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Energy transfer and trapping in Photosystem II core particles with closed reaction centers

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Abstract

Picosecond absorbance changes in the Q_y absorption region were measured on Photosystem II core particles with closed reaction centers by the one-color pump-probe method. The induced absorption changes are well described by three components with lifetimes of 21 ± 6 ps, 80 to 200 ps and 1.5 ns, in addition to a non-decaying component. The 1.5 ns lifetime component is assigned to recombination of the primary radical pair in equilibrium with the excited state. Since the lifetime of the intermediate component depends on the excitation wavelength and its spectrum differs from that of the 21 ps component, a rapid equilibration of the excitations over the whole antenna is excluded. The 21 ps and the intermediate component are discussed in terms of energy transfer and trapping processes.

Keywords: Picosecond transient absorption; Energy transfer; Trapping; Photosystem II; Core complex; Long-wavelength pigment

1. Introduction

In previous work [1] we have shown that in the isolated Photosystem II (PS II) reaction center (RC) the kinetics of excitation trapping depend on the wavelength of excitation: energy transfer from the 'short-wavelength pigments', with Q_y transitions around 670 nm, to the 'long-wavelength pigments', with Q_y transitions around 680 nm, was much slower than charge separation upon direct excitation of the long-wavelength pigments. We have argued [2] that such a slow energy transfer step would be inconsistent with the high quantum yield of intact PS II and is presumably bypassed by chlorophylls (Chls) of the core antenna transferring energy to the long-wavelength pigments more rapidly. In this context, a special role of the 690 nm Chl of the core antenna protein CP47 [3] was envisaged, mediating energy transfer from the core antenna to the 'active' pheophytin (Pheo) and hence to the primary electron donor, P680. In order to investigate these speculations, similar measurements of excitation-trapping kinetics in PS II particles retaining the core antenna would be needed.

Contrary to isolated reaction centers, core particles retain the secondary electron acceptor Q_A. The redox state of Q_A is of decisive importance for the fate of the charge separation. When neutral, Q_A can stabilize the charge separation by oxidizing $Pheo^-$ in about 300 ps [4,5] and fluorescence and absorbance measurements in the picosecond time domain on PS II core particles show biphasic kinetics [6–8]. Thermal equilibration of the excited state over the antenna pigments is thought to be very fast (< 10 ps [9]) and the first observed phase, with a time constant of 30–100 ps, is attributed to trapping, while the second phase, with a time constant of a few hundred of picoseconds, is assigned to electron transfer from $Pheo^-$ to Q_A.

When Q_A is in the reduced state, Q_A^-, no charge stabilization can occur and the primary radical pair, in equilibrium with the excited state, decays in about 2 ns to the ground state. In the ps time domain a single component of about 200 ps is observed and attributed to trapping, somewhat slowed down by the presence of Q_A^- [6,9].

The assumption [9] that thermal distribution of the excited state over the antenna pigments is attained in less than 10 ps implies that selective excitation of the various spectral forms should always lead to the same charge separation kinetics. Here we describe a first attempt to

Abbreviations: Abbreviations: Chl, Chlorophyll *a*; DCM, 4-(dicyanomethylene)-2-methyl-6-(*p*-dimethylaminostyryl)-4*H*-pyran; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; P680, primary electron donor of Photosystem II; Pheo, pheophytin *a*; PS II, Photosystem II; PS I, Photosystem I; Q_A, primary quinone electron acceptor.

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verify this prediction. We used spinach PS II core particles isolated according to van Leeuwen et al. [10], which have only about 35 Chl per RC. Q_A was kept in the reduced state (Q_A^-) so that electron transfer was limited to the formation and recombination of the primary radical pair, which processes were expected to take about 200 ps and 1.7 ns, respectively, as reported for PS II core particles from *Synechococcus sp.* with Q_A^- present [6]. Absorbance transients in this time domain were recorded upon monochromatic excitation at wavelengths from 670 to 706 nm with 7 ps flashes. Although so far only measurements at the wavelength of excitation have been carried out, the results clearly indicate that the charge separation is more rapid for selective excitation of the long-wavelength pigments than for that of the short-wavelength pigments.

