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Pheophytin reduction and fluorescence quenching in reaction centres of Photosystem II

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Introduction

Primary photochemical reaction in photosystem II comprises electron transfer from the primary donor, P680, to pheophytin (Pheo). At low redox potential, when secondary electron acceptors (primary quinone, Q_A, and cytochrome (cyt) b_{559}) are reduced, $Pheo^-$ can be accumulated. In this photochemical reaction, electrons can be transferred to Pheo from an external donor, such as dithionite, water-oxidising complex (Klimov et al. 1985) or from cyt b_{559} (Shuvalov et al. 1989). Accumulation of $Pheo^-$ is accompanied by 2 to 3 fold decrease of chlorophyll fluorescence intensity (Klimov et al. 1977). In this contribution we compare the amount of $Pheo^-$ to the extent of fluorescence quenching throughout the range of temperatures from 77 K to 277 K. The results indicate that the accumulation of reduced Pheo is accompanied by an additional change in reaction centre that substantially enhances non-radiant dissipation of excitation energy.

Material and Methods

Reaction centres of Photosystem II were isolated according to Vácha et al. 1995. Reaction centres contain 5 chlorophylls, 2 pheophytins and 1 β-carotene. Activity of the preparation was tested by measuring the pheophytin reduction in the presence of dithionite and by assaying the P680 oxidation in the presence of silico molybdate (for detail description of the methods see). Reaction centres were mixed with 2 aliquots of glycerol and bubbled with nitrogen. Metylviologen (10 μM) and dithionite (1 mg/ml) were added 5 min before the freezing of the sample in a temperature controlled cryostat (Oxford, UK). Absorbance of samples at 677 nm was 0.5 – 0.6. Temperature of the sample was checked by a thermocouple frozen directly in the sample. Reduced Pheo was accumulated by exposure of the sample to strong light (1200 μmol $m^{-2}s^{-1}$) from a halogen lamp source (KL 1500, Walz, Germany) with fibre optics. Fluorescence intensity was measured with a PAM fluorimeter (Walz, Germany). Fluorescence was excited by a blue LED diode (Nichia, Japan) with a filter Corning 4-96 (380-580 nm) and measured through a filter RG645 (Schott, Germany). Light-induced absorbance difference spectra were measured with the laboratory build flash spectrophoto-meter composed of a microsecond Xe flash lamp (FX-1160, $EG_{\Sigma}G$, USA, flash duration of 1 μs), an imaging monochromator (MS257, Oriel, USA) and an acquisition and triggering unit FL100 (Photon System International, Czech Republic). Detector was assembled from two large area photodiode arrays (S4111, Hamamatsu, Japan) for sample and reference signals. This experimental set gives noise about $2 . 10^{-4}$ A per one flash with spectral resolution of 2.1 nm per one pixel. Light-minus-dark absorption spectra were measured as the difference between the spectrum captured at the end of light exposure (above 200 K), or 2 s after switching off of actinic light (below 200 K), and the spectrum taken before the light exposure.

Results and Discussion

Irradiation of reaction centres in the presence of dithionite at temperatures above 240 K resulted in well known absorbance changes corresponding to the Pheo reduction. Light-minus-dark difference spectrum shows bleaching at 681 nm and a shoulder at 670 nm (Fig. 1, left) in the red region. At shorter wavelengths, a bleaching at 545 and 422-426 nm and

