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Fluorescence kinetics of Photosystem I core particles with different low energy chlorophyll content: A unified quantitative analysis

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Introduction

The PS I core is a large protein complex consisting of at least 11 protein subunits, the largest two of which, PsaA and PsaB, form a heterodimer to which the largest fraction of the core antenna Chls as well as most of the reaction center (RC) co-factors are bound. Structural data indicate that the core antenna and RC contain 96 chlorophyll *a* (Chl*a*) and 22 b-carotene molecules in total (Jordan et al., 2001).
The PS I core absorption spectrum is spectrally highly heterogeneous, and varies from species to species (van Grondelle et al., 1994). A conspicuous feature of all (intact) PS I cores is the presence of a relatively small number of red-shifted Chl*a* states that absorb at energies lower than that of the primary electron donor P700. It has been proposed that these low energy or "red" Chls represent closely coupled dimers or larger aggregates of Chl*a* (Gobets et al., 1994). The amounts and energies of these low energy Chls are highly species-dependent. We present room temperature time-resolved fluorescence data of five PS I core complexes containing different amounts of these long wavelength Chls, absorbing at different energies.

Materials and methods

The various PS I complexes were prepared as described in (Gobets et al., 2001). The samples, which were diluted to an OD_{680} of 0.6/cm, were contained in a spinning cell. The samples were excited using ~100 fs pulses at 400 nm with a 100 kHz repetition rate. Fluorescence was detected at magic angle (54.7^{o}) with a Hamamatsu C5680 synchroscan streak camera and a Chromex 250IS spectrograph. The full width at half of the maximum of the overall time-response of this system was 3-3.5 ps. The spectral resolution was 8 nm. All experiments were performed at room-temperature (293 K). All measurements were analysed using a model with a number of parallel compartments, which yields Decay Associated Spectra (DAS). A target analysis of the data (Holzwarth, 1996) yielded the Species Associated Emission Spectra

(SAES) of the different pools of red Chls. In order to obtain these spectra a constraint had to be put on the SAES of the long wavelength Chls which were put to zero at wavelengths shorter than ~680 nm (1st pool) and ~690 nm (2nd pool).

Results

Absorption spectra. In figure 1 the 6K absorption spectra of the various investigated PS I particles are displayed. In the region below 700 nm, which is dominated by the absorption of the bulk antenna Chls, the spectra of all particles are very similar. However, pronounced differences between the various PS I species are observed in the region above 700 nm, where the absorption of a relatively small number of red Chls dominates the spectra. Decomposition of the red part of the spectrum using Gaussian bands revealed that all these PS I species exhibit a red Chl pool with an absorption maximum at 708 nm (C708), and that the PS I from *Synechococcus* and *Spirulina* exhibits a second pool of red Chls peaking either at 719 nm (C719, *Synechococcus* trimers and *Spirulina* monomers) or 740 nm (C740, *Spirulina* trimers) (Gobets et al. 2001).

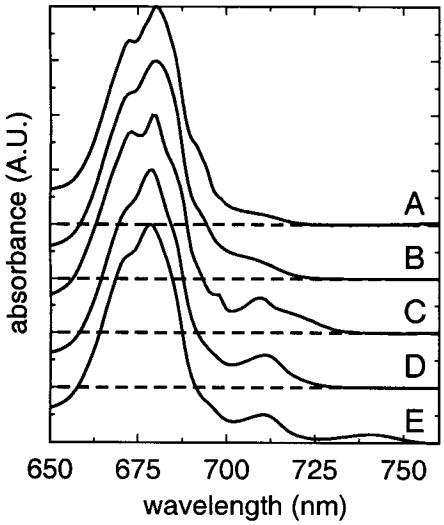

Figure 1. 6K absorption spectra of A) *Synechocystis* monomeric, B) *Synechocystis* trimeric, C) *Synechococcus* trimeric, D) *Spirulina* monomeric and E) *Spirulina* trimeric PS I core particles.

Time-resolved fluorescence measurements.

