Biochimica et Biophysica Acta 1837 (2014) 726–733

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Wavelength dependence of the fluorescence emission under conditions of open and closed Photosystem II reaction centres in the green alga *Chlorella sorokiniana*

Federico Rizzo, Giuseppe Zucchelli, Robert Jennings, Stefano Santabarbara *

Istituto di Biofisica, Consiglio Nazionale delle Ricerche, via Celoria 26, 20133 Milano, Italy
Dipartimento di Bioscienze, Università di Milano, via Celoria 26, 20133 Milano, Italy

ARTICLE INFO

Article history:
Received 20 December 2013
Received in revised form 10 February 2014
Accepted 12 February 2014
Available online 20 February 2014

Keywords:
Maximal photochemical efficiency
Variable fluorescence
Photosystem II
Photochemical yield
Excited state dynamics

ABSTRACT

The fluorescence emission characteristics of the photosynthetic apparatus under conditions of open (F_0) and closed (F_M) Photosystem II reaction centres have been investigated under steady state conditions and by monitoring the decay lifetimes of the excited state, *in vivo*, in the green alga *Chlorella sorokiniana*. The results indicate a marked wavelength dependence of the ratio of the variable fluorescence, $F_V = F_M - F_0$, over F_M, a parameter that is often employed to estimate the maximal quantum efficiency of Photosystem II. The maximal value of the F_V/F_M ratio is observed between 660 and 680 nm and the minimal in the 690–730 nm region. It is possible to attribute the spectral variation of F_V/F_M principally to the contribution of Photosystem I fluorescence emission at room temperature. Moreover, the analysis of the excited state lifetime at F_0 and F_M indicates only a small wavelength dependence of Photosystem II trapping efficiency *in vivo*.

1. Introduction

Photosystem II (PSII) catalyses the light-dependent oxidation of water and the reduction of plastoquinone to plastoquinol. It is composed of two functional moieties: the *core*, which serves both as the photochemical reaction centre as well as the internal antenna, and the external antenna, which only has a light harvesting function. Three principal pigment binding complexes are present in the *core*, CP43, CP47 and the D1–D2 heterodimer that coordinates all the cofactors active in primary photochemistry and successive electron transfer events (*e.g.* [1–6]). The core complex binds about 35 Chlorophyll (Chl) *a* and 12 β-carotene molecules [2,4]. In green plants, the external antenna is composed of several Chl *a*/*b* binding complexes (*e.g.* [5–7]) that also bind oxygenated carotenoids, known as xanthophylls. LHCII is the most abundant complex of the external antenna and is organised as a trimer [5–7]. Typical stoichiometries indicate the presence of three to four LHCII trimers per reaction centres. The other Chl *a*/*b*-binding complexes, CP29, CP26 and CP24, are monomers and are present as a single copy per reaction centre [5–7].

The fluorescence emission of PSII is markedly dependent on the redox state of its terminal electron acceptor (*e.g.* [5–18]), the plastoquinone molecule Q_A (and Q_B). When Q_A is oxidised (reaction centres are said to be in an "open state"), the fluorescence attains the minimal level, F_0, whereas when Q_A is reduced (Q_A^-) the fluorescence emission reaches its maximal level, F_M. Analysis of the excited state decay both in isolated thylakoids and in isolated PSII particles indicates that the average decay lifetimes (τ_{av}) vary as a function of active state of PSII: values in the range of ~300–450 ps (*e.g.* [16–27]) are reported for open states and in the range of ~1–2 ns (*e.g.* [16–22]) for closed reaction centres. Since the change of the average fluorescence lifetime parallels the intensity change observed at steady state, the low fluorescence emission under F_0 condition is interpreted in terms of singlet excited state quenching due to photochemical trapping (photochemical quenching). It is then easily demonstrated that the ratio between the variable fluorescence, $F_V = F_M - F_0$, and the maximal fluorescence level, F_M, represents the maximal quantum photochemical efficiency of PSII, (Φ_{PSII}^{max}) [10,11,14,15]. Values of Φ_{PSII}^{max} fall typically in the 0.75–0.85 interval [8–15].