2. Material and methods

PS II core complexes were isolated from spinach as described in [10]. The activity of the preparations was checked by measuring the oxygen yield and the photoreduction of Q_A. Before use the samples were diluted to an absorbance of about 1 at 675 nm in a buffer containing 20 mM Bis-Tris (pH 6.5), 400 mM sucrose, 5 mM $CaCl_2$ and 0.03% (w/v) *n*-dodecyl β-D-maltoside. Catalase, glucose and glucose-oxidase were added in that order to final concentrations of 65 μg/ml, 4 mM and 65 μg/ml, respectively, to remove oxygen. Presumably, no electron acceptor beyond Q_A is present in these particles [10], but 10 μM DCMU (final concentration) was added by way of precaution to ensure inhibition of the reoxidation of Q_A^-. All measurements were performed at room temperature.

Transient absorption differences were measured with a picosecond dye-laser system in the so-called one-color pump-probe configuration as described in [11]. In this configuration the sample is excited and probed at the same wavelength. The instrument response function, i.e., the width of the autocorrelation of pump and probe pulses, was 8 to 10 ps. The dye-laser was operated with DCM and was tunable from 668 to 706 nm. Fits to the measured kinetics were convolutions of exponential decays with the instrument response function, which was described by a squared secanthyperbolic function with an appropriate width. The fitting routine used the simplex algorithm.

The duration of a single laser pulse was about 7 ps with a spectral bandwidth of less than 1 nm allowing for selective excitation of the various pigments. Since the repetition rate of the dye laser is 76.6 MHz, the sample was placed in a rotating sample cell with a radius of 55 mm to avoid possible accumulation of triplet states. The sample cell rotated at 6000 rpm, and taking into account the spot diameter of 80 μm, a sample volume collected 80 pulses during one pass through the excitation beam. The time between successive passages of the same sample volume through the excitation beam, 10 ms, was short enough to avoid reoxidation of Q_A^-. The average excitation density was typically 2.5 μJ cm^{-2} per pulse, and the power of the probe beam was 30-times less. This excitation energy density corresponds to $(1.0 \pm 0.4) \cdot 10^{13}$ photons per cm^2 per pulse, resulting in the excitation of about 3.5% of the particles per pulse. Accumulation of the triplet state of P680 is not expected, taking into account its short lifetime and low yield [8].

According to Schatz et al. [6] an excitation energy-density of $0.7 \cdot 10^{13}$ per cm^2 per pulse or less should be used to avoid singlet-singlet annihilation in PS II core particles with Q_A reduced. However, the smaller antenna size of our particles, 35 vs. about 60 Chls, compensates the slightly higher excitation energy density used in our experiments. Furthermore, the most rapid component observed in our experiments was prominent at wavelengths above 700 nm where only 0.5% or less of the particles was excited per pulse. Thus, it is very unlikely that the fast component in our experiments is associated with singlet-singlet annihilation.

3. Results and discussion

The absorbance spectra of the PS II core complexes at room temperature and at 6 K are shown in Fig. 1. At room temperature the long-wavelength (Q_y) absorption band is broad and without any structure, with a maximum at 675 nm. At 6 K this broad absorption band is split into three bands with maxima at 669, 676 and 683 nm. The first two bands are mainly due to CP47 and CP43 [3,12], whereas the peak at 683 nm probably contains contributions from P680 [13] and the long-wavelength antenna Chls *a* of CP47 [3] and CP43 [12]. The Pheos *a* and accessory Chls *a* of the reaction center are expected to absorb at 675–680 nm and at 665–675 nm, respectively [13]. Although the individual absorption bands are not resolved at all at room temperature, some selectivity can be expected when tuning the excitation wavelength through the Q_y absorption band of the PS II core complexes.

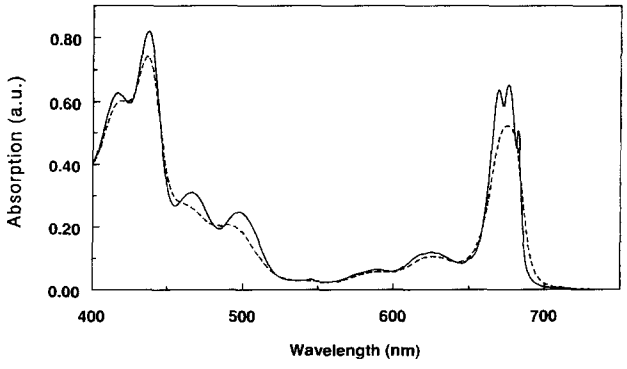

Fig. 1. Absorption spectrum of PS II core particles measured at room temperature (dotted line) and 6 K (solid line).