The Decay Associated Spectra resulting from the global analysis of the measurements of the 5 different PS I core particles are presented in figures 2a-e. With 400 nm light, initially the Chl*a* Soret band is excited, which relaxes to the Chl*a* Q_y state in a few hundred fs. In all PS I particles investigated in this study this is evident from a 0.4-0.9 ps component which exhibits an all-negative DAS (fig 2a-e, dotted). In all PS I particles one or two components are observed which are represented by a DAS which is positive at relatively short wavelengths and negative in the region of red Chl emission, and which reflect the transfer of excitation energy between the bulk and red Chl pools in the PS I antenna. The fast 3.4 to 4.7 ps equilibration process present in all PS I particles predominantly reflects energy transfer from the bulk Chls to the first (C708) pool of red Chls (fig 2a-e, dashed). In the PS I particles that exhibit two pools of red Chls, a second 9.6 to 15 ps equilibration process is observed. These components are highly non-conservative (fig 2c-e, dot-dashed), and represent mainly energy transfer from the bulk and first (C708) red Chl pool to the second red Chl pool (C719 or C740), as well as a considerable amount of non-equilibrium trapping. For all PS I particles a so-called trapping component is observed which exhibits a DAS that is clearly positive at all wavelengths (fig 2a-e solid). Both the trapping life-time, which varies between 23 and 50 ps, and the trapping spectrum depend strongly on the numbers and energies of the red Chls present in the various PS I particles, reflecting the differences in the equilibrium distribution of excitations, and the slower (uphill) energy transfer from the red Chls to P700. In all preparations a small decay component is found with a life-time of about 5 ns which

is assigned to a fraction of Chls in the preparation that is not attached to the protein. Hence, these slow components do not express a process in the intact systems.

Discussion

We further explored the differences and similarities between the different PS I species by performing a target analysis (Holzwarth, 1996) of the datasets using the model shown in figure 3. The model consists of 5 compartments, representing the Soret state (S) of all spectral forms, and the Q_y states of the bulk Chl pool (B), two red shifted Chl pools (1 and 2) and a pool accounting for free Chls that are not connected to the PS I core (F). For the modelling of *Synechocystis* PS I one of the red pools was omitted from the scheme. Some constraints had to be put on both the rate constants in the model and the spectra of the red Chl pools (Gobets et al., 2001). Under these constraints the target analysis always converged to a unique optimal solution.

SAES. The target analysis yields the individual SAES of the Chl pools of the different PS I particles. It was found that the spectra of each of the bulk, C708, C719 and C740 pools were practically identical for each of the PS I particles that contained them (Gobets et al., 2001). The average spectra of these pools are displayed in figure 4. The bulk spectrum (solid) peaks at 688 nm, and exhibits a spectrum similar to that of the free Chls (dotted) albeit 12 nm red shifted. The RT SAES of the C708 (dashed), C719 (dot-dashed) and C740 (double-dot-dashed) pools peak at 712, 722 and 733 nm, respectively, and are considerably broader than the bulk SAES reflecting the large (in)homogeneous broadening of these pools.

