TIME-RESOLVED FLUORESCENCE MEASUREMENTS OF PHOTOSYSTEM 1 FROM *SYNECHOCYSTIS* PCC 6803

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1.Introduction

The PS 1 core particle is a large pigment-protein complex that binds both antenna and reaction center (RC) and contains approximately 100 chl *a* in total. The antenna chls can be excited by (sun)light and transfer the energy to the the primary electron donor P700 from which a charge separation can take place.
It is presently unclear if the rate at which excitations are converted into a charge separation is limited by the intrinsic rate of charge separation (trap limited model) or if the rate at which excitations are transferred between antenna chlorophylls is limiting (diffusion limited model). The structure recently obtained by X-ray crystallography[1] shows that the RC is positioned relatively isolated from the bulk antenna, and therefore two distinctly different distance scales are present in PS1: the interpigment distance within the antenna, which is small and the distance from the antenna to the RC, which is large. Therefore a third, transfer-to-the-trap, limited model was proposed which suggests that the rate of excitation transfer from the bulk antenna to the RC is limiting [2,3].
The PS I absorption spectrum is spectrally highly heterogeneous due to differences in pigment-pigment and pigment-protein interactions. The most striking example of this is given by one or more pools of so-called “red” or “long wavelength” chls present in PS I that absorb at energies lower than P700. The number, size and absorption maxima of these pools are highly species-dependent [4,5]. In PS I from *Synechocystis* PCC 6803 one pool of red pigments is present, consisting of 2 chls, with an absorption maximum of 708 nm at 4 K. These two chls are thought to form a strongly coupled dimer.[4]
The purpose of these red chl species is still unclear. It is possible that they help to guide excitations to P700 by providing a nearby trap; therefore it has been suggested that the “linker” chls (fig. 1) are the red chls. It is however also possible that the red pigments increase the cross-section for absorption of red light, or that they are involved in photoprotection.

2.Materials and methods

The sample was prepared basically as described in [6]. For the fluorescence measurements the sample was diluted to an OD_{680} of 0.6/cm.
10mM sodium ascorbate and 10 µM PMS were added to avoid accumulation of $P700^+$. 150-200 fs pulses at 400, 506, 660, 702 or 710 nm were used to excite the sample. The fluorescence was detected at right angle to the excitation using a Hamamatsu C5680 synchroscan streak camera with a Chromex 250IS spectrograph. The full-width-at-half-maximum of the overall time response of this system was 3 ps. The spectral resolution was 8 nm. The recorded streak images represent 315 nm in the spectral domain and 200 ps in the time domain. (1018 3 1000 pixels on the CCD).
To avoid multiple excitation of the sample it was contained in a spinning cell The pulse energy was reduced to 0.2-0.3 nJ, thus avoiding singlet-singlet annihilation. All measurements were analyzed using a model with parallel decaying components, which yields Decay Associated Spectra (DAS). In some of the measurements in which a scattering contribution was present, a pulse-limited contribution was included in the fit.

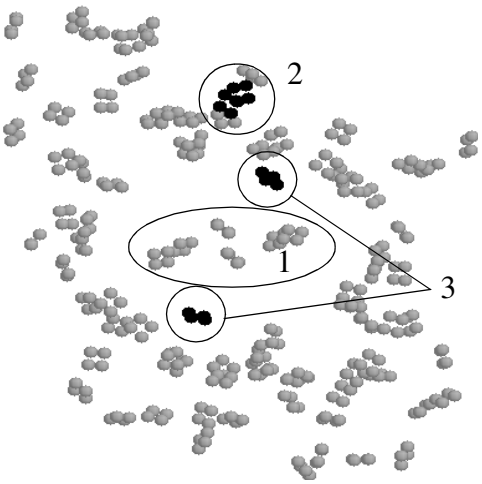

Figure 1. Topview of he positions of the chlorophylls in PS 1 according to [1]. Every 4 spheres represent the 4 nitrogen atoms to which the central Mg is ligated. 1: the reaction center, 2: the pair of chls used in the simulations (fig. 2 A' and C'), 3: the "linker" chls.