The transient absorption measurements were performed on PS II core particles in which Q_A was kept reduced (closed RC) by the excitation light. First of all, we checked if Q_A indeed remained reduced under these conditions. In fluorescence induction experiments on PS II core particles with continuous light we observed that the fluorescence rapidly increased to a maximum value and remained at that level, indicating that no protonation and double reduction of Q_A, resulting in its loss, occurred [14]. Also we found that under these conditions the reoxidation of Q_A^- took about two minutes. This time constant is in agreement with transient absorption measurements at 325 nm, at which wavelength the reoxidation of Q_A^- is observed. This implies that the rotational speed of the sample cell was high enough to keep all RCs closed even at excitation wavelengths where less than 0.5% of the particles was excited per pulse. Also, no differences were observed between ps kinetics measured with or without background illumination, confirming that the excitation light alone was already sufficient to close all RCs.

Fig. 2 shows transient absorption changes of PS II core particles with Q_A^-, excited and detected at 674 (A), 682 (B), 690 (C) and 700 nm (D), respectively, with parallel polarization between pump and probe beams. These changes and those at other wavelengths in the range of 670 to 706 nm could be very well fitted by a stable component and a set of three exponentials with lifetimes of 13–30 ps, 80–200 ps and 1.5 ns, the latter time constant being kept fixed. The lifetime of the intermediate component decreased from 200 ps around 670 nm to about 80 ps at wavelengths above 700 nm. The lifetime of the fast component did not show any clear dependence on the excitation wavelength. When the fit results obtained at different wavelengths are averaged, the lifetime of the fast component is 21 ± 6 ps.

When all traces were fitted with a 21 ps, a variable intermediate lifetime, a 1.5 ns component and a stable component, the amplitudes shown in Fig. 3 were obtained. A and B show the amplitude for parallel and perpendicular polarization between the pump and probe beam, respectively. The amplitudes are normalized to the amount of absorbed photons and because of the low absorption of PS II core at wavelengths longer than 700 nm the amplitudes in that region may be less reliable. Although the amplitudes are plotted as spectra, it should be noted that in general one-color pump-probe experiments cannot be used to obtain the spectrum of a kinetic component, unless that component is observed after the equilibrium distribution of the excited states over all pigments has been established.

The equilibrium condition may well be met for the 1.5 ns component. Its spectrum is similar for parallel and perpendicular polarization, suggesting that it is also not affected by photoselection effects, and resembles that of a corresponding lifetime component observed in dual wavelength measurements [4,6]. It is attributed to recombination of the primary radical pair in equilibrium with the excited state. A remarkable feature of the 1.5 ns component is its absence at longer wavelengths (695–700 nm). This suggests a radical pair-excited state equilibrium at which the 695 nm absorbance changes of $P680^+Pheo^-$ recombination and excited-state decay (including stimulated emission) compensate each other.

