ANALYSIS OF PIGMENT ORIENTATION IN PHOTOSYSTEM II AT DIFFERENT TEMPERATURES BY POLARIZATION FLUORESCENCE AND MOLECULAR EXCITON THEORY

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The effect of temperature on pigment orientation in photosystem II (PSII) was studied by fluorescence excitation and polarization fluorescence spectra of spinach thylakoid solution and molecular exciton theory. Experimental results showed that at 15 to 45°C, the absorption band of chlorophyll a at 436 nm at room temperature red-shifted with increased temperature. The excitation spectra intensity reached the maximum at 35°C but significantly dropped at 65 and 78°C. The polarization fluorescence spectra revealed that the fluorescence peak of PSII did not change at 15 and 45°C, and the calculated degree of fluorescence polarization increased with increased temperature. Spectral and molecular exciton theory analyses indicated that temperature affected pigment orientation in PSII, as well as the coupling strength between pigments or pigment and protein, thereby changing photosynthetic efficiency. These results can serve as a reference for studies on energy absorption, energy transmission, regulation mechanism, and prospective applications in solar cell materials.

Keywords: light-harvesting pigment, red shift, molecular exciton theory, fluorescence polarization.

Introduction. The ability of higher plants and alga to absorb light and transfer energy rapidly and efficiently is receiving considerable attention because of the decrease in nonrenewable resources. When light and temperature are considered, differences in the photosystem (PS) are found at the molecular level between higher and lower plants among the same and different kinds of species. However, the remarkable flexibility of the PSII structure, which is a multilevel regulatory mechanism, and its effects on photosynthetic functions are unclear. The regulatory function in the PSII system is generally believed to be caused by the feedback mechanism operated by the photosynthetic electron and proton transport system [1, 2]. The reversible aggregation of peripheral light-harvesting complex (LHCCI) and core antenna complexes (CP43 and CP47) is induced by thermo-optic activity, which is independent of the photochemical apparatus [3–5]. The orientation regulation of pigments in LHCCI, CP43, and CP47 self-aggregation is an economical and effective method to improve the photosynthetic efficiency and facilitate the protection function, which include dissipation of excess excitation energy [6].

The crystal structure of PSII is important in the study of photosynthesis. Chlorophyll (Chl) proteins in loosely stacked lamellar aggregates of LHCCI exist in the form of trimer. In addition, other models consider the existence of other aggregation states, which can transform under particular conditions to adapt to the changing environment [7–13]. CP43 and CP47 in PSII ensure effective power transmission, which is influenced partly by pigment arrangement. However, the acquired crystal structure of photosynthetic units is insufficient to explain the pigment-orientation adjustment of plants. In the present study, the orientation properties of pigments in PSII was examined with the use of molecular exciton theory and fluorescence excitation and polarization fluorescence spectra of spinach thylakoid solution treated at different temperatures.

Materials and Methods. Spinach seeds purchased from Nanjing Vegetarian and Seed Company were cultivated in a greenhouse and managed normally during the breeding period. The sixth and seventh leaves were gathered, washed, and placed in a refrigerator. Thylakoid membranes were isolated as described previously [14] and then suspended and stored in K solution (0.05 mol/l NaH2PO4–K2HPO4 containing 0.2 mol/l NaCl and 0.1 mol/l sucrose at pH 7.4). Thylakoid solution was divided into five portions labeled as S0, S1, S2, S3, and S4.

The thylakoid portions S1 to S4 were treated for 10 min in a water bath at 35, 45, 65, and 75°C, respectively, and then scanned with the use of a fluorescence spectrophotometer (HITACHI F-4600). The excitation spectra were recorded...
from 360 to 550 nm. The polarization fluorescence of the solution was scanned from 650 to 720 nm under 436 nm light, with polarizer at 0°, analyzer at 0° and 90°, and 240 nm/min scanning speed.

