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Time-resolved monitoring of flash induced changes of fluorescence quantum yield in spinach thylakoids

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Introduction

Measurements of the chlorophyll fluorescence provide an invaluable noninvasive tool for analyses of the functional properties of the photosynthetic apparatus in algae and plants (for a review see Renger and Schreiber 1986). The emission at room temperature mainly originates from Photosystem II and its yield depends on the strength of the photochemical and/or nonphotochemical quenching. Accordingly the population dynamics of these quenchers can be investigated by monitoring time resolved changes of the fluorescence quantum yield. After excitation with a short laser flash the primary radical pair $P680^{+\bullet}Pheo^{-\bullet}$ is formed and stabilized by rapid electron transfer from $Pheo^{-\bullet}$ to Q_A via a 300 ps kinetics (Eckert et al. 1988). The cationic and anionic chlorin radicals are strong nonphotochemical quenchers (Butler 1972, Klimov et al. 1977, Renger and Kayed 1987) while Q_A acts as a powerful photochemical quencher (Duysens and Sweers 1963). As a result of these properties the fluorescence quantum yield at about 1 ns after a strong actinic flash owing to population of the radical pair state $P680^{+\bullet}PheoQ_A^{-\bullet}$ is almost the same as before the excitation in state $P680PheoQ_A$. In addition to the components $P680^{+\bullet}$, $Pheo^{-\bullet}$ and Q_A also carotenoid triplets (^{3}Car) formed by rapid transfer from chlorophyll tripletts (^{3}Chl) (Schödel et al. 1998) act as strong quenchers (Schödel et al. 1999). Therefore, reduction of $P680^{+\bullet}$ by Y_Z (but not the recombination with $Q_A^{-\bullet}$) and ^{3}Car decay give rise to a fluorescence increase. The superposition of these contributions can be used to resolve the kinetics of both reactions as outlined in Steffen et al. (2001). The development of a new home built set-up offered the possibility to analyze the kinetics of $P680^{+\bullet}$ reduction with a time resolution of about 100 ns thus permitting a separation of the contributions of "fast" and "slow" components to the overall reaction in the ns time domain (Steffen et al. 2001). The present communication describes results obtained for the pH dependence of $P680^{+\bullet}$ reduction in isolated spinach thylakoids and transient ^{3}Car population in solubilized LHC II.

Materials and Methods

Thylakoid membranes and solubilized LHC II preparations were isolated from local market spinach as described by Winget et al. (1965) and Irrgang et al. (1988), respectively. All measurements were performed at room temperature in a 50 mM buffer medium (succinic acid for pH 4 to 4.5, MES for pH 5.5 to 6.5 and HEPES for pH 7.5 to 8) containing 10 mM NaCl and the sample material at a chlorophyll concentration of 10 µg/ml for thylakoid preparations and 5 µg/ml for LHC II. The samples in the cuvette were excited by actinic flashes (λ = 532 nm and FWHM = 10 ns) from a frequency doubled NdYAG laser with a maximum laser pulse energy of about 1.1 mJ/cm^2. The fluorescence transients were monitored with the

microchannel plate photomultiplier tube R5916U-51 from Hamamatsu with a red sensitive photocathode (multialkali element). The time course of flash induced changes of the relative fluorescence quantum yield was obtained by a method where signal differences were recorded. In the first run, the fluorescence emission caused by two arrays of LED's (λ_{max} = 660 nm) and passing through a cut-off filter (DT cyan spezial, $\lambda < 700$ nm) was monitored and stored followed by a second run with the LED's turned off. The difference between both signals reflect the flash induced fluorescence quantum yield as outlined in Steffen et al. (2001). The detector was gated in order to permit the monitoring of fluorescence signals within three different time domains with respect to the onset of the laser flash (see Figure 1). Data acquisition was performed in a digital storage oscilloscope with an electrical bandwidth of 100 MHz. The fluorescence emission collected at an angle of 90° with respect to the incident laser beam is passed through a 532 nm laser blocker and funneled into the detector unit by means of a light guide. A 730 nm interference filter in combination with a 685 nm long-pass filter was used to separate the fluorescence signal from the measuring light.

Results and Discussion

Figure 1 shows the traces of changes of the relative fluorescence quantum yield induced by a train of four laser flashes in dark adapted spinach thylakoids suspended in buffer solutions of different pH. The signal of each flash divides into three parts: a first section of about 2 µs determines the F_0-level of dark adapted samples followed by the second and third sections with time windows of about 5 µs and 2 µs, opening at 100 ns and 42 µs, respectively, after excitation with the actinic laser flash. An inspection of this data reveals several characteristic features. At 100 ns after the flash a kinetically unresolved positive change of the relative fluorescence quantum yield ΔF(100 ns) is observed at pH 6.5 and 8.0 whereas at pH 4.0 this change is of opposite sign. The extent of ΔF(100 ns) exhibits a striking oscillation as a function of the actinic flash number at pH 6.5 that is less pronounced at pH 8.0. In marked contrast, the "instantaneous" decrease ΔF (100 ns) at pH 4.0 is virtually independent of the flash number.

