Singlet Energy Dissipation in the Photosystem II Light-Harvesting Complex Does Not Involve Energy Transfer to Carotenoids
Mueller, Marc G.; Lambrev, Petar; Reus, Michael; Wientjes, Emilie; Croce, Roberta; Holzwarth, Alfred R.; Müller, Marc G.

Published in:
Chemphyschem

DOI:
10.1002/cphc.200900852

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2010

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Singlet Energy Dissipation in the Photosystem II Light-Harvesting Complex Does Not Involve Energy Transfer to Carotenoids


cphc_200900852_sm_mis_c33ellaneous_information.pdf
Supporting Information

(To be published online)

Estimation of expected maximal TA bleaching signals for Chl a as a function of excitation intensity

We assume an extinction coefficient $\varepsilon = 77000 \text{ mol}^{-1}\text{cm}^{-1}$ at the maximum of the Qy absorption of Chl a [1, 2] (Note that the extinction coefficient at the maximum absorption in proteins will most likely be somewhat higher due to less pronounced inhomogeneous broadening; thus the calculation performed here is on the conservative side). The excitation pulse for our low-intensity data (0.9 nJ/pulse focused into a 130 µm spot diameter in a 1 mm cuvette with absorption OD = 0.8/mm) centered at 680 nm excited at a wavelength where the excitation probability is about 85% from the maximum absorption. The excitation density is then 2.4·10^{13} photons/cm²/pulse. In our assumed quenching model the basic quenching unit is two trimers (see text) – independent of the actual physical size of the aggregates. We only need to consider the number of Chl a molecules in this two-trimer unit which is 48. The Chl b molecules can be ignored for this purpose when exciting at 680 nm. The excitation density then results in an excitation probability of 0.3 /particle/pulse, i.e. less than 1 out of 3 of the basic units (dimer of trimers) get excited per pulse. At these excitation conditions double excitation of the basic unit can be essentially ignored.

The difference extinction coefficient for the excited state of Chl a at the maximum of the absorption is nearly twice the ground state extinction coefficient due to the overlap of the bleaching with the essentially equally intense stimulated emission signal. We assume a difference extinction coefficient of 140000, i.e. about 90% of twice the ground state absorption extinction coefficient. For the sample conditions given above (OD = 0.8/mm at the excitation wavelength) we then expect a difference signal at the peak of the bleaching of $\Delta \text{OD}=8.8 \cdot 10^{-3}$/mm if the bleached species is a monomeric Chl a. The maximal signal in our data (Fig. 2C) is slightly higher than that. Apart from the general uncertainties in this calculation we do expect however a higher difference extinction coefficient than calculated above in case that we initially excite an excitonically coupled Chl pair which would have a difference extinction coefficient that could be higher than the one of the monomer by up to a factor of 2. Literature data actually suggest that under our conditions we would excite such an excitonically coupled state [3, 4]. Our measured difference signals (Fig. 2) are thus in the expected range.

The kinetic curves for a sample showing strong annihilation are given in Figs. S1A, S1B, and S1C. The kinetic shape of these curves is very similar to those shown in Fig. 2b of [5]. Fig. S2 shows the change of the kinetics due to annihilation at the peak of the ground state bleaching in the Qy range (682 nm) for different excitation intensities. The highest intensity corresponds to that used for the data shown in Fig. 1D.
**Fig. S1**: Kinetics for the high intensity data at 677 nm (A), 489 nm (B) and 537 nm (C) detection wavelengths (photon density $1.4 \times 10^{14}$ photons/cm²/pulse) identical to those shown in Fig. 1D. The data have been measured in two sets on the same sample with time resolutions of 25 fs per point (blue curve) and 1 ps per point (red curve). The data are shown on a linear time scale up to 10 ps and thereafter on a logarithmic scale for easy comparison with the curves provided by Ruban et al. [5].
Fig. S2: Development of annihilation in the kinetics of absorption changes at 682 nm as a function of excitation intensity on a short (top) and long (bottom) time scale. The signals are normalized at time = 0. The relative intensities of excitation are shown on the right-hand side. The highest intensity corresponds to the intensity used for Fig. 1D.
**Fig. S3:** Lifetime density maps of the TA kinetics measured under high excitation intensity (Fig. 1D) in the carotenoid (A), Chl Q_y (B), and near-IR (C) region. Note that the color coding represents different amplitude ranges in the three panels.
**Fig. S4:** Double difference absorption spectra of the kinetics at the indicated selected delay times for *npq1* (A) and *npq2* (B) aggregates. For calculating the double difference spectra the original spectra were normalized at the peak of the Qy bleaching. The original spectrum at 500 fs delay (normalized) is shown in red for comparison. The double difference spectrum for the very small amount of long-lived component in *npq2* is also shown (blue dashed). This difference spectrum is rather unspecific and it is not possible to decide whether it is due to a long-lived Chl excited state, a Chl triplet or a Car triplet, or a mixed signal.
Absence of a fast rise term in the blue range of the spectrum

