PS Ⅱ[3,4]. The predicted topology of 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]. CP47 binds chlorophyll a (Chl a) and β-carotene (β-Car) but no chlorophyll b. The pigment stoichiometry of CP47 is still not clearly known, and the spatial arrangement of the bound pigments has not been determined[5-7]. It is generally thought that the pigment content of CP47 is about 18-22 Chl a and 4 β-Car per CP47[5,6].

This paper investigates the effect of light and heat on the Chl a molecular state, the protein secondary structure and the Chl a interaction of CP47 using absorption and circular dichroism (CD) spectroscopy and discusses the relationships.

1 Materials and Methods

PS Ⅱ enriched membrane was prepared according to Kuwabura and Murata[8], and the oxygen evolving core complex was isolated and treated to remove the 33 ku extrinsic protein as discussed in Ghanotakis et al[9]. CP47 was then purified according to the method of Alfonso et al[2]. The purification of CP47 was verified by SDS-PAGE which showed one single polypeptide band. The fluorescence spectrum

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of purifed CP47 at 77 K upon excitation at 430 nm displayed a maximum at 693 nm.

CP47 was suspended in 10 mmol·L^{-1} Bis-Tris, pH 6.0, and mass fraction 0.05% β-dodecyl maltoside (DM). The sample concentration was adjusted so that the corresponding absorption maximum in the red region was 0.5. The sample was treated with light by illuminating with white light (intensity: 500 W/m^2). To study the effect of temperature, samples were heated in steps of 5 ℃ from 25 ℃ to 80 ℃. After each heating step, the samples were left to stabilize for 5 min before measuring the spectra.

Absorption and CD spectra were measured with a Jasco J-715 Spectropolarimeter at room temperature. Spectra were recorded at 100 nm/min scanning speed with 4 times accumulation, 2 nm bandwidth and 1 s response. Samples were in a 1 mm pathlength cell.

2 Results and Discussion

The optical absorption band in the red region of Chl a binding protein is due to the Chl a electronic $Q_y(0\text{-}0)$ transition, which reflects the electronic state of Chl a. Absorption spectrum of CP47 measured at room temperature had a maximum at 675 nm in the red region (640 – 710 nm). Figure 1 shows that the intensity of the absorption maximum continuously decreased during the illumination, which indicated that the Chl a molecular structure was damaged by the light.

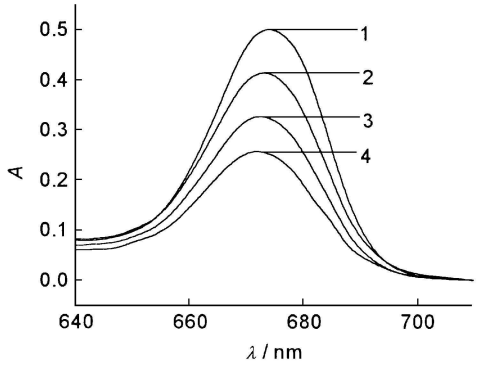

Fig. 1 Absorption spectra of CP47 during light treatment
1, 0 min; 2, 10 min; 3, 20 min; 4, 30 min

Far-ultraviolet CD spectrum is widely used to quantitatively assess the overall secondary structure content of the protein, and the ellipticity at 208 nm and 222 nm can be used to estimate the α-helix content[10, 11]. CD spectrum in the far-ultraviolet region (200 – 250 nm) of CP47 showed two negative peaks at 209 nm and 221 nm, respectively. The spectrum was not apparently changed during illumination (Fig. 2), which indicated that the protein secondary structure was not apparently changed.

