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Triplet States in Photosystem I Complexes from *Synechococcus elongatus*

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Introduction

Photosystem I of plants, algae, and cyanobacteria is a membrane-bound pigment-protein complex that mediates light-induced electron transfer from plastocyanin or cytochrome c_6 on the lumenal side to ferredoxin on the stromal side (for review, see Brettel, 1997). The recently published X-ray structure of photosystem I from *Synechococcus elongatus* at 2.5 Å resolution (Jordan et al. 2001) identifies twelve protein subunits, 96 chlorophylls (Chls), 22 carotenoids (car), two phylloquinones, three [4Fe-4S] iron sulfur clusters, four lipids, about 200 water molecules and a metal ion (presumably Ca^{2+}). The two largest subunits, PsaA and PsaB, bind most of the antenna pigments and the following redox cofactors involved in the electron-transfer process: the primary electron donor P700, an excitonically coupled heterodimer comprised of chlorophyll *a* (P_B) and *a´* (P_A), the primary acceptor A_0 (a Chl *a* monomer), the secondary acceptor A_1 (a phylloquinone), and F_X (a). The terminal electron acceptors F_A and F_B (two [4Fe-4S] iron sulfur clusters) are both coordinated by subunit PsaC, one of the three extrinsic subunits located on the stromal side. Transfer and trapping of excitation energy via charge separation occurs in PS I from *Synechococcus el.* with 35 ps at room temperature. Charge stabilization is achieved by subsequent electron transfer to the secondary acceptor, the phylloquinone A_1 in about 30 ps.

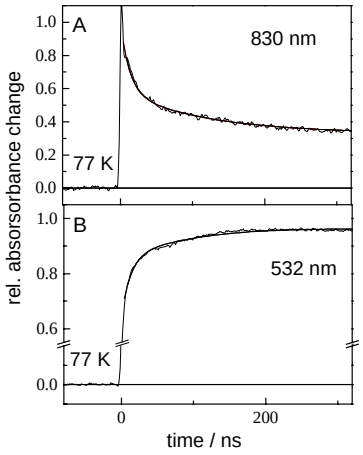
Materials and Methods

Trimeric PS I complexes with about 100 Chl/P700 were isolated from the thermophilic cyanobacterium *Synechococcus elongatus* as described by Fromme and Witt, (1998).
To study the triplet state of P700, PSI complexes were diluted to 15 µM Chl in 100 mM MES (pH 7) or CAPS (pH 10), 10 mM $MgCl_2$, 10 mM $CaCl_2$ and 0.02 % ß-DM. Glycerol was added to a final concentration of 65 % in order to obtain a transparent glass at low temperatures. 20 mM dithionite was added to this solution under argon. Before freezing in the cryostat, the samples were illuminated for a period of 5 min at 260 K with strong white light of a 250 W tungsten lamp filtered through water (10 cm). By this treatment prereduction of A_1 was achieved. When the electron transfer to A_1 is blocked, the primary radical pair, $P700^+A_0^-$, recombines with high yield to the triplet state of P700.
Very different sample conditions were used to study the dynamics of triplet excited states in the light harvesting system. These measurements were performed with "closed" PS I (P700 in the oxidized state) in order to prevent photochemistry. PSI complexes were diluted to about 70 µM Chl in 20 mM Tricine (pH 7.5), 20 mM $MgCl_2$, 0.02 % ß-DM, 65 % glycerol and 1 mM ferricyanide. Additionally, the samples were illuminated before freezing to achieve complete oxidation of P700.

Laser-flash induced absorption changes were measured as described previously (Schlodder et al., 1998). To follow the ^{3}Chl *a* $\rightarrow$ ^{3}Car in the ns time range, we used a cw laser diode (Hitachi HL 8318G) at 830 nm and a cw diode-pumped Nd:YAG laser (Coherent Compass 315M-20) at 532 nm as measuring light sources.The samples were excited by laser flashes of 3 ns pulse duration at 532 nm. The time course of the flash-induced absorbance changes was fitted to a (multi)exponential decay using an algorithm that minimizes the sum of the unweighted least squares.

Results and Discussion

Triplet excited states in the PS I antenna. The decay and rise of chlorophyll *a* and carotenoid triplet states were monitored at 830 nm and 532 nm, respectively. Fig. 1A shows the time course of flash-induced absorbance changes at 830 nm of PS I complexes with P700 oxidized at 77 K. In the presence of oxidized P700, photochemistry is blocked and the absorbance increase at 830 nm is attributed to the formation of ^{3}Chl *a*. In the antenna, Chl *a* triplet states arise from the lowest excited singlet state by intersystem crossing.