Fig S4 represents double difference absorption spectra, i.e. the TA spectra at delay times of 15 and 40 ps minus the TA spectrum corresponding to 500 fs. The 15 and 40 ps delay times were chosen with the following rationale: In the model of Ruban et al. [5] the maximal relative concentration of the proposed Lut1 S\textsubscript{1} state would occur at a delay time of 30-40 ps (depending on the actual lifetime of the Lut1 S\textsubscript{1} state; for our calculation we assumed lifetimes of 8 ps as reported by Ruban et al. and 14 ps, as found for the lutein S\textsubscript{1} absorption in intact LHCII [6]. The 15 ps delay time on the other hand corresponds to a time when the P state (assigned to a mixed Chl exciton/CT state, see main text) in our model (Fig. 3, fast/slow model) reaches its maximal concentration. Examination of both double difference spectra reveals only the broadening (rise term) of the difference spectrum on the blue and red sides of the Q\textsubscript{y} band for the P state. The relative increase in apparent bleaching on the red side that is seen in the double difference spectra agrees well with the development of an enhanced stimulated emission band on the red side (around 700-730 nm) for the CT state as compared to the initially excited state [4]. This stimulated emission band is substantially more pronounced in the npq2 aggregates as compared to the npq1 aggregates. This finding agrees also perfectly with the finding of a stronger red emission of the CT state for npq2 aggregates [4]. At still longer wavelength the signals are positive which indicates that a dominant contribution from absorption, most likely due to the CT state, develops.

No Car S\textsubscript{1} signal is indicated in the blue wavelength range in both double difference spectra. Rather this range, which shows quite featureless and nearly flat spectra, is characterized by signals that are mostly typical of excitonic Chl excited states, probably with some admixture of Chl radical difference spectra as indicated by the negative signal at short-wavelength end of the analyzed region (cf. [7] and discussion in main text). Even without any detailed kinetic analysis these double difference spectra provide strong evidence for ruling out any contribution from a Car S\textsubscript{1} state at these delay times. Furthermore, the double difference spectra and the comparison to the spectrum at 500 fs delay also indicate the absence of a rise term in the time range from 10-40 ps. Rather than a relative rise in the difference spectra in the blue range these data indicate the relative decrease (decay term) of the bleaching in the Q\textsubscript{y} band. The same conclusion is reached from our kinetic models shown in Fig. 3. The observation of a rise term in the blue range is however a necessary prerequisite for the Ruban et al. model (see main text). Thus the absence of such a rise term alone puts into question the proposed formation of a Car S\textsubscript{1} state.

The absence of a rise term in this region can also be observed directly in the kinetic traces. Fig. S5 shows the directly measured kinetic traces in the blue wavelength range for the low intensity data of the npq2 aggregates for two different time ranges. It is obvious that these kinetic data do not contain any rise term with a lifetime of a few ps. The absence of a rise term in the blue range can furthermore be deduced from the double difference spectra in Fig. S4. In contrast, the raw kinetic traces clearly reveal the decay of the positive absorption difference on the time scale of a few hundred picoseconds (Fig. S4B). The data for the npq1 aggregates are essentially the same (not shown). Note that the suggested Car S\textsubscript{1} state intermediate model [5] requires that the relatively narrow Car S\textsubscript{1} ESA spectrum (about 20 nm halfwidth) would have to appear as a negative DADS (rise term) associated with a lifetime of 4-8 ps in the blue wavelength range around 540-560 nm in the LFD maps. This signal would not be overlaid by any other signal since no Chl related signal would occur on this time scale according to the model of Ruban et al.. Likewise a corresponding decay term (positive
amplitude) with a lifetime of 200 ps but with the same spectrum and exactly the same absolute amplitude as for the negative DADS would have to appear. The 200 ps SADS would furthermore be expected to be overlaid by a smaller amplitude, but spectrally quite broad, background due to Chl excited state absorption in the same region. No signal due to a Car S1 state is visible in our data at 200 ps delay (Fig. 2, Fig. 4), but only a broad structureless Chl excited state type signal. Since we can clearly detect the positive amplitude signal, it follows that we would also be able to detect the predicted negative signal of similar amplitude in that wavelength range. However Fig. 2 shows that no such negative signal of a few ps lifetime occurs in the LFD maps in the blue wavelength range, in agreement with the difference spectrum (Fig. S4). In summary, several different types of observation clearly show that there is no indication for the occurrence of any Car S1 absorption contribution to these spectra.

