Energy transfer within the isolated light-harvesting chlorophyll *a*/*b* protein of photosystem II (LHC-II)

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Picosecond absorption spectroscopy has been used to study energy transfer within the isolated light-harvesting chlorophyll *a*/*b* protein complex of photosystem II (LHC-II). The results suggest: (i) a fast transfer ($\tau = 6 \pm 4$ ps) from chlorophyll *b* to chlorophyll *a*, (ii) during the lifetime of the chlorophyll *b* excited state there is no transfer of excitation energy between differently oriented molecules of chlorophyll *b*, (iii) a fast redistribution ($\tau = 20$ ps) of excitation energy between different chlorophyll *a* chromophores.

Light-harvesting complex *Chlorophyll* *Energy transfer* *Picosecond spectroscopy* *Absorption anisotropy* *Photosynthesis*

1. INTRODUCTION

The chlorophyll of higher plants is bound to certain proteins which reside in the chloroplast thylakoid membrane [1,2]. The most abundant chlorophyll-containing protein complex, binding more than half of the chlorophyll, is the light-harvesting chlorophyll *a*/*b*-protein complex of photosystem II. This complex, designated LHC-II, is composed of at least 2 polypeptides and contains chlorophyll *a* and *b* in approximately equal proportions. LHC-II is, under most physiological conditions, structurally associated with the photosystem II core to which it transfers the absorbed excitation energy [3]. The energy transfer within the light-harvesting apparatus has been studied by time-resolved spectroscopy of intact thylakoid membranes [4–6]. However, the complexity of organization of the system does not allow a detailed analysis of the energy transfer within a particular chlorophyll-protein complex such as the LHC-II. The main biophysical data concerning the energy transfer in LHC-II are: a low degree of polarization of light emitted from chlorophyll *a* at 695 nm when chlorophyll *b* is excited at 650 nm [7,8]; a strong circular dichroism (CD) at about 650 nm indicative of exciton interaction within a chlorophyll *b* trimer [7,9]. Moreover, there are reports on time-resolved fluorescence kinetics of LHC-II isolated by detergent fractionation [10–12]. Lifetimes of chlorophyll *a* emission in the range 1.2–5.2 ns were observed, depending on the type of detergent used. However, no fluorescence kinetics indicating a transfer of excitation energy between chlorophyll *b* and chlorophyll *a* or between chlorophyll *a* chromophores were reported in these experiments. A powerful method for studies on the energy transfer between pigment molecules is picosecond absorption spectroscopy which has recently been used to follow the transfer of excitation energy within phycobilisomes from cyanobacteria [13,14]. Here, we present the first picosecond absorption measurements on the energy transfer between chlorophyll molecules within an isolated light-harvesting complex of higher plants.

Abbreviations: SDS-PAGE, SDS-polyacrylamide gel electrophoresis; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine

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2. MATERIALS AND METHODS

LHC-II was isolated from spinach thylakoids by Triton X-100 solubilization and sucrose gradient centrifugation according to Burke et al. [15] as modified by Ryrie et al. [16]. The purity of the preparation was determined by SDS-PAGE which revealed only those polypeptides belonging to the complex. The final LHC-II preparation was suspended in 50 mM sucrose, 0.5 mM EDTA, 5 mM Tricine, pH 7.5, and kept at −78 or −196°C until use. For absorption measurements the samples were diluted with the suspension medium, containing 0.3% Triton X-100, to an absorbance of approx. 0.4 at 670 nm in a 1 mm thick sample cell. Unless otherwise stated all experiments were performed at 23–25°C on freshly thawed samples.

For time-resolved experiments we used the so-called pump-and-probe technique [17]. In this technique the cavity-dumped pulse (~12 ps) from a synchronously pumped dye laser system [18] is divided into 2 parts. The most intense pulse, which contains about 95% of the intensity, is used to excite the sample, while the weaker pulse is used to interrogate the absorbance of the sample as a function of time. The pulse repetition rate was normally 80 kHz. At the low excitation degree (about 2%) used in this work the signal is proportional to the absorbance change in the sample. To avoid damaging effects on the sample due to multiple excitation by the repetitive laser pulses we used a rotating sample cell. With this cell it is possible to change the excited sample volume completely between 2 consecutive pulses at 80 kHz.