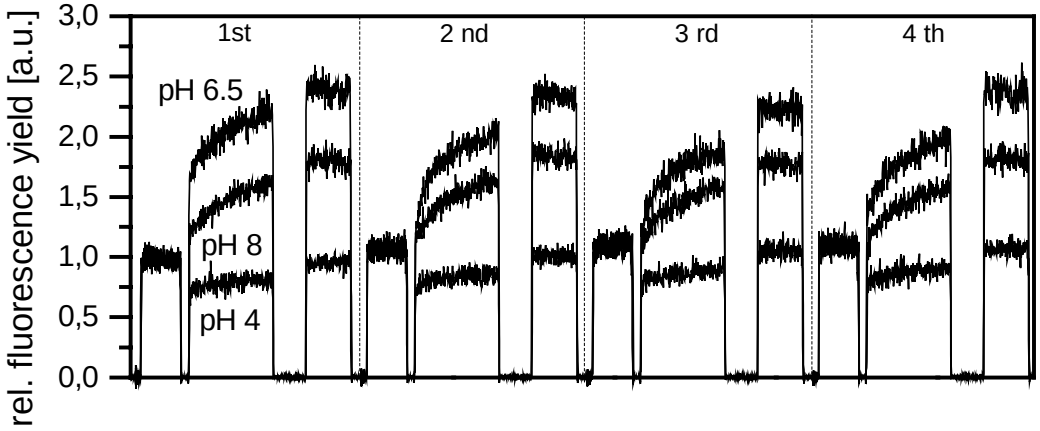

Figure 1. Changes of relative fluorescence quantum yield induced by a train of actinic laser flashes at different pH of the suspension. Each signal is an average of 80 measurements. The F_0 level of the first flash is normalized to 1.0.

These phenomena are explainable by the superposition of two reactions, i.e. in case of dominating $P680^{+\bullet}$ reduction by Y_Z via the "fast" ns kinetics with τ of about 30 ns a positive ΔF(100 ns) is observed, while in the opposite case of preponderance of ^{3}Car triplet formation ΔF(100 ns) is negative (for a discussion, see Steffen et al. 2001). Accordingly, the traces

measured at pH 4 reveal that the contributions owing to "fast" $P680^{+\bullet}$ reduction are negligibly small. At all pH values ΔF(100 ns) (positive or negative) is followed by a rise kinetics in the µs time domain that is the superposition of kinetics owing to " slow" ns-$P680^{+\bullet}$ reduction and ^{3}Car decay. A closer inspection reveals that the overall kinetics is faster at pH 6.5 and 8.0 than at pH 4 because in the latter case only the markedly slower ^{3}Car decay is responsible for the fluorescence rise between 100 ns and 5 µs. This feature perfectly fits the conclusion gathered from the ΔF(100 ns) data (vide supra). The levels reached at about 45 µs, ΔF(45 µs), are also strongly dependent on pH. The extent of ΔF(45 µs) is assumed to reflect the population of state $P680Q_A^{-\bullet}$ because the $P680^{+\bullet}$ reduction by Y_Z is almost complete and the extent of $Q_A^{-\bullet}$ reoxidation by Q_B comparatively small at pH 6.5 (for a discussion, see Christen et al. 1999). On the contrary, the ΔF(45 µs) at pH 4.0 is close to zero. Since in the time domain of about 45 µs the population of ^{3}Car is vanishingly small (Schödel et al. 1998) it has to be concluded that the state $P680Q_A^{-\bullet}$ is not formed to a significant extent, i.e. at pH 4 the reduction of $P680^{+\bullet}$ by Y_Z is not fast enough for efficient competition with the recombination reaction $P680^{+\bullet}Q_A^{-\bullet} \rightarrow P680Q_A$. The high retardation of $P680^{+\bullet}$ reduction by Y_Z cannot simply be explained by an elimination of the functional competence of the water oxidizing complex (WOC) because at pH 5.0 the reaction in tris-washed thylakoids was found to be of the order of 30 µs (Conjeaud and Mathis 1979). It rather shows that at pH 4 not only oxygen evolution is suppressed but also the $P680^{+\bullet}$ reduction by Y_Z becomes highly retarded. This conclusion is highly supported by our previous studies on flash induced absorption changes at 690 nm (Renger et al. 1977). In order to illustrate the effect of ^{3}Car formation and decay on the relative fluorescence quantum yield measurements were performed with solubilized LHC II complexes at different pH-values. Typical traces normalized on $F_0 = 1$ are shown in Fig. 2. Virtually no differences were observed and therefore only one curve is shown. The relaxation kinetics with $t_{1/2}$ of 3-5 µs is typical for the decay of ^{3}Car (Schödel et al. 1998). It seems most likely that analogous features also arise owing to ^{3}Car formation in thylakoids. Therefore the $P680^{+\bullet}$ reduction kinetics by Y_Z can be "extracted" from the overall fluorescence rise by subtracting the pH-independent contribution due to transient ^{3}Car population. The method described in this report will be used for further studies of $P680^{+\bullet}$ reduction kinetics in thylakoids and whole cells.

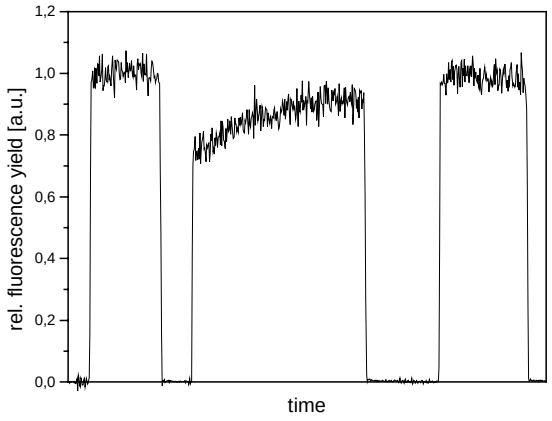

Figure 2. Laser flash induced changes of the relative fluorescence quantum yield of solubilized LHC II at pH 6.5. For the sake of clarity only one trace is shown because the curves are virtually the same at pH 4, 6.5 and 8.

Acknowledgement

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