The F_V/F_M parameter is routinely employed in physiological investigations, particularly under *in vivo* conditions [14,15]. Nevertheless, deriving Φ_{PSII}^{max} from the F_V/F_M ratio is correct only when assuming negligible Photosystem I (PSI) emission in the wavelength range of measurement. Room temperature measurements of PSI isolated from higher plants show that for wavelengths shorter than 700 nm the emission is significantly weaker than that of PSII and it is characterised by an average decay lifetime of 20–40 ps (*e.g.* [28–39]). On the other hand, PSI shows

Abbreviations: PSI(II), Photosystem I (II); LHC, Light Harvesting Complex; Chl, chlorophyll; DAS, Decay Associated Spectra; F_0, fluorescence level at open PSII reaction centres; F_M, fluorescence level at closed PSII reaction centres; F_V, variable fluorescence; Φ_{PSII}^{max}, maximal photochemical yield of PSII; $\tau_{(av)}$, (average) lifetime; CP, chlorophyll protein complex

* Corresponding author at: Istituto di Biofisica, Consiglio Nazionale delle Ricerche, via Celoria 26, 20133 Milano, Italy. Tel.: +39 02 50314857.
E-mail address: stefano.santabarbara@cnr.it (S. Santabarbara).

http://dx.doi.org/10.1016/j.bbabio.2014.02.009
0005-2728/

a maximal fluorescence emission at about 720 nm, where PSII emission is minimal. Moreover the average excited state lifetime of isolated PSI increases in monotonic fashion up to 60–80 ps in the long wavelength range [28,34,37,38]. Thus PSI might be a source of error in the estimation of Φ_{PSII}^{max} if F_V/F_M is monitored at wavelengths above ~710–720 nm.

The use of the F_V/F_M ratio as an unbiased estimator of Φ_{PSII}^{max} also requires that this parameter is independent of both the excitation and the emission wavelength. This condition is attained if the band-shape of the PSII fluorescence emission spectrum is independent of both the excitation wavelength, and the active state of the reaction centre. Although it was shown that the emission spectrum of the isolated PSII–LHCII complex is substantially independent of excitation wavelength and, hence, that the system can be considered, to a good approximation, thermally equilibrated [40,41], it was also shown that the value of the F_V/F_M ratio (data were presented as F_0/F_M) is not constant through the emission spectrum [42–44]. Minimal values of F_0/F_M (corresponding to a maximal value of F_V/F_M) were observed in the 685–690 nm interval and interpreted in terms of an increase of photochemical quenching in the reaction centre complex, due to a partial kinetic bottleneck of excited state energy transfer from CP43 and CP47 [44,45]. This suggestion has been, in part, supported by kinetic modelling studies based on the crystallographic structures [46,47]. At the same time, spectroscopic studies performed either on the isolated core complex of PSII [48–52] or in PSII–LHCII complex [52–54], point towards a limited contribution of kinetic bottlenecks for energy transfer to the overall trapping kinetics.

Possible underestimations of the value of Φ_{PSII}^{max} obtained from F_V/F_M due to PSI emission have been already discussed in the literature (*e.g.* [15,53,55–57]). However, the dependence of Φ_{PSII}^{max} through the emission band has not been investigated under conditions in which PSII is embedded in its native membrane.

Therefore, we have performed a detailed *in vivo* investigation of both steady state and time resolved fluorescence emission under F_0 and F_M conditions in the green alga *Chlorella sorokiniana*. Both spectroscopic approaches demonstrate a pronounced variation of F_V/F_M across the emission band, with maximal values observed in the 660–680 nm interval and minimal values in the 700–720 nm window. The analysis of data indicates that PSI fluorescence emission is the principal cause of F_V/F_M spectral dependence, leading to an underestimation of the value of Φ_{PSII}^{max} between 5 and 20%, depending on the fluorescence detection wavelength.