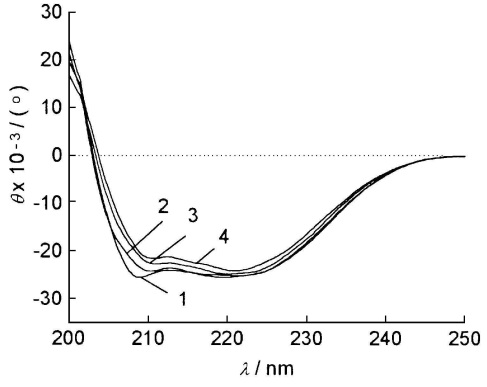

Fig. 2 CD spectra in far ultraviolet region of CP47 during light treatment
1, 0 min; 2, 10 min; 3, 20 min; 4, 30 min

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 in the red region of the CD spectrum was caused by the excitonic interaction between Chl a which is very important for the energy transfer function of the antenna complex. The CD spectrum in the red region (640 – 710 nm) of CP47 had a doublet with a negative peak at 681 nm and a positive peak at 667 nm. Both the negative and positive magnitudes decreased during illumination (Fig. 3), which suggested that the excitonic interaction of Chl a was destroyed and the energy transfer function was lost.

The absorption spectrum of CP47 was not

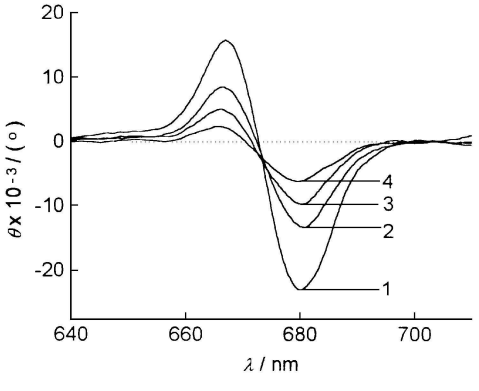

Fig. 3 CD spectra in red region of CP47 during light treatment
1, 0 min; 2, 10 min; 3, 20 min; 4, 30 min

apparently changed after high temperature incubation (Fig. 4), which indicates that the molecular structure of Chl a was not affected over the experimental temperature range. The CD spectrum of CP47 was clearly changed by heat treatment in both the far-ultraviolet and red regions (Figs. 5, 6). The two negative peaks in the far-ultraviolet region and the exciton splitting signals in the red region decreased during heat incubation. The CD spectrum in the far-ultraviolet region showed a single negative peak at about 226 nm and the CD spectrum in the red region showed a small negative peak at about 668 nm at 70 ℃. The results suggested that both the secondary structure and the excitonic interaction of Chl a were affected by heat with a decrease of the α-helical content and destruction of the excitonic interaction of Chl a.

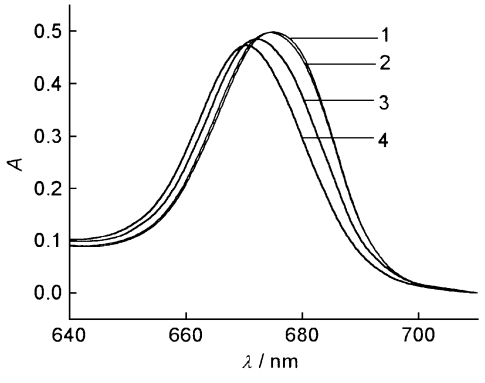

Fig. 4 Absorption spectra of CP47 during heat treatment
1, 25 ℃; 2, 50 ℃; 3, 60 ℃; 4, 70 ℃

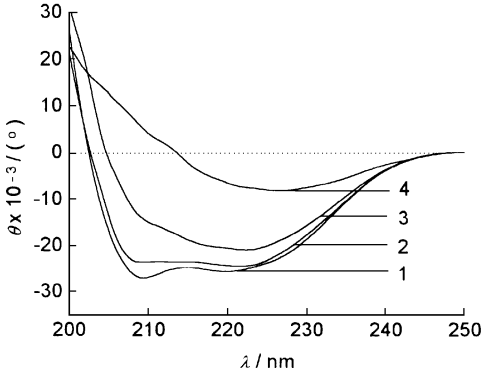

Fig. 5 CD spectra in far-ultraviolet region of CP47 during heat treatment
1, 25 ℃; 2, 50 ℃; 3, 60 ℃; 4, 70 ℃

The light and heat induced modification of CP47 can be clearly observed when the absorption maximum, the 222 nm ellipticity and the sum of the doublet signals were plotted against time and temperature (Fig. 7). Light caused the absorption maximum and the doublet in the red region of the CD spectrum to decrease with no apparent change of the 222 nm ellipticity; while heat caused the 222 nm ellipticity and the doublet in the red region of the CD spectrum to decrease with no apparent change of the absorption maximum. It was also found that the relative decrease of the CD activity in the red region was greater than that of the absorption maximum

