Biochimica et Biophysica Acta 1827 (2013) 779–785

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Photochemical trapping heterogeneity as a function of wavelength, in plant photosystem I (PSI–LHCI)

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ARTICLE INFO

Article history:
Received 9 January 2013
Received in revised form 12 March 2013
Accepted 20 March 2013
Available online 28 March 2013

Keywords:
Photosystem I
Light Harvesting Complex I
Fluorescence decay
Trapping rate heterogeneity
Red spectral forms

ABSTRACT

In the present paper the marked changes in photochemical trapping time over the absorption/fluorescence band of isolated PSI–LHCI are studied by means of time resolved fluorescence decay measurements. For emission at 680–690 nm the effective trapping time is close to 17–18 ps, and represents the effective trapping time from the bulk antenna. At wavelengths above 700 nm the effective trapping time increases in a monotonic way, over the entire emission band, to attain values in the range of 70–80 ps near 760 nm. This is argued to be caused by "uphill" energy transfer from the low energy states to the core antenna and reaction centre. These data, together with the steady state emission spectrum, permit calculation of the overall trapping time for maize PSI–LHCI, which is estimated to be approximately 40 ps. The wavelength dependence of the trapping time indicates, that in PSI–LHCI there exists at least one red form which emits at lower energies than the 735 nm state. These data indicate that Photosystem I is about 55% diffusion limited.
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1. Introduction

Photosystem I (PSI) of higher plants is a supramolecular pigment–protein complex, localized in the non-appressed regions of thylakoid membranes. The catalytic activity of PSI is that of a light-dependent oxido-reductase, which uses the diffusible electron carries plastocyanin as the electron donors and ferredoxin as the electron acceptors [1]. The complex has two functional moieties, which are the central chl *a*-binding core complex and a peripheral antenna, which consists of chlorophyll *a*/*b* binding proteins known collectively as LHCI. The core complex binds approximately 95 chl *a* and probably about 20 β-carotene molecules [2,3] as well as the cofactors involved in primary photochemical events and the successive electron transfer reactions. The LHCI complexes seem to be arranged on one side of the core [2,4] and, taken together, they bind about 66 chl $a + b$ according to the crystallographic structure [2] and 80–100 according to biochemical analysis [5], and 20 xanthophylls, with the number of complexes surrounding the core probably being four according to the crystallographic structure and around 10 according to biochemical analysis [5]. The LHCI complexes are now thought to bind around 15–16 chlorophylls per monomer [2].

The PSI–LHCI supercomplex can be isolated from maize in an intact form, that is, without detergent-solubilized chls present [6]. In the region of the lowest lying electronic transition (Q_y), the complex has a broad absorption band with its maximum at 680 nm. This band is associated with the so-called "bulk" antenna chl *a* and accounts for about 90–95% of the Q_y oscillator strength. A peculiarity of the PSI–LHCI absorption spectrum is the presence of significant absorption in the low-energy tail, indicating the presence of red spectral chl *a* forms, or states, absorbing at energies lower than that of the primary electron donor pigments from which photochemistry takes place, including the electron donor P_{700}. In PSI–LHCI the combined oscillator strength of the "red forms" is approximately equivalent to 7–8 chls [6] which, at room temperature, in the steady state, are highly populated, with 80–90% of the PSI–LHCI excited states being associated with them [6]. These low energy states have been demonstrated to be associated with excitonic dimers in the case of the cyanobacterium *Spirulina platensis* [7], in which the interaction energy was estimated to be 330–340 cm^{-1}, and also for the approximately 735 nm emission states in the external antenna complexes of PSI–LHCI [8,9]. It has been experimentally demonstrated that the red forms, located primarily in the peripheral antenna in plant PSI–LHCI [10–12], are kinetically limiting for the transfer of energy from the antenna to the reaction centre [13,14], indicating that PSI is partially diffusion limited, though there is some disagreement on this point [15].

The most widely used technique to study PSI–LHCI photochemical trapping involves measurement of the fluorescence lifetime decay, either by means of streak camera or of single photon counting techniques, which are subjected to global lifetime analysis. It is a common

Abbreviations: PSI, Photosystem I; LHC, Light Harvesting Complex; RC, reaction centre; DAS, decay associated spectra; SOI, spectral overlap integral; OGP, octyl-gluco-pyranoside; DM, dodecyl-maltoside

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http://dx.doi.org/10.1016/j.bbabio.2013.03.008

practice to associate the trapping time to decay associated spectra (DAS) [e.g. 13–18] that display positive amplitude across the whole fluorescence emission spectrum. As there are often two such DAS components this has led to the conclusion of two distinct trapping kinetics, which occur roughly in the 40–60 and 80–130 ps time range. In order to explain these results it has been suggested that the 40–60 component may be associated with trapping from the core and the longer decay with trapping from the LHCI complexes of the external antenna. On the other hand, it was initially shown [14], and subsequently confirmed [18], that the effective trapping time in maize PSI–LHCI, calculated from the DAS, varies significantly as a function of the emission wavelength, increasing in a monotonic fashion from 700 nm to 760 nm across the emission band. This demonstration suggests that the trapping dynamics require a somewhat more detailed explanation than that previously suggested, based on the analysis of two "positive" DAS components only. Moreover, whereas these previous analyses [14,18] clearly demonstrate the wavelength dependence of the effective trapping times, they seem to have considerably underestimated the extent of this phenomenon. In order to clarify the situation we have re-examined the wavelength dependence of the trapping times in a preparation of PSI–LHCI prepared from maize by the solubilisation of thylakoid membranes using the mild detergent octyl-glucopyranoside (OGP) [6,14,18]. The results permit determination of

i) the intrinsic trapping time from the directly excited bulk.
ii) the large effective trapping time variation, as a function of wavelength, associated with the low energy chlorophylls in the wavelength range 700–760 nm
iii) the overall PSI–LHCI effective trapping time
iv) the marked contribution of excited state diffusion limitation to the effective photochemical trapping in PSI–LHCI.