Fig. S5: Original TA signals for npq2 aggregates (the excitation intensity is 1.2·10^{13} photons/cm^2/pulse) in a short (A) and long (B) time range for selected blue wavelengths. Note the absence of a rise term with a few ps lifetime (left) which would be expected to have a similar amplitude as the 200 ps decay component (right).

Fluorescence kinetics of LHCII oligomers

Time-resolved fluorescence measurements and data analysis were performed according to [4]. In contrast to the published experimental data, in this work the oligomers of the two mutants were prepared by a different method (using Bio-beads, as in [5]). Moreover, we chose experimental conditions that are close to the conditions of the TA measurement: The sample concentration was adjusted to give an OD 5-8 (per cm) at the absorption maximum which is much higher than what was used in our previous fluorescence measurements [4]. The sample
was placed in a moving glass cuvette of 0.1 mm optical pathlength in order to minimize self-absorption and was excited at 663 nm. Fluorescence was detected in front face mode.

**Fig. S6:** Decay-associated fluorescence emission spectra of oligomers of Arabidopsis npq1 (A) and npq2 (B). The fluorescence is registered using single-photon counting as described in [4]. Samples were concentrated to OD 5-8 and measured in cuvette of 0.1 mm optical pathlength. The kinetics at all measured wavelengths were fitted simultaneously to a 5-exponential function convoluted with the instrument response.

The fluorescence kinetics was fitted with five exponential components using global analysis of the 680-740 nm wavelength region. The component with the highest amplitude had a lifetime of 160-170 ps. A fast equilibration component was also found with lifetime of 4 ps in npq1 and 10 ps in npq2. These results are thus in good agreement with the TA. Compared to the data in [4] though, the fluorescence in the presently analyzed oligomers decayed with faster lifetimes. Accordingly, the rate constants of energy equilibration and decay determined by target analysis of the TA and fluorescence measurements on these oligomers are somewhat faster than those reported in [4]. For this reason we also remeasured the fluorescence kinetics of the present (high concentration) samples (see above for details) (Fig. S6). The resulting fluorescence lifetimes are in good agreement with the TA data.

LHCII oligomers prepared in vitro are possibly heterogeneous [8] and the distribution of fluorescence lifetimes varies depending on the details of the preparation procedure [9, 10] and other factors that modulate the aggregate size such as e.g. cation concentration [11]. The concentration of LHCII itself – which for the TA measurements is more than a factor of 10 higher than used previously for the fluorescence lifetime measurements [4] – may also influence the size distribution and the exact type of distribution of aggregates formed and hence the measured lifetime. This can explain the somewhat different lifetimes and rates presented here (both for transient absorption and fluorescence (see above) and those previously reported [4]. We should note that also in [4] faster lifetime components were resolved, including a 5-8 ps rise component. In particular if the data were analyzed using a heterogeneous CT model (see supporting online material in that paper) an equilibration component of 6.5 ps lifetime was determined, in good agreement with the present data.
The variability in the organization of different preparations of *in vitro* LHCII oligomers is also demonstrated by the TA spectra shown in Fig. S7. The SADS represent data obtained from oligomers prepared by the method used in [4]. In comparison with the SADS shown in Fig. 3 in this article, the spectrum of the product state (ChlCT) is considerably broader, which also reflects the internal heterogeneity of the sample. However, despite of the variability in the LHCII preparations and normal measurement conditions (for fluorescence and TA measurements), the general results and the conclusions with respect to the quenching mechanism and intermediates are the same in all cases.

![Fig. S7](image-url) **Fig. S7:** Normalized species-associated absorption difference spectra of oligomers of *npq1*-LHCII. A. Oligomers prepared from trimers by diluting below the CMC of the detergent and concentrating to OD 8 by centrifugation through a 30 kDa cutoff filter. B. Oligomers prepared with Bio-beads (data from Fig. 3)

**References**