To follow the anisotropy of the kinetics [19] we recorded the signal intensity with the polarization of the excitation light put parallel ($I_{\parallel}(t)$), perpendicular ($I_{\perp}(t)$) or at 54.7° ($I_{iso}(t)$) relative to the polarization of the analyzing light. The isotropic kinetic terms are then given by $I_{iso}(t)$ or $I_{\parallel}(t) + 2I_{\perp}(t)$, while the anisotropy, $r(t)$, is given by $r(t) = (I_{\parallel}(t) - I_{\perp}(t))/(I_{\parallel}(t) + 2I_{\perp}(t))$. This anisotropy term is of special importance when transfer between identical but differently oriented chromophores is studied.

3. RESULTS AND DISCUSSION

The chlorophyll *a/b* ratio of the LHC-II preparation was 1.1 and its absorption spectrum showed a maximum at 672 nm and a distinct shoulder, due to chlorophyll *b*, at 652 nm. One of the main objectives of this study was to follow the energy transfer from chlorophyll *b* to chlorophyll *a* in the isolated complex. For this purpose samples were excited at 652 nm using the 3 different polarizations of the excitation light and the signals obtained are shown in fig.1. In all cases a very fast recovery of the signal back to a level close to the initial state was observed. This demonstrates a very efficient radiationless process, i.e. transfer of excitation energy to molecules with only minor absorption coefficients around 650 nm. This observation is taken as evidence for an efficient transfer of energy from chlorophyll *b* to chlorophyll *a*. The plots of the isotropic signal (fig.1) indicate lifetimes of about 10 ps. Since this is the limiting time resolution imposed by the laser pulse, 10 ps should be taken only as an upper limit for the energy transfer between chlorophyll *b* and chlorophyll *a*. However, a lower time limit can be estimated from the shape of the isotropic signal. In a previous study on bacteriorhodopsin [19] a perfectly symmetric signal was observed for a lifetime of approx. 2 ps. Since the isotropic signal of fig.1 is slightly unsymmetric we estimate the lifetime of the energy transfer from chlorophyll *b* to chlorophyll a to be in the range 2–10 ps.

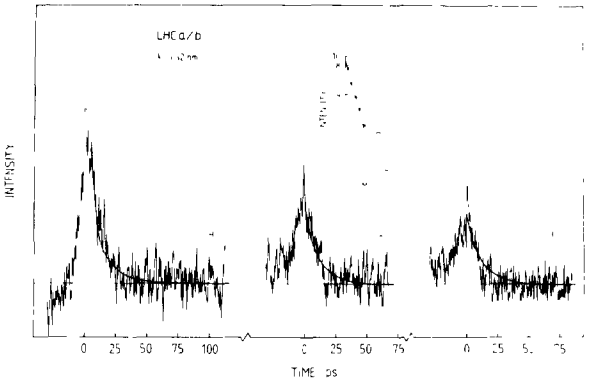

Fig.1. Picosecond absorption recovery measurements at 652 nm on fresh samples of LHC-II. The angle between the polarization of the excitation and analyzing light was (from left to right) 0, 54.7 and 90°, respectively. The logarithmic plot shows the isotropic signal. The lifetime of the decay is ~10 ps.

The anisotropy, $r(t)$, is constant (0.42 ± 0.05) in the time interval 5–25 ps, where it could be accurately measured. This demonstrates that during the lifetime of the chlorophyll *b* excited state there is no transfer of excitation between differently oriented chlorophyll *b* molecules. This is in contrast to CD measurements on isolated LHC-II which suggest the existence of a trimer of functionally interacting chlorophyll *b* chromophores with their transition dipoles arranged in a C_3 symmetry [7,9]. Such organization would lead to a decrease of anisotropy from $r(t) = 0.4$ unless the transition dipoles of the chlorophyll *b* chromophores are oriented in parallel. The latter possibility, however, is not very likely.

After the LHC-II preparation had been standing for 24 h at room temperture or had been used in picosecond laser experiments for 4–8 h the kinetics of excitation transfer from chlorophyll *b* were completely changed. The fast recovery was lost and instead the isotropic signal could be divided into 2 exponential components with lifetimes of 25 ± 5 ps and about 2 ns, respectively (fig.2). This shows that the ageing of LHC-II severely inhibits the energy transfer from chlorophyll *b*. This change in lifetime of the chlorophyll *b* excited state was paralleled by a decrease in the absorbance at 652 nm and a broadening of the absorption peak of chlorophyll *b* (not shown).