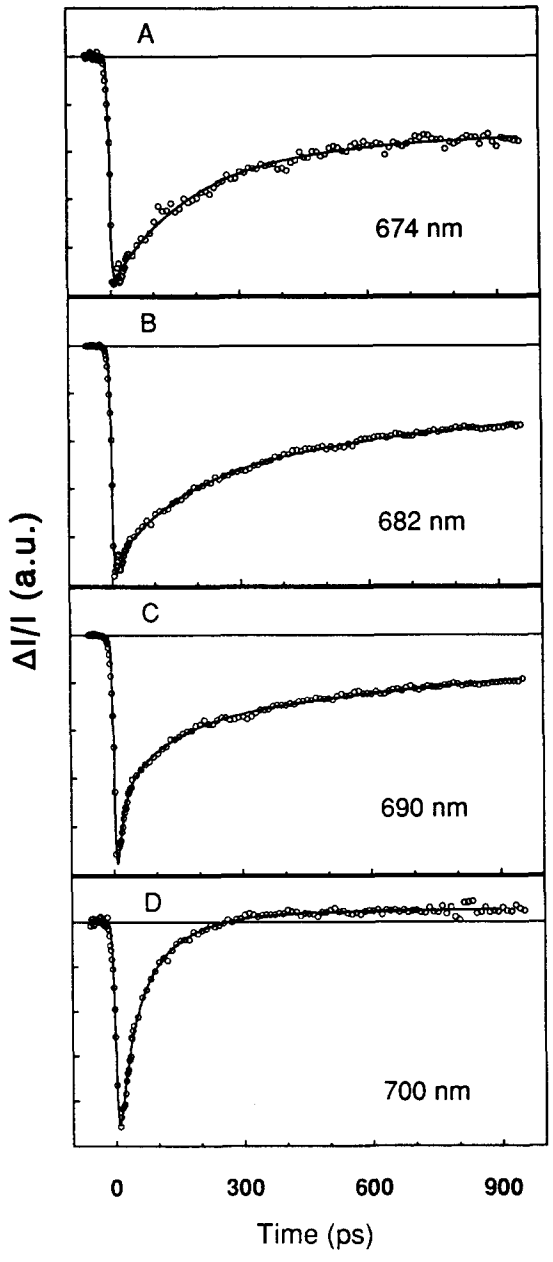

Fig. 2. Transient absorption changes in PS II core particles in the presence of Q_A^- at room temperature induced and detected with pump and probe parallel at 674 (A), 682 (B), 690 (C) and 700 nm (D). The data are plotted as circles and the solid line represents a fit of an offset and three exponentials, one of which had a fixed lifetime of 1.5 ns. The following time constants, with their relative contribution to the total decay given between brackets, were found: at 674 nm: 23 ps (0.01), 182 ps (0.75) and 1.5 ns (0.24), at 682 nm: 16 ps (0.15), 174 ps (0.39) and 1.5 ns (0.46), at 690 nm: 13 ps (0.43), 110 ps (0.24) and 1.5 ns (0.32), and at 700 nm: 29 ps (0.49), 95 ps (0.47) and 1.5 ns (0.04).

The end level of the absorption changes (triangles in Figs. 3A and B) also seems to be independent of the polarization of the probe beam. In both cases it has a significant (but small) contribution only at wavelengths shorter than 680 nm. Van Mieghem et al. [8] assigned a 7 ns fluorescence component observed in PS II core particles with closed RCs to radical pair recombination in part of the RCs. However, in our measurements the maximum of the end level is around 670 nm, and clearly not around 682 nm. Therefore, it is more likely that the end level in our measurements is caused by a triplet or excited state of chlorophyll uncoupled from the energy transfer process, and does not involve P680.

The amplitudes of the fast (21 ps, solid circles) and the intermediate (80 to 200 ps, open circles) components are also shown in Fig. 3. In view of the presence of at least two components and the wavelength dependence of the lifetime of the intermediate component, which is shown in Fig. 4, thermal equilibration of the excitations in less than 10 ps, as postulated by Schatz et al. [9], can be excluded. These components are responsible for the complete decay of the absorbance changes at wavelengths longer than 695 nm. At wavelengths above 700 nm the initial absorbance change is larger than can be explained by ground-state bleaching, and therefore we attribute part of the change to

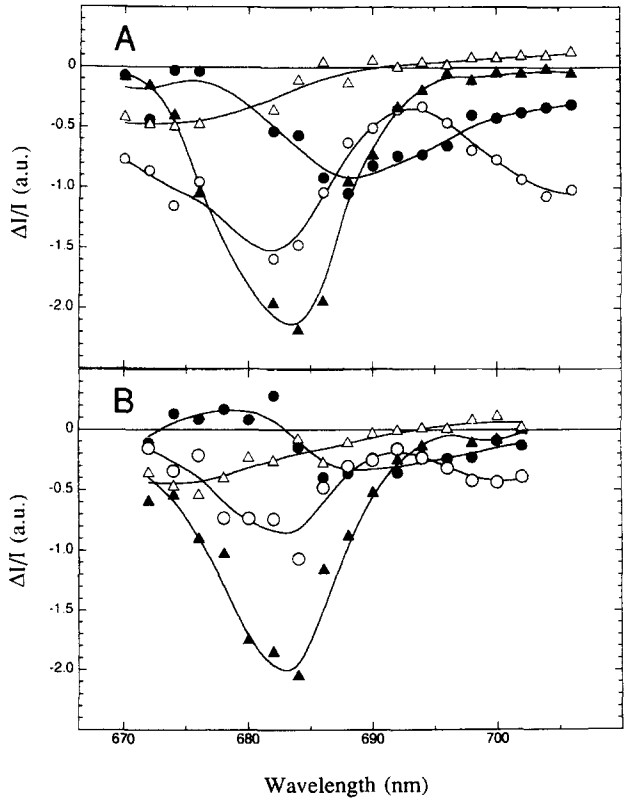

Fig. 3. Contributions of the 21 ps (●), 80 to 200 ps (○), and 1.5 ns (▲) components and the end level (△) to the absorption changes induced and probed with pump and probe either parallel (A) or perpendicular (B) in the Q_y absorption band of PS II core particles in the presence of Q_A^-.