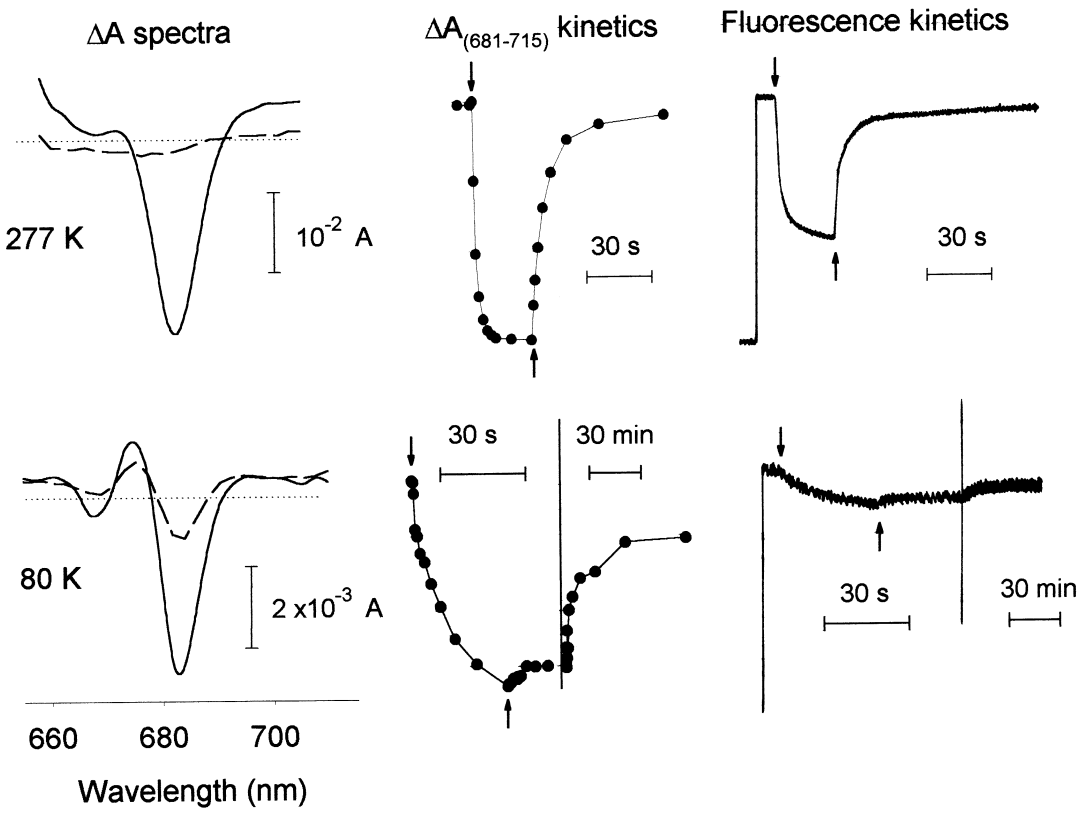

Fig. 1. Comparison of absorption and fluorescence changes in reaction centres of photosystem II upon light-induced accumulation of reduced Pheo at 80 and 277 K. Light-minus-dark difference absorbance spectra (full lines) and the spectra after dark relaxation (broken lines) are shown (left). Samples contained 1 mg/ml of dithionite and 10 μM of metylviologen. The scaling for absorbance and fluorescence in the lower part of the figure is five times expanded relatively to the upper part.

an increase around 450 nm is observed (Fig. 2). The low temperature spectrum differed from that measured above 240 K by the presence of additional negative bands at 557, 530 and 426 nm showing cyt b_{559} oxidation (Shuvalov et al. 1989) upon light exposure of frozen samples (Fig. 2). Kinetics of light induced absorbance change ($\Delta A_{(681-715)}$) and light-induced fluorescence changes are presented in the central and left parts, respectively, of the Fig. 1. At high temperature both the changes were fully reversible. In contrast to kinetics at 277 K, low temperature kinetics show slow reversible phase lasting several tens of minutes and an irreversible part which corresponds to an the spectrum electrochromic shift at 680 nm (Fig. 1, left-bottom). The irreversible component of fluorescence quenching could results from a charge accumulation on a non-pigment molecule similar as in thylakoids or PS II particles (Šiffel et al. 2000).

Freezing of the sample resulted in a depression of both absorbance and fluorescence changes. The ratio of the amplitude of light-induced changes at 681 nm and absorbance in the

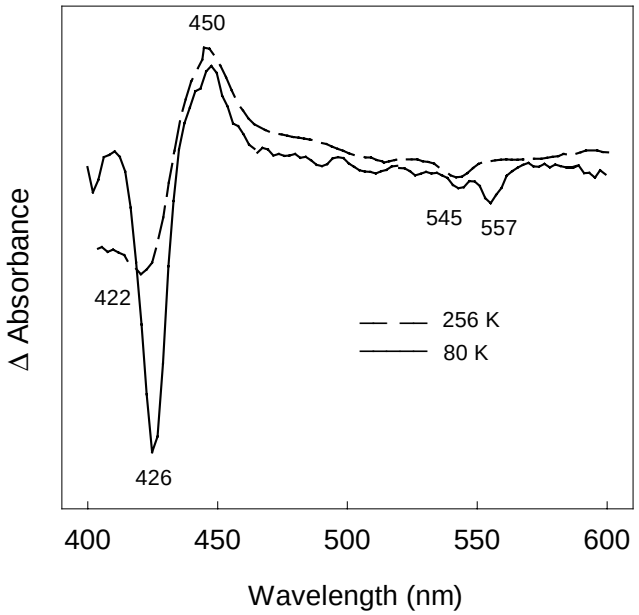

Fig. 2. Comparison of light-minus-dark absorption difference spectra of reaction centres complex measured at 256 (broken line) and 80 (solid line) K. Samples contained 1mg/ml of dithionite. "Dark" spectra were measured before exposure of sample to actinic light. "Light" spectrum was taken at the end of light exposure (256 K), or 2 s after switching off of actinic light (80 K). Spectra are normalised at the red maximum.