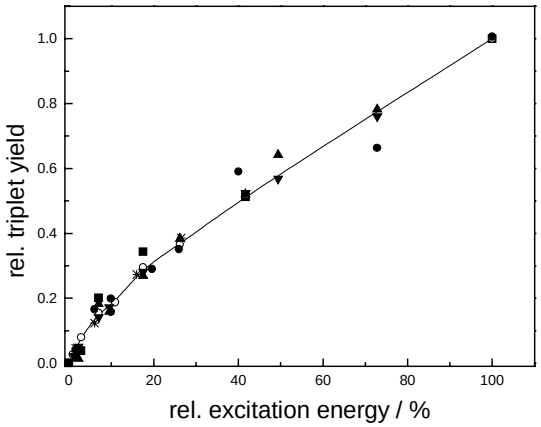


At least two exponentials are required for a satisfactory fit of the Chl *a* triplet decay on the depicted ns timescale. We obtained the following half-lives and relative amplitudes: $t_{1/2}$ ≈ 10 ns (60%) and ≈ 80 ns (40%).

Fig. 1: Flash-induced absorbance changes at 830 nm(A) attributed to the decay of ^{3}Chl *a* and at 532 nm (B) attributed to the rise of ^{3}Car

The absorbance change at 830 nm which is not decaying on the ns timescale is due to a phase with a half-life of about 180 µs (not shown). This phase can be attributed to the decay of $P700^+A_1^-$ indicating that a small fraction of P700 could not be oxidized. The rise of the carotenoid triplet state has been followed at 532 nm (Fig. 1B). The rise kinetics that is clearly resolved on the timescale depicted in Fig. 1B can be described using virtually the same half-lives as obtained for the Chl *a* triplet decay. The initial absorbance increase can be explained by the fact that excited states of Chl *a* also cause an absorbance increase at this wavelength. However, it can not be excluded that part of the carotenoid triplet formation is too fast to be resolved by our experimental set-up. Identical kinetics of the decay of ^{3}Chl *a* and the rise of ^{3}Car demonstrate that the carotenoid triplet is formed by a direct triplet-triplet exchange reaction with the Chl *a* triplet state. Within the error limits, the kinetics of the triplet-triplet energy transfer were found to be independent of temperature between 5 K and 300 K. The decay of ^{3}Car showed a weak temperature dependence. At T = 5 K, the decay kinetics are biphasic with half-lives of about 6 µs and 28 µs and relative amplitudes of 70% and 30%. Due to the slowing down of spin-lattice relaxation at 5 K, the triplet sublevels are uncoupled and the different decay kinetics from the zero-field sublevels can be partially resolved. Above 50 K, the decay was more monophasic with $t_{1/2}$ =7.1 µs at 77 K and 3.2 µs at 280 K.

Fig. 2 shows the triplet yield as a function of the excitation flash energy at low temperature.



With a molar extinction coefficient of 200000 $M^{-1}cm^{-1}$ for ^{3}Car at 532 nm (Land and Swallow, 1971), a maximum triplet yield of ≈ 0.5 carotenoid triplets per PS I complex at about 40 mJ/cm^2 (=100 %) has been calculated.

Fig. 2: Triplet yield as a function of the excitation flash energy at 77 K and 5 K. Measurements at 532 nm and 830 nm gave the same results for both temperatures.

The temperature dependence of the triplet yield resembles that of the fluorescence yield in trimeric PS I with P700 being oxidized (Pålsson et al., 1998; Byrdin et al., 2000). The triplet yield increases by factor of about 5 as the temperature is decreased from 290 K to 5 K. The half-maximum value is observed at ≈ 140 K. The increase of the fluorescence and the triplet yield upon lowering the temperature can be explained by the presence of "red" chlorophylls that absorb at wavelengths above 700 nm, i.e. at energies below that of the primary donor P700. Efficient uphill energy transfer becomes impossible at cryogenic temperatures and the excitation is trapped on the energetically lowest-lying Chls. In this case, the excited singlet states decay predominantly by fluorescence and intersystem crossing.

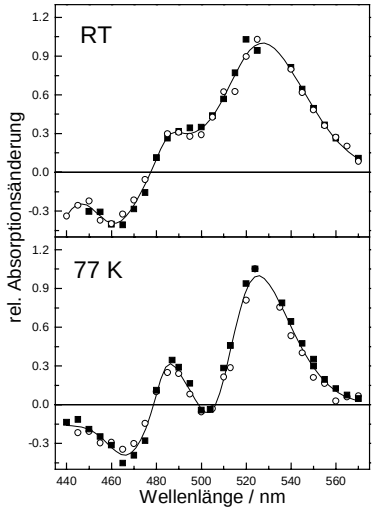


Fig. 3: Triplet-minus-singlet (T-S) absorbance difference spectra of the carotenoids. The absorbance changes decaying with $t_{1/2} \approx 3$ µs at 295 K and with $t_{1/2} \approx 7$ µs at 77 K have been measured as a function of wavelength.