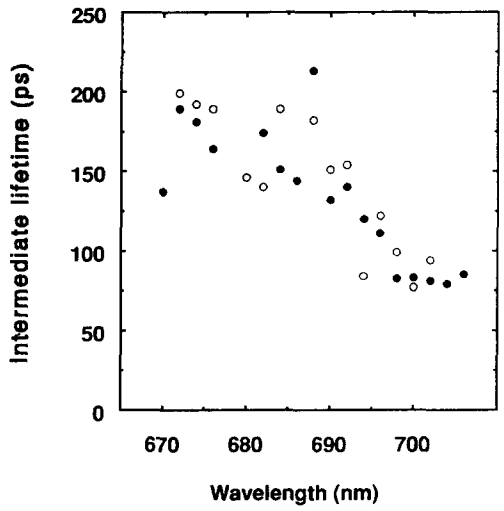

Fig. 4. Wavelength dependence of the lifetime of the intermediate component that is obtained when all absorption changes are fitted with fixed 21 ps and 1.5 ns components, the intermediate and a non-decaying component. Pump and probe beams parallel (●) and perpendicular (○).

stimulated emission. Hence, we attribute the fast and intermediate components to processes by which excitations are redistributed or trapped.

Schatz et al. [6] used 675 nm excitation and found trapping to take about 200 ps in the presence of Q_A^-. This is not inconsistent with our data, but we find that the lifetime ranges from about 200 ps at 670 nm to about 80 ps beyond 700 nm. Without normalization to the amount of absorbed photons, the 80 ps component was very small. At present we cannot guarantee that a PS I contamination to this extent can be excluded, but we have no indications for it. If the 80 ps component is due to PS II, perhaps the average trapping time varies with wavelength because longer-wavelength pigments are positioned closer to the trap and the 'first passage time' is reduced. However, with open centers (Q_A) Schatz et al. [6] found a 2-times faster trapping, which would suggest that the 200 ps in the presence of Q_A^- is at least partially trap-limited. These findings can be reconciled by the assumption of two pigment pools: with open centers trapping would be limited to 100 ps by the transfer from the pool excited at 675 nm to pigments absorbing at longer wavelengths, while in the presence of Q_A^- this transfer would be followed by a trap-limited charge separation. The 80 ps time constant we find upon direct excitation of the long-wavelength pigments would reflect this trap-limited charge separation.

A third pigment pool is required to explain the 21 ps phase we observe upon long-wavelength excitation. In the above interpretation its time constant would have to be ascribed to energy transfer to the pigment pool which gives charge separation in 80 ps. On the other hand, if the intermediate lifetime component in this material turns out not to depend on the redox state of Q_A, a simpler interpretation would be that the 21 ps component reflects charge

separation after direct excitation of a small pool of long-wavelength pigments, including P680. In that case the intermediate component would reflect energy transfer from the antenna to this small pool. The spectrum of the 21 ps component and its pronounced polarization dependence suggests that this pool comprises only a few pigments, absorbing selectively on the long-wavelength side, with a significantly different average orientation with respect to the other antenna pigments. This might support the hypothesis proposed in [2], but two-color pump-probe experiments and measurements with open RCs will be needed to arrive at an unambiguous assignment of the fast and intermediate components.

In RCs (D1-D2-cyt*b*559 particles) isolated from these core particles according to [10] we do not find a 21 ps component upon long-wavelength excitation, but charge separation in 3 ps [1]. It is tempting to speculate that the 21 ps charge separation time upon 694 nm excitation of isolated RCs reported in [15,16] has the same origin as the 21 ps component reported here, and is due to selective excitation of a small fraction of the centers retaining (part of) the core antenna. The 25 ps trapping time observed by Freiberg et al. [17] in RC-CP47 complexes may support this view.

In summary, we conclude that in PS II core particles with Q_A^- no rapid equilibration of the excitations over the entire antenna occurs. At least two or three pigment pools can be distinguished on the basis of the observed energy transfer and trapping kinetics in the 20–200 ps time range. After trapping of the excitations, radical-pair recombination in equilibrium with the excited state occurs in 1.5 ns.

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