**Results and Discussion.** In our experiment, the fluorescence peak of PSII was 682 nm, and thus we adopted 682 nm as the emission wavelength. Figure 1 shows the typical excitation spectra of the thylakoid membranes after water-bath treatment at different temperatures. At room temperature, the excitation peak at 436 nm corresponds to the absorption band of Chl \(a\), and the shoulder peak on the right hand is the absorption of Chl \(b\). The absorption spectrum of carotene at 420 to 520 nm is concealed by Chl \(b\), thereby indicating low carotene content [15, 16]. The band at 350 to 400 nm is from the scattered light of background solution absorbed by pigments [17]. Figure 1 also indicates that with increased temperature, the absorption peaks of Chl \(a\) show slight red shift, which is caused by the variation of pigment orientation in LHCII [18]. The intensity of excitation spectrum reaches its maximum at 35°C and significantly decreases at 65 and 75°C. The photosynthetic efficiency is expected to enhance under certain temperatures, whereas the structure of pigment–protein complex (PPC) in PSII is damaged at high temperature. In PSII, the solar energy absorbed by LHCII is delivered to the reaction center (RC) through CP43 and CP47. The highly organized LHCII complexes form monomers, including 12 to 14 pigments, which aggregate into trimers by noncovalent interaction. The changes in the absorption spectra of pigments in LHCII are due to the different aggregation states. For example, the spectrum of pigments in a trimer shows red shift in comparison with those in monomer and ethanol solution [19].

The crystal structures of the PPC indicate the presence of dipole interaction between pigments. The operator is [20]

\[
V_{ij} = \frac{(\mu_i \mu_j)}{R^3} - 3(\mu_i R \mu_j R)/R^5,
\]

where \(\mu_i\) is the transition moment operator of the \(i\)th molecular, and \(R\) is the distance between two centers of transition moment. We apply the molecular exciton theory to investigate the LHCII trimer composed of monomers \(A, B,\) and \(C\) (Fig. 2a).

The monomer has average dipole orientation with ratio of dipole length to the side of triangle at 1 and \(\beta\) between 0 to 90°. The wave function of LHCII trimer in the ground state is

\[
\Psi_0 = \phi_A \phi_B \phi_C.
\]

For the same excitation probability, the monomer in this equation couples with each other between different states because of the resonance effect. If the monomer has two states (ground and excited), then the excited state wave function of the ring-like trimer belongs to the \(D_{3h}\) group in exciton space. On the basis of group theory, the LHCII trimer has a one-dimension irreducible representation at coupled excited state

\[
\Psi_1 = \frac{1}{\sqrt{3}} (\phi_A^* \phi_B \phi_C + \phi_A \phi_B^* \phi_C + \phi_A \phi_B \phi_C^*),
\]

and a two-dimensional irreducible representation:

\[
\Psi_2 = \frac{1}{\sqrt{6}} (2\phi_A^* \phi_B \phi_C - \phi_A \phi_B^* \phi_C - \phi_A \phi_B \phi_C^*),
\]

\[
\Psi_2 = \frac{1}{\sqrt{2}} (\phi_A \phi_B^* \phi_C - \phi_A \phi_B \phi_C^*),
\]

where \(\phi_N^*\) denotes that the \(N\)th monomer is in the excited state and the others are in the ground state. The transition matrix element is described as

\[
M = \langle \Psi_0 | \sum_i \mu_{Ai} + \sum_i \mu_{Bj} + \sum_i \mu_{Ck} | \Psi_0 \rangle.
\]

The summation extends all over the pigments in the LHCII trimer. Coupling among excited states splits the energy level. The splitting value is proportional to the coupling strength:

\[
\varepsilon = \langle \phi_A^* \phi_B \phi_C | V_{AB} | \phi_A \phi_B^* \phi_C \rangle = \int \int \int \int \int \phi_A^* \phi_B \phi_C d\phi_A d\phi_B d\phi_C dV_A dV_B dV_C,
\]