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Spectral dependence of irreversible light-induced fluorescence quenching: Chlorophyll forms with maximal emission at 700–702 and 705–710 nm as spectroscopic markers of conformational changes in the core complex

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ABSTRACT

The spectral dependence of the irreversible non-photochemical fluorescence quenching associated with photoinhibition *in vitro* has been comparatively investigated in thylakoid membranes, PSII enriched particles and PSII core complexes isolated from spinach. The analysis of the fluorescence emission spectra of dark-adapted and quenched samples as a function of the detection temperature in the 280–80 K interval, indicates that Chlorophyll spectral forms having maximal emission in the 700–702 nm and 705–710 nm ranges gain relative intensity in concomitance with the establishment of irreversible light-induced quenching, acting thereby as spectroscopic markers. The relative enhancement of the 700–702 nm and 705–710 nm forms emission could be due either to an increase of their stoichiometric abundance or to their intrinsically low fluorescence quantum yields. These two factors, that can also coexist, need to be promoted by light-induced alterations in chromophore-protein as well as chromophore-chromophore interactions. The bands centred at about 701 and 706 nm are also observed in the PSII core complex, suggesting their, at least partial, localisation in proximity to the reaction centre, and the occurrence of light-induced conformational changes in the core subunits.

1. Introduction

Photosystem II (PSII) is a large macromolecular pigment-protein complex, composed of over 20 subunits, that catalyses the light-dependent oxidation of water and the reduction of plastoquinone (*e.g.* [1–4] for reviews). From a structural perspective PSII can be seen as being composed of two moieties: i) the core complex, which contains the photochemically active pigments and the cofactors involved in electron transfer reactions, bound to the reaction centre proteins, as well as the chromophores involved in light harvesting, that are bound to the so-called inner or proximal antenna complexes [1–3] and ii) the external antenna (*e.g.* [4–7]). Whereas the structure and biochemical composition of the core complex are overall well conserved in different oxygenic photosynthetic organisms, those of the external antenna vary greatly, also in terms of chromophore composition. In higher plants and green algae the external light harvesting antenna is composed of several Chlorophyll (Chl) *a*/Chl *b*-binding proteins [4–7], collectively known as light harvesting complex II (LHCII) and which are coded by the nuclear gene family known as *Lhcb*. The most abundant pigment-protein complex in the external antenna, is a trimer composed of different combinations of the *Lhcb1-2-3* gene products that coordinate, each, 8 Chl *a*, 6 Chl *b* as well as four oxygenated carotenoids which are part of the xanthophyll family [4–7]. Typical stoichiometries indicate the presence of 3 to 4 *Lhcb1-2-3* trimers per core complex. Because of their dominant stoichiometry, these trimers are often simply referred to as LHCII. The other complexes of PSII outer antenna are the gene products of *Lhb6*, *Lhb5* and *Lhcb4*, and are also known as CP24, CP26 and CP29, according to their apparent molecular weight [4–9]. These antenna complexes are isolated as monomers and present as a single copy per reaction centre.

Abbreviations: Chl, chlorophyll; DBMIB, 2,5-dibromo-6-isopropyl-3-methyl-1,4-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHC, light harvesting complex; PS (II, I), Photosystem (II, I); RC, Reaction Centre; F_M, maximal fluorescence emission intensity; F_0, minimal (dark adapted) fluorescence emission intensity; F_V, variable fluorescence (F_M–F_0); F_q, fluorescence emission level after light-induced irreversible quenching; NPQ, non-photochemical quenching; qE, energy dependent component of NPQ; qI, irreversible component of NPQ

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The yield of PSII fluorescence emission is dependent on the redox state of the cofactors involved in the electron transfer reactions, particularly on the reduction level of the quinone acceptor Q_A (*e.g.* [10–11]). When Q_A is in its oxidised state, the PSII centres are said to be in an open state, that is photochemically active, and the fluorescence yield is low. This intensity level is denoted as F_0 and measurements of the excited state relaxation revealed that the average lifetime under these conditions is 250–400 ps (*e.g.* [12–15]). On the other hand, when Q_A is in the semi-quinone state, the centres are said to be in a closed state, denoted as F_M, the maximal fluorescence level. The fluorescence yield, as well as the lifetime of the excited state decay, are 4–5 folds larger under F_M conditions than at F_0 (*e.g.* [10,11,13,14]). The quenching of the maximal fluorescence level observed at F_0 is due to photochemical reactions and is therefore known as photochemical quenching.

The yield of maximal PSII fluorescence emission is also modulated by processes other than photochemical trapping, collectively known as non-photochemical quenching (NPQ). These are principally observed when the photosynthetic apparatus is exposed to actinic light close to, or exceeding, the saturation of the thylakoids electron transport chain (e.g. [16–18] for reviews). NPQ is a complex phenomenon that comprises several components and effectors, which can be distinguished on the basis of their formation and relaxation kinetics [16–18].

Under most illumination conditions NPQ is dominated by the energy-dependent qE component, which is rapidly reverting, being characterised by half lives in the 1–2 min range [16–18]. This is the most intensively investigated NPQ component. qE was shown to be dependent on the formation of the transmembrane pH gradient, on the presence of the PsbS subunit of PSII and to be strongly correlated to the enzymatic conversion of the xanthophyll violaxantin to its epoxide zeaxanthin [16–18]. Although the molecular mechanism of qE is still debated, there is a general agreement that the quenching site is localised in PSII external antenna [16–19]. It has been suggested that singlet excited state quenching might be caused by energy transfer to the so-called forbidden S_1 state of carotenoids (Car), principally lutein, as a result of a change in the Chl-Car coupling that would favour such energy transfer pathways in the quenched state of the LHCII complex [20]. This would require subtle conformational rearrangements of the complex, which were observed as a distortion of the neoxanthin molecule bound to LHCII (e.g. [19]) and supported by thermodynamic analysis of the quenching temperature [21] and pressure [22] dependences, as well as single molecule investigations [23]. An alternative mechanism has been suggested to take place in the minor antenna complexes, particularly CP29, and it involves the formation of a Car-Chl charge transfer complex acting as the effective quenching site [24]. Evidences for the involvement of multiple quenching centres and mechanisms have also been presented [25].

