A Circular Dichroism Spectroscopic Study Revealing the Cause of the Changes of Chlorophyll a Fluorescence Induction of Photosystem II During Heat Treatment

LI Dong-Hai1, RUAN Xiang1, XIU Qiang1, GONG Yan-Dao1, ZHANG Xi-Fang1, ZHAO Nan-Ming1*
WANG Ke-Bi2, KUANG Ting-Yun2

(1. State Key Laboratory of Biomembrane and Membrane Biotechnology, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, China;
2. Photosynthesis Research Center, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China)

Abstract: Chlorophyll a fluorescence and circular dichroism (CD) spectra of photosystem II (PSII) membrane were measured after heat treatment. The chlorophyll fluorescence parameter Fo* remained stable after treatment at the temperatures from 30 °C to 40 °C and then reached a maximum after treatment at 55 °C. In PSII membranes and LHCII (light-harvesting chlorophyll a/b binding complex)-enriched complexes, anomalous CD signals with extremely large amplitudes occurred during the heat treatment. The temperature corresponding to the maximum anomalous CD intensity peaking at 677 nm was 40 °C. The results indicate that the aggregation state of the LHCII in PSII is related to the anomalous CD signal, and can be an important factor influencing Fo* in the heat treatment of PSII membrane.

Key words: chlorophyll; circular dichroism (CD); photosystem II (PSII); light-harvesting chlorophyll a/b binding complex (LHCII)

Circular dichroism spectroscopy is a powerful and noninvasive technique to obtain structural information in samples of biological origin. In molecular complexes or small aggregates, CD signal is generally induced by short-range interactions, e.g., due to excitonic interactions between molecules or neighboring particles, and a distortion by differential scattering. However, the large aggregation-induced CD could not be explained by above theory. Theory predicts that in polymer- and salt-induced aggregates the magnitude of the anomalous CD, at a constant density of chromophores and a constant pitch of the macro-helix, is controlled by the volume of the aggregate (Keller et al., 1986). Barzda et al. (1994) have investigated the CD of pigment-protein complexes in different aggregation states both in thylakoid membranes and in isolated LHCII, and provided direct experimental evidence that the magnitude of the major CD bands increases with the size of the aggregates. In their experiments, they treated the materials with MgCl2 and Triton X-100 in order to cause the anomalous CD with extremely large amplitudes. Then, are there other ways that can also induce the anomalous CD? In photosynthetic systems, what causes the anomalous CD?

Chlorophyll II (Chl) a fluorescence is a useful and routine probe for information on the various aspects of photosynthesis, and a good non-destructive technique for evaluating the heat-induced effects on photosystem II (PSII) photochemical activities (Schreiber et al., 1977; Berry et al., 1980). For the plant leaf and chloroplast, the effects of heat treatment on the Chl a fluorescence parameters have been studied by many researchers, and an increase of Fo and decrease of both Fv and Fv/Fm have been reported (Yamane et al., 1997; Yamane et al., 1998; Pospisil et al., 1998). Mechanisms about changes of the chlorophyll a fluorescence parameters have also been suggested and testified. So far, some reports have shown that the increase in the Fo level by heat can be attributed to the dissociation of light-harvesting chlorophyll a/b binding complex (LHCII) from PSII core complex (Schreiber et al., 1978) and inhibition of electron flow from QA to QB (Bukhov et al., 1990). However, the cause of the little change of the Fo level at lower heat level (30–40 °C) has not been well explained.

In the present experiment, PSII membrane was adopted to study the heat-induced changes of CD and chlorophyll a fluorescence. The results indicated that during the heat treatment of PSII membrane anomalous CD signals was strongly related to the aggregation state of LHCII which could be a factor influencing the chlorophyll fluorescence parameter Fo*.
1 Materials and Methods

1.1 Preparation and heat treatment of samples
PS II membranes were prepared from the leaves of market spinach (*Spinacia oleracea* L.) as described by Kuwabara and Murata (1982). The samples were suspended in SMN buffer containing 0.4 mol/L sucrose, 40 mmol/L Mes-NaOH (pH 6.0) and 10 mmol/L NaCl with a concentration of 5 mg/mL Chl, and then stored in liquid nitrogen. LHC II was prepared from spinach as previously described (Lou et al., 1995).