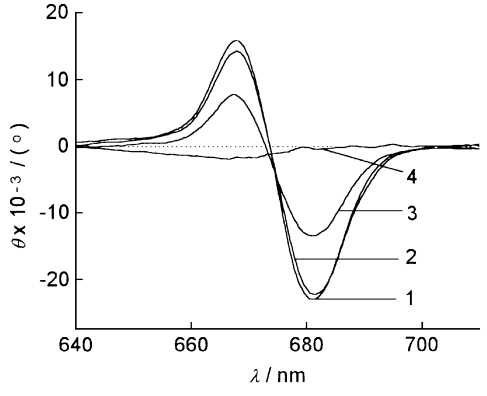

Fig. 6 CD spectra in red region of CP47 during heat treatment
1, 25 ℃; 2, 50 ℃; 3, 60 ℃; 4, 70 ℃

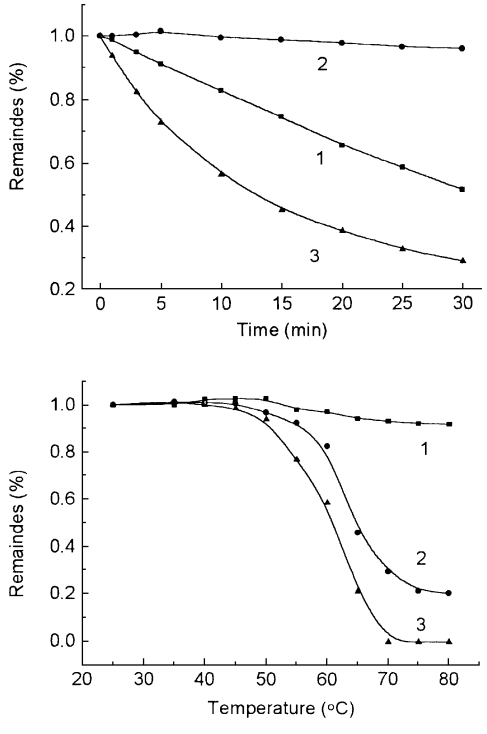

Fig. 7 Dependence of the absorption maximum (1), the 222 nm ellipticity (2) and the sum of the doublet signals (3) on light illumination time (a), and temperature (b)

during illumination and the 222 nm ellipticity during heat treatment, suggesting that small changes in the Chl a molecular or protein structure induced large decreases of the CD activity in the red region. The result suggests that excitonic interaction of Chl a is very sensitive to Chl a molecular structure and protein conformation.

The destruction of the Chl a molecules by light caused the chlorin ring to open and caused further decay to a product without significant absorption in the visible region, which caused the bleaching of absorption in the red region. As a consequence, the previous excitonic interaction of Chl a was lost causing the CD signal in the red region to decrease. Although the Chl a molecular structure was not changed by heat treatment, the distance and/or the orientation of the chromophores were affected by the protein structure modification, which caused the loss of excitonic interaction of Chl a. Since the doublet signal of CP47 mainly arose from the excitionic interaction of Chl a, and the intrinsic CD signal of monomeric Chl a is very weak in the red region[12], loss of excitonic interaction by thermal treatment resulted in destruction of the doublet in the red region of CD spectrum.

Both light and heat treatment destroyed the excitonic interaction of Chl a in CP47 and thus the energy transfer function, but the mechanisms were different. Light induced loss of Chl a excitonic interaction was due to the destruction of the Chl a molecule during illumination, but the protein secondary structure was not apparently changed. Heat induced inactivation was due to the conformational modification of the protein with a decrease of the α-helix content, while the Chl a structure was not changed during the procedure.

3 Conclusions

Both destruction of Chl a by light illumination and modification of protein structure by heat incubation caused the loss of the excitonic interaction of Chl a and the energy transfer function of CP47. The results show that both the Chl a structure and the protein native structure are necessary to the Chl a excitonic interaction and the energy transfer function of the antenna complex, CP47.

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