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Conformational changes and their role in non-radiative energy dissipation in photosystem II reaction centres†

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Accumulation of reduced pheophytin in photosystem II under illumination at low redox potential is known to be accompanied by a pronounced decrease of a chlorophyll fluorescence yield. Simultaneous measurement of this fluorescence quenching and absorbance changes in photosystem II reaction centres, in the presence of dithionite, showed each event to have a different temperature dependence. While fluorescence quenching was suppressed more than 20 times when measured at 77 K, pheophytin accumulation decreased only 5 times. At 77 K, the fluorescence was quenched considerably, but only in those reaction centres where reduced pheophytin had been accumulated at room temperature before sample freezing. This showed that the accumulation of reduced pheophytin above 240 K was accompanied by an additional, most probably conformational, change in the reaction centre that substantially enhanced non-radiative dissipation of excitation energy.

Introduction

Sunlight radiation is the main source of energy for planet Earth, where this energy is converted into the energy of chemical bonds by the process of photosynthesis. Sunlight is therefore essential for all life; however, in excess it can substantially damage even photosynthetic organisms.[1] To deal with such high light stress, photosynthetic organisms have developed several regulatory mechanisms, where readily measurable manifestation of these mechanisms can be made by fluorescence quenching, the light-induced depression of a chlorophyll fluorescence yield that is found in thylakoid membranes,[2] photosystem II (PSII) particles[3] or in aggregates of the light-harvesting complex of PSII.[4] The function of this mechanism is to efficiently match the input of absorbed solar energy to the photosynthetic capacity of organisms.

The key regulatory site in photosynthetic light reactions is PSII, a pigment-protein complex of the thylakoid membrane in higher plants, algae and cyanobacteria. The primary photochemical reaction in the PSII reaction centre (PSII RC) is a separation of charge, with the transfer of electrons from a primary donor, P680, to pheophytin (Pheo). The electron goes from reduced Pheo to the primary and secondary plastoquinone electron acceptors, Q_A and Q_B, respectively. Under certain conditions, when the Q_A is reduced or missing, the electron can alternatively go to a cytochrome (cyt) b_{559} molecule.[5]

Reduced Pheo can be accumulated in PSII RC by illumination when secondary electron acceptors (primary quinone, Q_A, and cyt b_{559}) are reduced or missing. Such accumulation of reduced Pheo is known to be accompanied by fluorescence quenching, a two- to three-fold decrease of chlorophyll fluorescence intensity. It was proposed that the accumulated molecule of reduced Pheo is the actual fluorescence quencher.[6–8]

In this work, we have compared the amount of reduced Pheo and the extent of chlorophyll fluorescence quenching, under normal (277 K) and low (77 K) temperatures, in isolated reaction centres of PSII. Our results indicate that Pheo reduction in the PSII RC is accompanied by additional changes in the reaction centres that enhance non-radiative dissipation of the excitation energy.

Experimental

PSII reaction centres were isolated according to Vácha *et al.*[9] Activity of the preparation was tested by measuring Pheo reduction and P680 oxidation in the presence of dithionite and silicomolybdate, respectively.[10] For accumulation of reduced Pheo, reaction centres were bubbled with nitrogen and then mixed with methyl viologen (10 μM) and dithionite (1 mg ml^{-1}). When measured at 77 K, glycerol was added to the sample to a final concentration of 65% (v/v). The final concentration of reaction centres was adjusted to an absorbance of 0.5–0.6 at 677 nm. The mixture was left to equilibrate in a dark on ice prior to measurements. Low temperature spectra were measured in an Oxford Optistat Bath cryostat (Oxford Instruments, UK), temperature in the cryostat was controlled by an Oxford Temperature Controller ITC 503 (Oxford Instruments, UK). Samples were frozen slowly to get a transparent amorphous glass. To enable precise temperature measurements a temperature sensor (thermocouple) was frozen directly in the sample. Reduced Pheo was accumulated by an exposure of the sample to a strong light (1200 μmol m^{-2} s^{-1}) guided from a halogen lamp source (KL 1500, Walz, Germany) by fibre optics. Chlorophyll fluorescence intensity was measured with a PAM fluorimeter (Walz, Germany). Fluorescence was excited by a blue LED diode (Nichia, Japan) filtered by a Corning 4–96 filter (380–580 nm) and measured through a RG645 filter (Schott, Germany). Light-induced absorbance difference spectra were measured with a laboratory-built flash spectrophotometer composed of a FX-1160 Xe flash lamp (EG & G, USA, 1 μs flash duration), MS257

† Dedicated to Professor James Barber on the occasion of his 65th birthday.

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imaging monochromator (Oriel, USA) and an acquisition and triggering unit FL200 (Photon Systems Instruments, Czech Republic). The detector was assembled from two large area S4111 photodiode arrays (Hamamatsu, Japan), for sample and reference signals. Light-minus-dark absorption spectra were measured as a difference between the spectrum captured at the end of the light exposure and the spectrum taken before the light exposure.