red maximum, *i.e.*, $\Delta A_{(681-715)}/A_{677}$, decreased from 0.05 – 0.06 at 277 K to 0.08 –0.012 at 80 K. Light-induced decrease of fluorescence intensity declined from 50-60 % at 277 K to 2-4 % at 80 K. It shows that fluorescence changes were depressed at low temperature to much higher extend (~20 times) than the absorbance change (~5 times). Temperature dependence of the fluorescence quenching normalized to the absorbance chance at 681nm is depicted in Fig. 3. The decrease of this ratio upon sample cooling indicates decrease of the efficiency of fluorescence quenching upon $Pheo^-$ accumulation with decreasing temperature. Freezing of the sample during a light exposure (with $Pheo^-$ accumulated) resulted in a "freezing of the quenching state". The resulted fluorescence quenching at 80 K was comparable or even higher ($\Delta F/F=0.67$) than the quenching observed above 240 K (Fig. 4). The fluorescence quenching

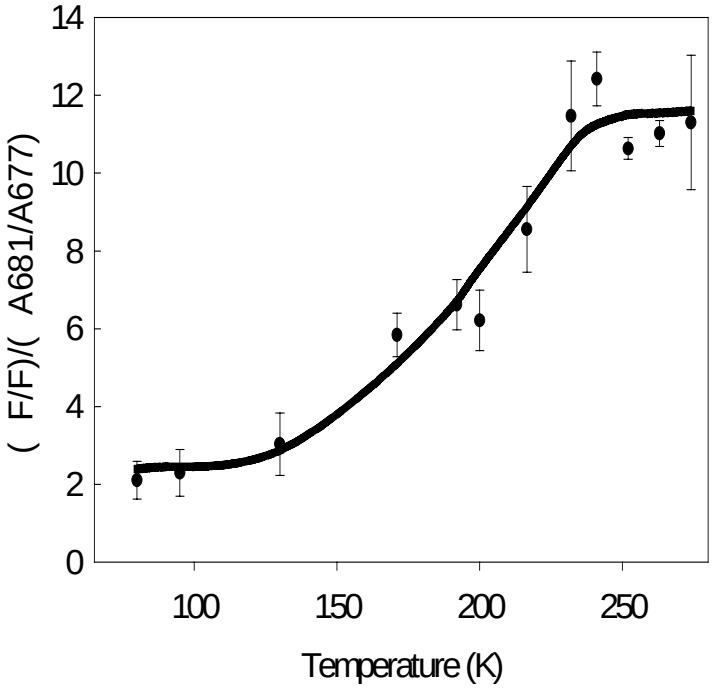

Fig. 3. Temperature dependence of the fluorescence quenching normalised to the absorbance change at 681 nm. Fluorescence quenching was calculated as $(F_{DARK} - F_{LIGHT})/F_{DARK}$. Absorbance change, $\Delta A_{(681-715)}$ was normalised to the red absorption maximum at 677 nm for each sample and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

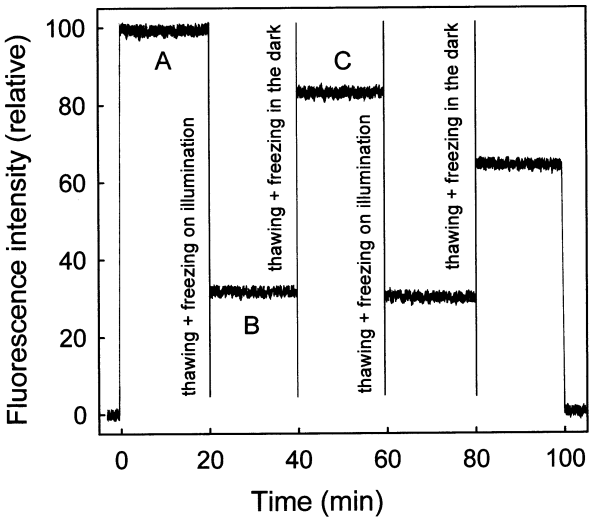

Fig. 4. 77 K fluorescence quenching upon accumulation of reduced Pheo at -10 °C. First the fluorescence intensity was measured at 77. Afterwards the sample was thawed to -10 °C, irradiated for 10 s to accumulate reduced Pheo and then, upon irradiation, quickly frozen to 77 K by pooling liquid nitrogen onto the sample. At the end, the sample was warmed to -10 °C, to allow its relaxation, and frozen again to 77 K in the dark. This cycle was repeated two times. Fluorescence was measured above 650 nm.

was reversible upon warming up the sample. It shows that the depression of the quenching efficiency at low temperature does not results from temperature dependence of the fluores-cence quenching itself but it is related to the inhibition of the formation of a "quenching state" produced upon $Pheo^-$ accumulation at high temperature. It indicates that some changes in reaction centers enhancing the fluorescence quenching accompany the $Pheo^-$ accumulation above 240 K.

Similar temperature dependence as for the efficiency of fluorescence quenching was found for light-induced changes of circular dichroism in the region of chlorophyll *a* absorption. The changes were measured on same preparation of reaction centers in the presence of dithionite (see Vácha et al., this volume). This indicates a conformation change accompanying $Pheo^-$ accumulation in reaction centers.

Acknowledgements

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