To study the energy transfer between chlorophyll *a* molecules measurements were also performed with excitation at 665 nm (fig.3). The decay is double exponential, dominated by a component with a lifetime of approx. 2 ns. This lifetime is equivalent to the fluorescence lifetime of chlorophyll *a* of LHC-II observed earlier [10–12]. In addition, we resolved a fast component of lower amplitude with a lifetime of 20 ± 5 ps. The existence of such a fast component suggests energy transfer between chlorophyll *a* chromophores having different absorption spectra. Moreover, it follows that the excitation energy is transferred to a chlorophyll *a* chromophore with a lower extinction coefficient at 665 nm than the initially excited chlorophyll *a* chromophore. It has been suggested that the 672 nm chlorophyll *a* absorption band of LHC-II is composed of at least 2 major components with maxima at 670 and 677 nm [9]. According to these spectral studies the 677 nm chlorophyll *a* component should absorb very little at 665 nm. A complete transfer of the excitation energy from the 670 nm to the 677 nm form should therefore result in an almost complete fast recovery of the signal at 665 nm (fig.3). Since this is contrary to our observations we have to conclude that a fast equilibration of the excitation energy between the spectroscopically different chlorophyll *a* chromophores occurs rather than a complete transfer from the 670 nm chlorophyll *a* component to the 677 nm component. This situation is likely to change, however, when energy transfer to the photosystem II core is in operation.

The anisotropy at 665 nm also showed a fast lifetime of approx. 20 ps before a steady-state

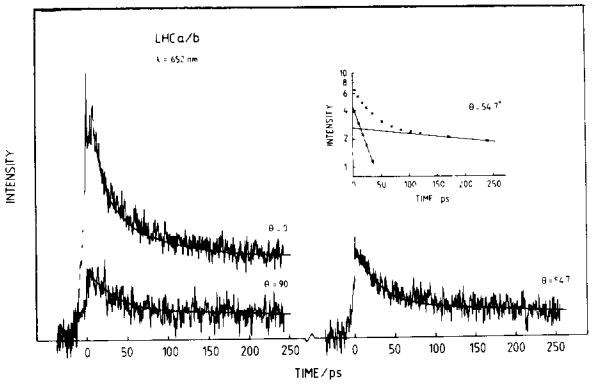

Fig.2. The same experiments as in fig.1 performed at 652 nm on samples of LHC-II that had been aged for about 6 h at room temperature.

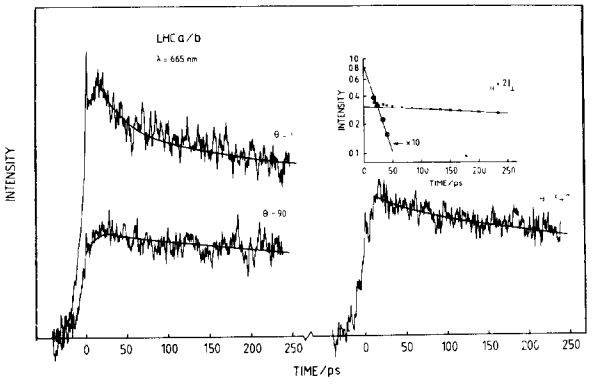

Fig.3. Picosecond absorption recovery experiments at 665 nm on fresh LHC-II samples. The plot shows the isotropic signal ($I_{\parallel} + 2I_{\perp}$, see text). For clarity, the fast component has been magnified × 10.

value of 0.28 ± 0.04 was reached. This relatively high anisotropy still present at 665 nm after the initial fast decay indicates that the 670 nm chlorophyll *a* chromophores are not randomly distributed within the LHC-II, but have a rather high order. By comparison with the emission polarization data [7,8] ($p = 0.14$ or $r = 0.10$) we can conclude that there must be some additional change of anisotropy in the energy transfer from the 670 nm to the 677 nm component and/or an energy transfer between chlorophyll *a* 677 nm chromophores. At 665 nm no effects upon ageing of the isolated LHC-II could be detected in contrast to the observation at 652 nm. This may suggest that the chlorophyll *a* molecules reside in a more protected environment within the complex compared to chlorophyll *b*.

How do our measurements relate to the situation in situ where LHC-II is embedded in the thylakoid bilayer in close association with the photosystem II core? An inevitable problem is that the addition of detergents and the time required for preparation may cause some alterations in the organization of the complex. However, the isolation procedure required only low amounts of the non-ionic detergent Triton X-100 and relatively short preparation time. The dissociation of LHC-II from its energy sink, the photosystem II core complex, might also influence the energy transfer within the complex. Picosecond absorption measurements on LHC-II in association with the photosystem II core are in progress to address this problem.

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