Results and discussion

Fig. 1 shows the absorbance difference spectra of PSII RC in the presence of dithionite and methylviologen upon irradiation by actinic white light at 277 and 77 K. Irradiation at temperatures above 240 K results in well-known absorbance changes attributed to a Pheo reduction:[11] bleaching at 681 nm and a shoulder at around 670 nm in the red region of the light-minus-dark spectrum. The shape of the difference spectra measured at 77 K resembles the spectra at 277 K. The key difference is the more pronounced bleaching in the region of 670 nm at 77 K as opposed to 277 K.

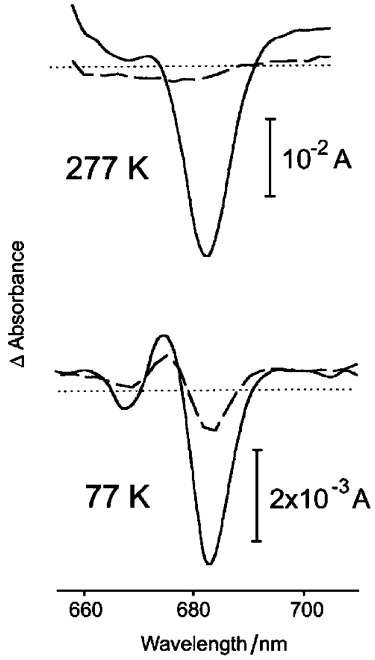

Fig. 1 Light-minus-dark absorbance difference spectra of PSII RC accompanying light-induced accumulation of reduced Pheo at 77 and 277 K (full lines) and the difference spectra after dark relaxation (dashed lines). Samples contained 1 mg ml^{-1} of dithionite and 10 μM of methyl viologen.

Fig. 2 data are the kinetics of simultaneous measurements of light-induced absorbance and fluorescence changes. Absorbance kinetics are presented as a difference between absorption change at 681 and 715 nm, being normalised to the absorbance at 677 nm of the actual sample ($\Delta A_{((681,715)/677)}$). At high temperature, absorbance and fluorescence changes were fully reversible. The low temperature absorbance kinetic shows slow reversible phase lasting several tens of minutes and an irreversible part which corresponds to a spectrum of an electrochromic shift at 680 nm (see Fig. 1(b) dashed line). Such irreversible change in fluorescence kinetics at low temperature may result from a charge accumulation on a non-pigment molecule similar to that which has been suggested in thylakoid membranes or isolated PSII particles.[12] Both the amplitude of the negative absorption difference at 681 nm and the extent of the fluorescence quenching are much lower at cryogenic temperatures than at 277 K. The amplitude of light-induced changes at 681 nm normalised to the absorption maximum (*i.e.* ratio of $\Delta A_{(681,715)}/A_{677}$) decreased from about 0.06 at 277 K to 0.012 at 77 K. The light-induced

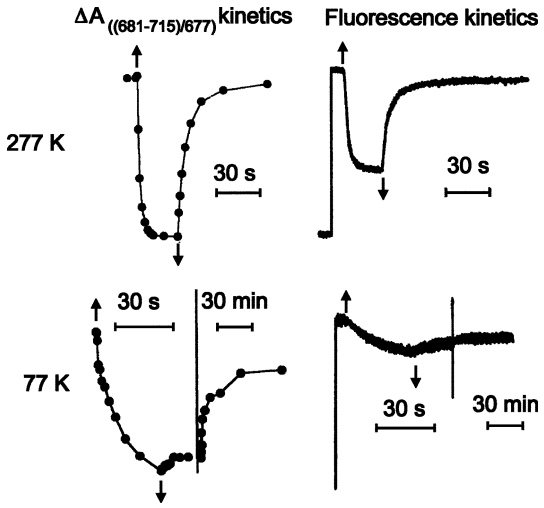

Fig. 2 Absorption and fluorescence kinetics in PSII RC upon light-induced accumulation of reduced Pheo at 277 and 77 K in presence of dithionite. The scaling for absorbance and fluorescence measured at 77 K (in the lower part of the figure) is five times expanded relatively to the upper part. Upward arrows: light on; downward arrows: light off.

decrease of fluorescence intensity declined from about 60% at 277 K to 3% at low temperature. This indicates that while the absorption bleaching at 681 nm decreased about five times upon the temperature decrease from 277 to 77 K, the light-induced fluorescence quenching decreased about 20 times upon the same temperature drop.

To further demonstrate the different extent of spectral changes and the dependence between the accumulation of reduced Pheo and the fluorescence quenching upon temperature decrease, the temperature dependence of quenching efficiency of the accumulated reduced Pheo was measured (Fig. 3). The fluorescence quenching was normalized to the absorbance change at 681 nm upon accumulation of reduced Pheo. The decrease of quenching efficiency of reduced Pheo during the sample cooling is evident. Similar temperature dependencies, as for the efficiency of fluorescence quenching shown in Fig. 3, were also found regarding the kinetics of electron transport and charge recombination in purple bacterial reaction centre[13] or for light-induced changes of circular dichroism in the red region of the absorption spectra[10] and interpreted as possible conformation changes accompanying primary processes in photosynthetic reaction centres.

