Photosystem II from spinach can be driven by far-red light

Fredrik Mokvist, Anders Thapper, Stenbjörn Styring and Fikret Mamedov

Molecular Biomimetics, Department of Photochemistry and Molecular Science, Uppsala University, Ångström Laboratory, P.O. Box 523, S-751 20 Uppsala, Sweden,
Email: Fredrik.Mokvist@fotomol.uu.se

Charge separation in Photosystem II (PSII) using excitation light up to 800 nm has been reported using PSII enriched membranes and PSII core preparations from spinach [Thapper et al., (2009) Plant Cell 21, 2391-2401]. In this study fluorescence emission (between 650 and 730 nm) and excitation (measured at 685 nm, F685) spectra were recorded when excited (λex) with far-red light between 700 and 800 nm. In addition, charge separation related reactions and the $S_1 \rightarrow S_2$ transition using far-red wavelengths as λex has been studied by EPR spectroscopy.

Fluorescence excitation spectra recorded at room temperature revealed that detection limit for fluorescence emission is around 770 nm in both PS II membranes and PSII core preparations. The fluorescence intensity differs between preparations and, more interestingly, with excitation wavelength. When exciting from the blue-side (λex =440 nm), PSII cores F685 is 4 times more intense if compared to PSII membranes, but 7 times less when excited from the far-red-side (λex =750 nm). This shows the importance of the intact antenna in order to observe the far-red light induced fluorescence.

The fluorescence emission spectra recorded using λex <715 nm, were identical in shape at F685 peak when normalized and compared to the blue-side induced emission spectra using λex =440 nm. Time resolved fluorescence decay measurements were also performed using 750 nm light and no fundamental difference could be established if compared to 640 nm excitation, in terms of number of time-constants.

The temperature dependence between 295 K and 77 K for F685 and $S_1 \rightarrow S_2$ transition was also studied in PSII membranes using far-red excitation. In fluorescence measurements the emission intensity at 77 K was 150 times lower if compared to intensity at room temperature. When comparing the emission intensity at F685 and F695 at 77 K the intensity was found to be much stronger at F695 upon far-red excitation revealing a biased energy transfer. The emission was completely eliminated >735 nm in spite of their non in-common emission origin. The shape of excitation spectra was changing with increasing temperature – a weak band was appearing at 710-715 nm between 180 K and 240 K. Thus, temperature dependence of the far-red induced fluorescence shows no similarities with temperature dependence of the blue-side induced fluorescence.

Temperature dependence of the charge separation and the $S_1 \rightarrow S_2$ transition in PSII induced by the far-red light is also investigated. The half-inhibition temperature ($T_{1/2}$) for the $S_1 \rightarrow S_2$ transition induced by 532 nm and 750 nm light differs significantly. The 532 nm induced transition have a $T_{1/2}$ of 135 K [Styring et al., (1988) BBA 933, 378-387] while the corresponding value for 750 nm induced transition is 200 K. Upon loss of $CaMn_4$-cluster oxidation a small oxidation of Cytochrome b_{559} is observed, as a sign of charge separation and sustained functionality of the reaction center. At 5 K, split S_1 can be induced with 730 and 750 nm light. Further experiments are underway to clarify these phenomena.