Moreover they also

i) provide evidence that the red spectral states are not quenching states.
ii) indicate the presence in PSI–LHCI of at least one spectral form which emits at lower energy than the F735 form.

These conclusions are reached directly from the experimental data, without the need to use the usual compartmental type modelling, on which there is still significant debate in the literature.

2. Materials and methods

PSI–LHCI and LHCI were prepared by the method of Croce et al. [6,10] which employs the mild detergent OGP to solubilise the thylakoid membranes. Steady-state fluorescence emission spectra were measured at 90° with respect to the excitation beam using a CCD camera (Princeton, Applied Physics) as a detector, with a spectral resolution of 0.25 nm and were corrected for the instrument wavelength sensitivity. The excitation beam was provided by a xenon lamp passed through a Jasco monochromator with the transmittance half-width adjusted to either 1 or 2 nm, depending on the excitation wavelength. The emission spectra were measured across a Schott (Wayzata, MN) OG 530 filter. When excitation was into the Q_y absorption band, the emission spectrum was corrected for the stray excitation "spike" by means of measurements performed either on the same sample, but after complete fluorescence quenching by addition of DBMIB (2,5-Dibromo-6-methyl-3-isopropyl-1,4-benzoquinone, 80 mM), or on a scattering suspension of glycogen. In this way it was possible to completely eliminate the scattering spike to within ±5 nm of the spike maximum for all excitation wavelengths.

Time-resolved fluorescence measurements with picosecond resolution were performed using the time-correlated single-photon counting (TCSPC) technique, as previously described [18]. In brief, the excitation source was a pulsed diode laser (PicoQuant GmbH, Berlin, Germany), controlled by a PicoQuant PDL 800-B unit, peaking at 632 nm and operating at a repetition rate of 20 MHz. The emission were passed through a monochromator (Jasco CT-10, Tokyo, Japan) and detected by a cooled microchannel plate photomultiplier tube (Hamamatsu R3809U-51, Hamamatsu, Japan). The instrument response function was measured using the reference dye DCI′, as previously described [19], resulting in an overall response of about 80 ps (FWHM) which after deconvolution yielded a time resolution of 10–20 ps. The emission decays were recorded at 10 nm intervals between 680 and 760, corrected for instrument wavelength sensitivity, and analysed globally by means of an algorithm developed in the laboratory [19].

The effective trapping time for antenna excited states, as a function of the detection wavelength, was determined from the decay associated spectra (DAS), by calculating the average lifetime which is defined as $\tau_{av}(\lambda) = \left(\sum_i A_i(\lambda)\cdot\tau_i)/\sum_i A_i(\lambda)\right)$. As previously described [14] this parameter has been demonstrated to be the effective trapping time. It is worth noting that the interpretation of the physical meaning of $\tau_{av}(\lambda)$ is independent of the details of the primary photochemical reactions, which are still a matter of debate.

The spectral overlap integral (SOI) for a bulk chlorophyll, acting as donor, and a red chl form, acting as acceptor, in a native protein environment was approximated on the basis of the calculated absorption spectrum both for weak (bulk pigment, S = 0.8) and strong coupling (red form, S = 4) to a phonon bath of mean frequency $\nu_m = 20\ \mathrm{cm}^{-1}$, as previously described [20]. In short, we have used the set of vibrational frequencies and Franck–Condon factors reported in hole burning studies by Gillie et al. [21] as the input data for a Fast Fourier Transform algorithm used to numerically estimate the normalised chlorophyll a bandshape. The normalised fluorescence spectrum was obtained by the mirror image of the absorption bandshape and the Stokes shift determined by the Stepanov relation [20]. When a donor molecule, D, having the absorption maximum at ν_0^D and florescence spectrum $F(\nu_0^D,\nu)$, and an acceptor molecule, A, with absorption maximum at ν_0^A and absorption spectrum $\varepsilon(\nu_0^A,\nu)$ are considered, the SOI was determined by:

$$SOI\left(\nu_0^A - \nu_0^D\right) = \int_0^\infty d\nu\,\nu^{-4} F\left(\nu_0^D,\nu\right)\varepsilon\left(\nu_0^A,\nu\right), \quad (1)$$

as a function of the energy difference between the acceptor absorption maximum and the donor absorption maximum $\Delta E = \nu_0^A - \nu_0^B$.

3. Results and discussion

3.1. Fluorescence decay analysis of maize PSI–LHCI

The excited state trapping kinetics were studied in a PSI–LHCI supercomplex isolated from *Zea mais* and purified as described by Croce et al. [6,10]. Fluorescence decay, recorded in the 680–760 nm interval, are described in a global fit analysis, by three dominant components characterized by lifetimes of 13 ± 2 ps, 41 ± 5 ps and 74 ± 6 ps, and a minor component with an associated lifetime of 785 ± 30 ps. The confidence limits are the standard deviation of the fit parameters obtained from five independent measurements made on the same preparation. In our experience, the global fit minimum is rather broad, in agreement with others [22], and the standard deviations for measurements made on the same sample are an indication of this. The present results are very similar and within the experimental uncertainties of the solutions previously obtained by Engelmann et al. [18] for the same type of preparation, and using the same experimental set-up. Therefore, we have decided to average the results reported in ref. [18], with those obtained in the new PSI–LHCI preparation in order to extend the statistics. It is these averaged results which will be analysed in the present study. In most

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