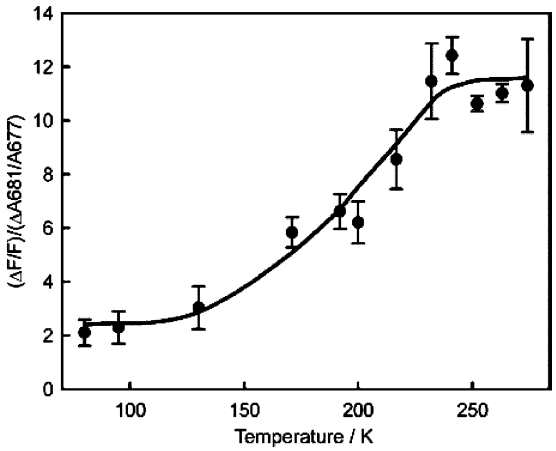

Fig. 3 Temperature dependence of quenching efficiency of accumulated reduced Pheo plotted as the fluorescence quenching normalized to the absorbance change at 681 nm *vs.* temperature. Fluorescence quenching was calculated as $(F_{DARK} - F_{LIGHT})/F_{DARK}$. Absorbance change, $\Delta A_{(681,715)}$ was normalized to the red absorption maximum at 677 nm for each sample and each temperature ($\Delta A_{(681,715)}/A_{677}$). Fluorescence was measured above 650 nm.

Temperature dependence studies of protein reactions may reveal aspects of conformational changes that accompany these processes. At low temperatures many degrees of freedom are thermally arrested. Charge separation is a substantial perturbation of the protein that results in conformational changes that can be studied on dark adapted and pre-illuminated samples. Conformational changes were demonstrated mainly in long range electron transfer studies in reaction centres of purple bacteria,[14–17] however, evidence exists for a crucial role of protein conformation in a stabilisation of the separated charge in these reaction centres.[18] The conformation changes associated with the charge separation in the PSII RC were also proposed.[10,19–22]

The pre-illumination effect on the chlorophyll fluorescence quenching in PSII RC is demonstrated in Fig. 4. When the reduced Pheo was accumulated prior to the sample cooling, the fluorescence quenching was "frozen". The resulting fluorescence quenching at 77 K was comparable or even higher (up to 67%) than the quenching observed above 240 K. The strong pre-illumination effect on the temperature dependence of fluorescence quenching efficiency of reduced Pheo suggests a formation of a "quenching state" at physiological temperatures. We assume that the "quenching state" is related to some conformational changes that accompany the accumulation of reduced Pheo and enhance the non-radiative energy dissipation in PSII RC.

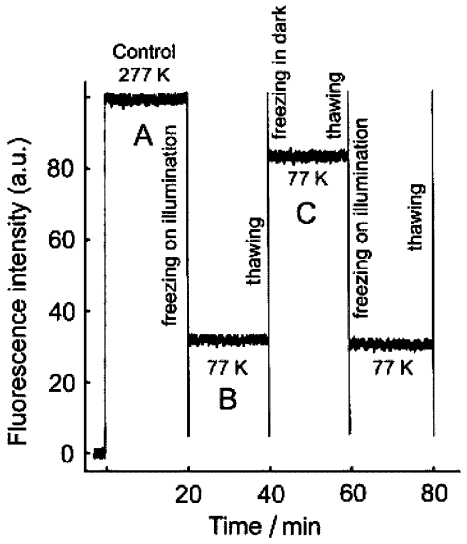

Fig. 4 77 K chlorophyll fluorescence quenching upon accumulation of reduced Pheo at 263 K prior to freezing. At first the fluorescence intensity was measured at 277 K (A). Afterwards the sample was cooled to 263 K, irradiated for 10 s to accumulate reduced Pheo and then, upon irradiation, quickly frozen to 77 K (B) by pooling liquid nitrogen onto the sample. Then, the sample was warmed to 263 K, to allow its relaxation, and frozen again to 77 K (C) in the dark. This cycle was repeated. Fluorescence was measured above 650 nm. It is clear from comparison of fluorescence intensity in parts B (freezing on illumination) and C (freezing in the dark) that the chlorophyll fluorescence quenching efficiency is much higher, when reduced Pheo is accumulated at high temperature.

The matter of the conformational change that generates the quenching state may have in principle two backgrounds, in a protein dynamics or in Pheo molecule conformation change itself. It was shown that not only protein conformation plays a role in the charge transfer reactions but that at least two conformational states of Bacteriopheophytine in the active branch of purple bacteria reaction centres can be freeze-trapped at 100 K after light induced formation of separated charge. These conformational changes of the chromophore lead to a rearrangement of the pigment protein interactions.[23–26]

Quenching of PSII RC chlorophyll fluorescence emission upon Pheo reduction was already observed some time ago. Here we have demonstrated that the quenching efficiency of reduced Pheo decreases with falling temperature, which suggests a conformation-dependent chlorophyll fluorescence quenching state of PSII RC. The physiological consequences of the processes of non-radiative energy dissipation that protect PSII against photoinhibition are obvious. The role of protein dynamics in these processes is now more readily evident.

Acknowledgements

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