2. Material and methods

2.1. Cell culture

C. sorokiana cells were grown under continuous illumination (80 μE m^{-2} s^{-1}) and shaking, at 25 °C in TAP medium, containing acetate as a carbon source. The culture was harvested during the logarithmic growth phase by centrifugation for 5 min at 1000 *g* and suspended in minimal media at an optical density of ~1 O.D. cm^{-1} at 680 nm. After 2 h of incubation under growth conditions, the cells were diluted to an optical density of 0.1 O.D. cm^{-1} in minimal medium supplemented with the non-osmotic polymer Ficoll (20% w/v) and further adapted for half an hour. The measurements were performed after a further 2 min of dark adaptation, for F_0 conditions. For measurements performed at F_M the PSII inhibitor DCMU (15 μM) was added to the dark-adapted sample.

2.2. Fluorescence emission spectroscopy

Fluorescence emission spectra were recorded using a laboratory assembled spectrometer using a liquid nitrogen cooled CCD camera (Princeton Applied Research, LN/CCD-ST138), coupled to a spectrograph (PAR, SpectraPro 300i) as a detector/analyser system, that has been described previously in detail [58]. The excitation source is a 250 W xenon lamp, filtered through a spectro-polarimeter (Jasco J500), a depolariser, a CS4-96 (Schott) band-pass filter and neutral density filters (Balzers) to control the excitation intensity. The excitation wavelength was set at 435 nm with a bandwidth of 1.5 nm, and it was sufficiently weak to maintain the cells in a state close to F_0, as judged from i) the F_V/F_M measured on the same sample after the addition of DCMU and ii) parallel measurements in a conventional fluorescence induction set-up. The spectra presented are the results of ten independent replicates (cell batches), each of which has been analysed three times on the same day of measurement. All spectra are corrected for the sensitivity of the detector, using a normalised instrument response function that maintains the experimental count number at the peak channel.

2.3. Measurement of the excited state lifetime

The decay of the excited state was monitored by the time-correlated single photon counting (TCSPC) technique in a home assembled set-up which has been previously described [34,52]. The excitation source was a diode laser (PicoQuant 800B) centred at 632 nm (FWHM 3 nm), with a pulse width of ~20 ps and operating at a repetition frequency of 20 MHz. The laser spot-size was about 1 mm in diameters and the pulse intensity is ~2 picoJ/pulse, which is sufficiently low to avoid the build-up of a significant population of closed centres at F_0 and of meta-stable states, such as (carotene) triplets. The emission was monitored at discrete wavelengths, selected by a monochromator (Jasco, JT-10), in the 660–750 nm interval. The detector was a cooled microchannel plate photomultiplier (Hamamatsu, R5916U-51). The overall response function of the instruments is 110 ± 10 ps, which allows resolving decay components which are an order of magnitude faster after numerical deconvolution. Measurements under F_0 and F_M conditions were performed on the same sample before and after DCMU was added. The sample was changed periodically during the measurement period. Moreover for each condition, measurements were performed by initially scanning from low to high wavelengths and subsequently in the reverse order. The instrument response function was determined using the dye DCI′ in ethanol as a reference standard, as previously described [52]. The results presented are the averages of eight independent cell batches.

2.4. Global analysis of TCSPC data

The kinetics of the excited state decay collected under F_0 and F_M conditions were analysed by iterative reconvolution of the instrument response and a sum of exponential functions, by a global fitting routine based on the MINUIT package that minimises the reduced χ^2 as previously described [52]. In brief the decay lifetimes (τ_i) are analysed as global parameters, whereas the pre-exponential amplitudes associated with each decay lifetime are treated as a local parameter and are therefore a function of the emission wavelength ($A_i(\lambda)$). The plot of the amplitude as a function of the emission wavelength yields the decay associated spectra (DAS). The quality of the fit was judged by the value of χ^2, inspection of the fit residuals and their autocorrelation and the band-shape of the DAS. It is a well known problem that such an analysis does not always lead to an unequivocal description of the data. As a rationale for selection amongst different possible fit solutions, we opted for those that were characterised by a minimal number of parameters and displayed stability through the independently tested cell batches.

The average lifetimes is defined as $\tau_{av} \equiv \left(\sum_{i=1}^{n} A_i \cdot \tau_i\right) / \sum_{i=1}^{n} A_i$.

3. Results and discussion

3.1. Steady state fluorescence emission spectra

The fluorescence emission spectra in whole cells of *C. sorokiniana*, under conditions in which the reaction centres of PSII are either open