The T-S absorbance difference spectrum of the carotenoids has been measured at 295 K and 77 K. The spectra exhibit the maximum triplet absorption for the ß-carotenes in PS I at 528 nm. The better resolved 77 K spectrum shows a positive band at 486 nm and smaller negative bands at 465 nm and 502 nm. The spectrum at room temperature (RT) resembles closely the T-S spectrum of all-*trans*-ß-carotene in hexane (Land and Swallow, 1971) except that the maximum is redshifted by about 13 nm in PS I.

Triplet state of P700. To study the triplet state of P700, measurements were performed with PS I complexes under reducing conditions with the secondary electron acceptor A_1 in the reduced state. Therefore, the electron transfer to A_1 is blocked and the primary radical pair, $P700^+A_0^-$, recombines to the triplet state of P700 with high yield. Fig. 4 shows the flash-

induced triplet-minus-singlet absorbance difference spectrum of P700 (dots) obtained with high spectral resolution at 5 K and, for comparison, the microwave-induced T-S spectrum of P700 in PS I from *Synechococcus elongatus* (solid line) obtained by an ADMR study (D. Carbonera and E. Schlodder, unpublished results).

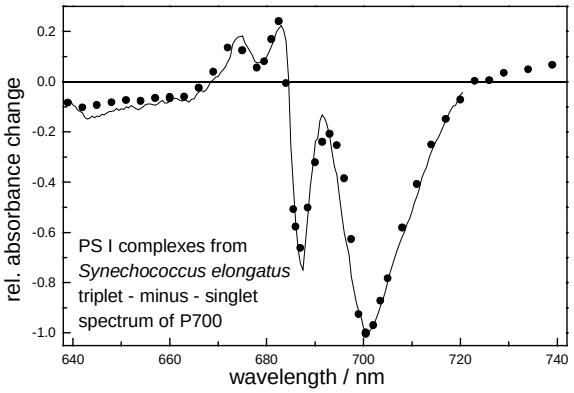


Fig.4: Spectrum of flash-induced absorbance changes at 5 K attributed to ^{3}P700 formation (dots) and the ADMR monitored T-S spectra of P700 at 1.8 K (solid line) (for experimental set-up, see Carbonera et al., 1997).

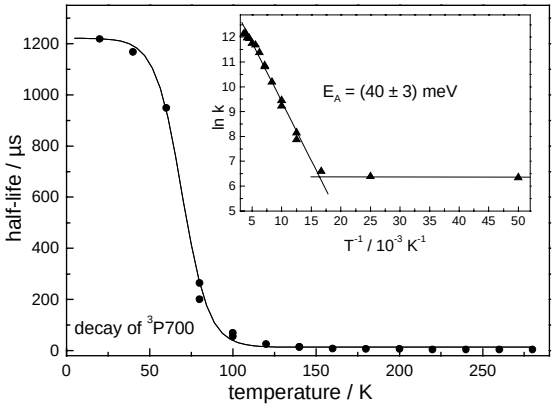


Fig.5: Half-life of the decay of ^{3}P700 as a function of temperature. The inset shows an Arrhenius plot of the decay rate.

The T-S spectrum of P700 shows the main bleaching at 701 nm which is attributed to the disappearance of the low-energy exciton band of P700. The sharp negative band at 687 nm and positive bands at 674 and 683 nm presumably reflect the loss of excitonic interactions with neighboring Chls upon the formation of the triplet state of P700.

Fig. 5 shows the half-life of the decay of ^{3}P700 as a function of temperature between 20 K and room temperature. At 5 K, the decay kinetics of ^{3}P700 are similar to that of the chlorophyll *a* triplet state *in vitro* ($t_{1/2} \approx 0,.7$ ms (65 %) and 7 ms (35%)) indicating that the triplet state is localized on one Chl *a* molecule, probably P_B (Krabben et al. 2000). Above 20 K, the decay could be fitted with a single exponential with $t_{1/2} \approx 1.1$ ms at 40 K, 260 µs at 80 K and 4.5 µs at 220 K. In the range between 60 K and 220 K, the data are reasonably described by a straight line yielding an activation energy of (40 ± 3) meV which is in good accordance with results obtained with SDS particles from tobacco (Mathis et al.,1978). The mechanism of the activated decay which is not observed for ^{3}Chl *a in vitro* remains to be elucidated.

Acknowledgements

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