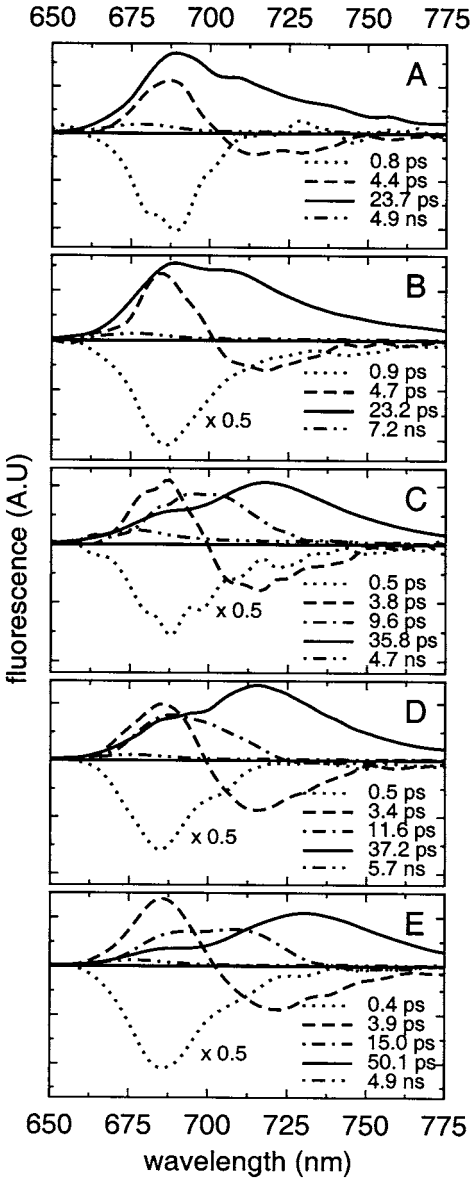

Figure 2. Decay Associated Spectra of fluorescence decay of different cyanobacterial PS I core particles upon excitation at 400 nm. A) monomeric PS I core of *Synechocystis*, B) trimeric PS I core of *Synechocystis*, C) trimeric PS I core of *Synechococcus*, D) monomeric PS I core of *Spirulina*, E) Trimeric PS I core of *Spirulina*.

Energy transfer and trapping.

The target analysis also yields the values of the various rate constants in the model. Using the concept of detailed balance, the rate constants of energy transfer between the red and the bulk Chl pools can be combined with the stoichiometric estimates of each pool (as obtained from the 6K absorption spectra) to estimate the room temperature absorption maxima of the various red pools (Gobets et al., 2001). It was found that at room temperature the C708, C719 and C740 pools absorb around 702, 708 and 715 nm respectively. Clearly all pools are significantly blue shifted at room temperature as compared to their 6 K absorption maxima, indicating a strong coupling of these Chls to their local environment.

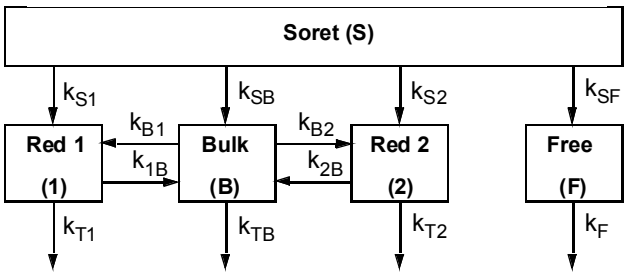

Figure 3. Compartmental model describing the kinetics in PS I

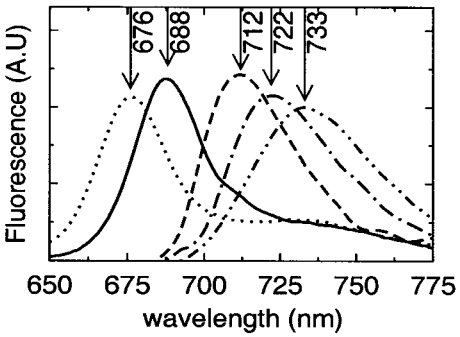

Figure 4. Average Species Associated Emission Spectra (SAES) of the Chl pools in figure 3. Solid: Bulk Chls, dashed: Red 1 (C708), dot-dashed: Red 2 (C719, in *Synechococcus* trimers and *Spirulina* monomers), double-dot-dashed: Red 2 (C740, in *Spirulina* trimers), dotted: Free.

It was found that the dynamics in all five PS I particles could be described using the same value for the bulk trapping rate k_{TB} of 18 ps^{-1}, indicating that all the observed differences in the kinetics is solely due to the differences in the amounts and energies of the red Chls between the different PS I species (Gobets et al., 2001). A comparison of the trapping rates k_{T1} and k_{T2} with k_{TB} and the relative overlap integrals of the bulk and red Chl emission spectra with the spectrum of P700 indicated that the C708 pool is located on an intermediate distance from the RC, whereas the C719 and C740 pools may occupy a more remote location in the structure (Gobets et al. 2001).

Acknowledgments

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