Heat inactivation was performed by incubating aliquots of PS II membrane with a concentration of 5 mg/mL Chl in a water bath. After incubation for 5 min at desired temperatures from 25 °C to 65 °C, the PS II samples were immediately diluted to the measured concentration with an SMN buffer, fully equilibrated at 25 °C. After 5 min, the Chl a fluorescence and circular dichroism (CD) spectra of the PS II samples were measured at 25 °C. All operations were done in the dark.

1.2 Measurement of photosynthetic activities of PS II membranes
Time courses of the variable chlorophyll fluorescence were obtained at 25 °C with a modulation fluorometer, PAM101 (Walz) equipped with PAM103 and KL 1500, a light source for saturating pulses (150 W halogen lamp, Schott). The duration of the saturating pulse was 0.8 s. The concentration of each sample corresponded to 20 μg/mL Chl for measuring photosynthetic activities.

1.3 Measurement of circular dichroism spectra of samples
CD spectra were measured with a Jasco J-715 Spectropolarimeter at a scanning speed of 100 nm/min, a bandwidth of 2 nm and response time of 1 s. CD spectra in the red region (620–720 nm) were measured in cells with path length of 2 mm. The concentration of each sample in measurement of CD in the red region was 0.1 mg/mL Chl.

2 Results

2.1 Effect of heat treatment on fluorescence Fo' of the PS II membranes
Figure 1 shows the modifications of Fo’ from 25 °C to 65 °C. Fo is the minimal fluorescence of PS II with open reaction centers excited by weak measuring light. Here we adopt Fo’ instead of Fo, since the electron transport chain in the PS II membrane, being different from leaf or chloroplast, does not contain photosystem I (PS I) and cytochrome b6f. The changing pattern of Fo’ was similar to that of Fo for leaf and chloroplast shown in previous reports (Yamane et al., 1997; Pospšíl et al., 1998), indicating that there was a similar mechanism for the changes in the chlorophyll fluorescence parameter of both PS II membrane and those more complicated systems during heat treatment. For the temperature lower than 40 °C, Fo’ remained stable. Above 40 °C, Fo’ increased and reached a maximum at 55 °C.

2.2 Effect of heat treatment on the circular dichroism spectra in each samples
CD spectroscopy is a powerful technique for structural analysis of biological systems, and can provide insight into the molecular architecture of the photosynthetic antenna systems (Garab et al., 1991). CD is generally induced by short-range interactions, e.g., excitonic coupling between chromophores. Excitonic interactions give rise to a conservative band structure. Figure 2a shows that the CD spectra in the red region of PS II membrane are remarkably altered by heat treatment. The CD spectrum in the red region of the native PS II membrane contained three components with peaks at 650 nm (–), 664 nm (+) and 677 nm (–). The excitonic interaction of Chl b and Chl a brought about the negative peak at 650 nm and the doublet signal peaking at 664 nm and 677 nm, respectively. The bands located at 650 nm (–) and 664 nm (+) had no apparent changes except for the temperature range from 55 °C to 60 °C, but the band located at 677 nm (–) red-shifted to 680.5 nm with the increasing temperature. During heat treatment, an interesting phenomenon occurred that CD intensity for the wavelength range from 620 nm to 720 nm dramatically increased at temperatures from 30 °C to 40 °C and decreased over 40 °C (Fig. 2a). The magnitude of peak at 677 nm (–) changed much more obviously. At 40 °C it was almost twice that for the native state and reduced for the temperature range from 55 °C to 60 °C.
and CD spectra were measured as in the procedure for the PS II membrane. The anomalous CD signals occurred significantly for the LHC II-enriched part (Fig.2b), but did not occur obviously for the OECC-enriched part (Fig.2e). Figure 3 also shows the CD intensity of the LHC II-enriched part changed more significantly than that of the OECC-enriched part for the temperature lower than 40°C. The CD intensity of PS II membrane and LHC II-enriched part changed almost in parallel and both reached maximum after treatment at 40°C. These results proved that the anomalous CD signal can be induced by the changes of LHC II.