3.(sub) picosecond timeresolved fluorescence results

In fig 2A the Decay Associated Spectra (DAS) for 400 nm excitation of monomers are shown. The fastest component is a 0.7 ps ingrowth, which we attribute to Soret-Q_y relaxation. The second component is positive in the blue and negative in the red region of the spectrum and decays in 4.6 ps. This indicates a redshift of the fluorescence, caused by relaxation of the initially excited distribution to a more thermally equilibrated distribution. A similar contribution is present for 702 and 710 nm excitation (fig 2 B,C), but here the relaxation is much faster (0.9 - 1.3 ps) and mainly reflects a blueshift of the fluorescence.
For all wavelengths of excitation a spectrally identical all positive component is present decaying in 22.9-23.8 ps. This reflects trapping from the relaxed distribution. We like to point out that trapping also occurs on shorter timescales from the non-equilibrated distribution, which can account for the equilibration components being non-conservative.The 4.6 ps redshifting and 23 ps trapping components were observed before, in contrast to the much faster blueshifting component upon selective excitation of the red chls[7,8].
For 702 nm and, to a lesser extent, 710 nm excitation a second all-positive spectrum is present, decaying in ~11 ps, that to our knowledge has not been observed before. The

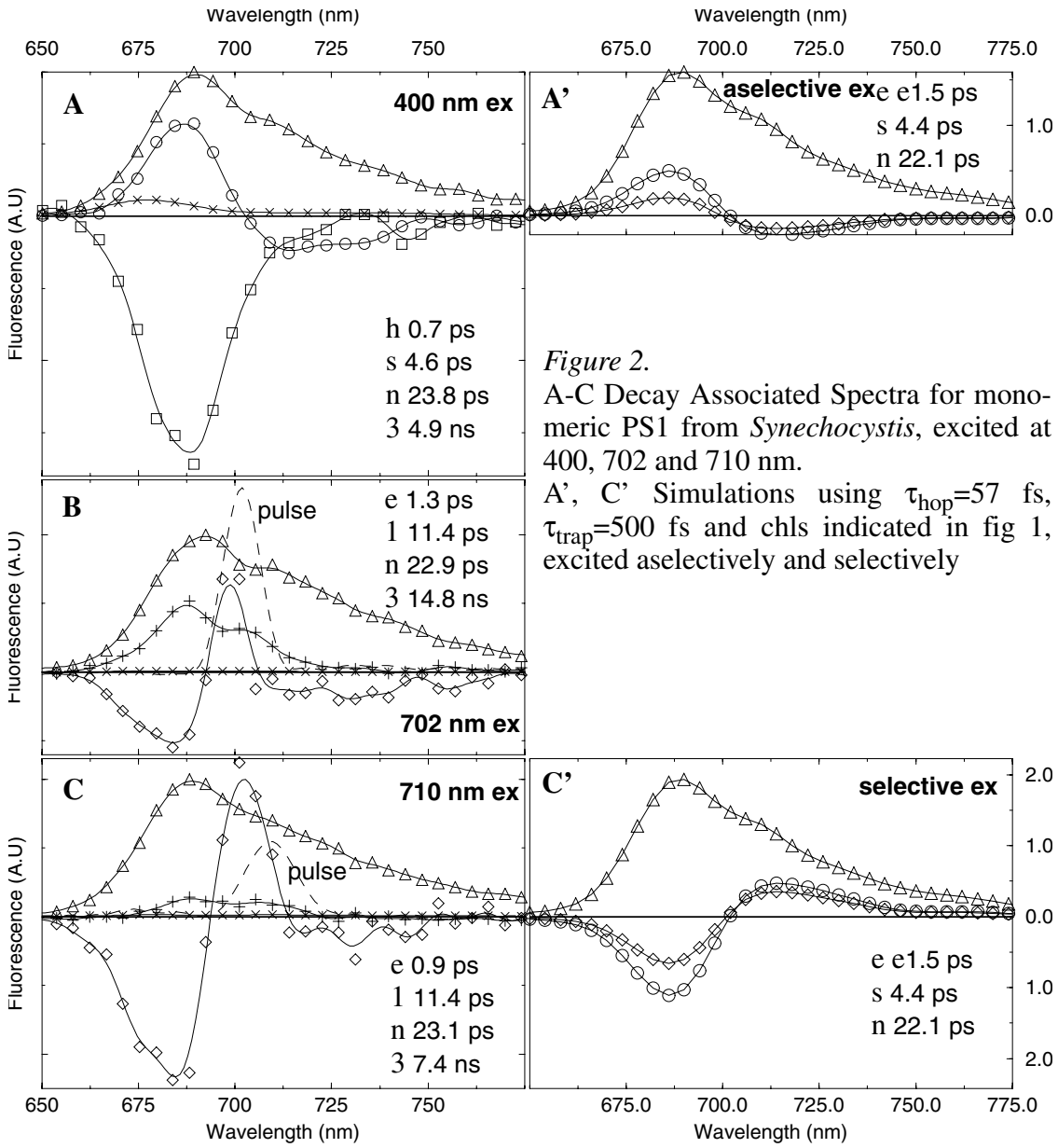

Figure 2.
A-C Decay Associated Spectra for monomeric PS1 from *Synechocystis*, excited at 400, 702 and 710 nm.
A', C' Simulations using τ_{hop}=57 fs, τ_{trap}=500 fs and chls indicated in fig 1, excited aselectively and selectively

exact nature of this component is presently unclear; it may involve fast trapping due to direct excitation of P700.
We note that results obtained for 506 and 660 nm excitation (not shown) are similar to those for 400 nm excitation and that results for the trimeric complexes show only small differences reflecting a slightly larger number of red chls. For all wavelengths of excitation a weak 4-15 ns contribution was present which we attribute to a small number of uncoupled chls present in the preparation.

4.Simulations