An NPQ component that relaxes with lifetimes in the 5–15 min interval has been associated with a process known as state-transitions that leads to a lateral redistribution of LHCII complexes between PSII and PSI (e.g. [26,27] for reviews). State transitions are not a pure quenching process of PSII, rather reflect a reduction of its absorption cross section [26–30]. However, involvement of quenching processes during the onset of state transitions has been discussed [31,32].

The most slowly relaxing component, having half lives that often exceed the hour and that becomes irreversible *in vitro*, is also commonly observed when the photosynthetic apparatus is illuminated with saturating actinic fluxes [16–18]. This component is called qI, for inhibition-associated or irreversible component of NPQ. While a large and significant body of information has been gathered concerning the molecular mechanisms leading to qE, much less is known about the sustained qI component (*e.g.* [33–37]). *In vivo*, part of qI was shown to be mechanistically related to qE, depending on the accumulation of zeaxanthin, leading to a very slow quenching reversibility [36–38]. Another component of qI is due to F_M quenching occurring in concomitance with the onset of photoinhibition, which also leads to the loss of PSII quantum efficiency (*e.g.* [39,40]). The photoinhibition-associated component of qI, *in vivo*, is even slower in terms of reversibility with respect to the sustained zeaxanthin-associated process. In isolated thylakoids, where qI is irreversible, it was demonstrated to be strongly correlated to the light-induced loss of maximal photochemical efficiency of PSII as it shares the same action spectrum [41,42]. However, the molecular nature of the quencher involved in this process remains to be elucidated.

To gain more information on light-induced irreversible quenching *in vitro*, the process has been here comparatively investigated in thylakoids, PSII-LHCII (BBY particles) and isolated PSII core complexes.

2. Material and methods

2.1. Biochemical preparations

Thylakoid membranes were freshly isolated from spinach leaves as previously described [43] and suspended in 30 mM Tricine/NaOH pH 7.8, 5 mM $MgCl_2$, 10 mM NaCl and 0.1 M Sucrose. The average Chl *a/b* ratio in the thylakoid membranes was 2.78 ± 0.04 as determined from water/acetone extraction, using the extinction coefficient given by Lichtenthaler [44]. BBY particles, which are highly enriched in PSII-LHCII supercomplexes, were purified by Triton X-100 fractionation of isolated thylakoids according to Berthold et al. [45], with the modification described by Dunahay et al. [46], and suspended in 30 mM Tricine/NaOH pH 7.8, 50 mM NaCl and 0.1 M Sorbitol. The average Chl *a/b* ratio in the BBY membranes was 2.0 ± 0.1. The PSII core complex was purified by octylglucopiranoside (OGP) solubilisation of the BBY membranes, as described by Ghanotakis et al. [47] and suspended in 30 mM Hepes pH 7.8, 0.1 M Sorbitol, 0.003% w/v β-DM. Chl *b* was undetectable in the isolated PSII core, indicating the preparation is not contaminated by external antenna complexes. This was also confirmed by SDS-PAGE analysis (Further details are presented in the Supplementary Material, Appendix A, Fig. S1). DCMU and DBMIB, when necessary, were added to the samples from methanol and ethanol stock solutions respectively, keeping the final alcohol volume below 1%.

2.2. In vitro photoinhibition treatment

The set-up used for inducing photoinhibition *in vitro* has been previously described in detail [41,42]. In brief, the samples, suspended in a 1 cm path-length cuvette at a concentration equivalent to 4 μg ml^{-1} of Chl (corresponding to an optical density of ~0.1 at the maximal absorption) and in the presence of the inhibitor DCMU (10 μM), were illuminated, at 4 °C and under constant stirring, by the white light from a 900 W Xenon arc lamp (Applied Photophysics). The beam size was defocused to a diameter of ~6 mm to obtain a homogeneous illumination of the sample. The light was filtered by a combination of a Calflex C and 5 cm of water to remove infrared and UV radiation. The intensity at the sample level was 150 mW cm^{-2} of visible radiation corresponding to ~3000 μmole of photons m^{-2} s^{-1}.

Photoinhibition is here defined as the irreversible loss of maximal PSII quantum efficiency, assessed by monitoring the decrease of the F_V/F_M ratio. Thus, the efficiency of the photoinhibitory treatment *in vitro*, and the kinetics of its reversibility in a period of dark, were estimated by measuring the fluorescence induction parameters in an home-built apparatus described in ref. [42]. The results are presented in the Supplementary Material, Appendix B, Figs. S2 and S3. The comparison of treatments performed in the absence of DCMU as well as in the presence of saturating concentrations of the uncoupler nigericin is also presented in the Supplementary Material, Fig. S4. For the conditions employed in this study, neither DCMU, nor nigericin, affected the kinetics of photoinhibition *in vitro*, as determined by the F_V/F_M ratio. Light-induced quenching of the (dark) F_M level was also unaffected, indicating that qE does not contribute to any significant extent to the