There is a misapprehension problem in Fig.3, and trace c shows the CD intensity of the OECC-enriched part increased at 30°C. It appeared certain that OECC could induce the anomalous CD signals. In fact, the size of OECC is too small for the anomalous CD signals (Keller et al., 1986). Because OECC-enriched part includes only a few LHC II complexes in the present experiment, the anomalous CD signals of OECC-enriched part at 30°C can be owed to the changes of LHC II.

3 Discussion

Maestre et al. (1982), Livolan and Maestren (1988), and Reich et al. (1991) observed that much stronger CD signals with nonconservative, anomalously shaped bands
in sperm heads, condensed chromatin, and DNA aggregates, respectively. In general thylakoid membranes and in macroaggregates of LHC II, nonconservative CD signals with extremely large amplitudes have been observed. In the present experiment, in PS II membranes, anomalous CD signal with extremely large amplitudes also occurred during the heat treatment. The anomalous increase of CD signal could not be due to the increase of short-range interaction, e.g. excitonic interactions between chlorophyll molecules, and a distortion by differential scattering (Garab et al., 1988). Such anomalous CD may be associated with the state of aggregation of the PS II complexes (Gregory et al., 1980), and attributed to long-range interactions of aggregation (Simidjiev et al., 1997). According to the results shown in Fig. 2, the anomalous CD signals were not induced by the state of aggregation of the PS II membrane, but that of the LHC II during the heat treatment. Barzda et al. (1994) also observed the Triton X-100-treated anomalous CD of LHC II and provided direct experimental evidence of the size dependency of CD in macroaggregates. The results indicated that though the treatments were different in these experiments, the mechanism of the changes of LHC II could be accordant. In PS II membranes and LHC II enriched complexes, the anomalous CD may be involved in the changes of the aggregation state of LHC II.

During the heat treatment, the increase of Fo' with the rise of temperature was usually attributed to detachment of LHC II from PS II core complex (Schreiber et al., 1978) and/or inhibition of electron flow from Qa to Qb (Bukhov et al., 1990). Since Fo' maintained stable for the temperature range from 30 °C to 40 °C in the present experiment (Fig. 1), some other factors might exist to influence Fo' value. Fig. 2a shows that the CD signal rapidly increased at the temperatures from 30 °C to 40 °C, indicating that the changes of the aggregation state of LHCII occurred, in other words, the size of aggregation of LHCII in the heat-treated PSII could be bigger than that in the native LHC II (Barzda et al., 1994; Simidjiev et al., 1997). The increase of the aggregation of LHC II could quench its chlorophyll fluorescence (Mullet et al., 1980; Ruban et al., 1992). In view of such decreasing effect on fluorescence, the stableness of Fo' at the temperatures from 30 °C to 40 °C is reasonable. Figure 2a also shows the reduction of CD intensity after treatment at temperatures from 40 °C to 55 °C, indicating the size of aggregation of LHC II became smaller, which would increase the fluorescence Fo' value. This is consistent with the results shown in Fig. 1. Therefore, our results showed that the state of aggregation of LHC II can be a factor influencing Fo' in the heat-induced denaturation of PSII. During the heat treatment of PSII membrane for the temperature range from 40 °C to 55 °C, the decrease in the excitonic interaction between chlorophyll molecules may also be involved in the change of the CD signals.

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