In order to understand the observed processes and the time scale on which they occur, we

performed a simulation calculating Förster transfer rates using the locations of the 89 chls found in the crystal structure. Overlap integrals were calculated from the absorption and emission spectra of chl *a* in solution as a function of the energy difference between donor and acceptor. All other factors in the Förster formula were contained in one parameter of the simulation, determining the median hopping time, τ_{hop}. The trapping time τ_{trap} from P700 was the only other parameter that was varied. All antenna pigments were taken to absorb at 680 nm, both chls representing P700 were taken at 700 nm, and two red chls were taken at 708 nm.

We calculated the eigenvalues of this system and their contributions to the dynamics. We found that for reasonable parameters τ_{hop}~50-100 fs and τ_{trap}~0.4-1 ps, we can reproduce the time-scales of ~23, ~4.5 and ~1 ps, the latter being the sum of 87 eigenvalues shorter than ~1.5 ps, which will produce one time constant with the time resolution of our equipment. This does however require that the red pigments are at some distance from the RC. If we take the "linker" chls (fig 1) to be the red chls it is not possible to reproduce the 4.5 equilibration and 23 ps trapping time constants simultaneously.

Although we find that the relative amplitude of the 1 ps component is larger for selective than for aselective excitation (fig 2 A', C'), it has not been possible so far to find a configuration of the red pigments in which the 4.5 ps component is dominant for aselective excitation and the 1 ps component is dominant for selective excitation of the red pigments.

If, for a certain realization of our model we make τ_{trap} infinitely short, we find that the 23 ps trapping time typically reduces only to around 15 ps, supporting the transfer-to-the-trap limited model.

We conclude that the transfer-to-the-trap limited model is the most appropriate view of energy transfer and trapping in PS 1 and that the red chls are not in direct contact with the RC.

5.References

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