Chapter 5

Effects of flash energy on PS II kinetics

The multiphasic decay of flash-induced 830 nm absorption of O$_2$ evolving PS II preparations sometimes include components with time constants of less than 10 ns. This is particularly so where high irradiances are required to achieve a good signal to noise ratio. Under these conditions, most of the RCs are excited, P680$^+$ formation is maximized and the PS II sample is saturated.

The level of flash intensity required to cause saturation is found by measuring the flash-induced absorption change at increasing flash intensities.
and then plotting the amplitudes of the multiphasic components due to P680+ as a function of flash intensity. At low flash intensities, the gradient of this function is high. At higher intensities, the gradient decreases sharply and further increases in flash intensity do not significantly increase the amplitude of the P680+ absorption signal.

This chapter describes the flash-induced absorption changes of BBYs and PS II core complexes at increasing flash intensities. These signals have been analysed by fitting a sum of exponential decays to the decay kinetics. The amplitudes of the multiphasic kinetics are then plotted as a function of flash intensity. The amplitude of a rapidly decaying component was found to continue to increase beyond where P680+ formation is maximum. In order to investigate the origin of this component further, the flash induced absorption changes were fitted with the kinetic model described in Chap. 4.

5.1 Effects of flash energy on BBYs

The effects of total flash energy on the transient 830 nm absorption changes of BBYs are shown in Fig. 5.1(a). At low flash energies, the absorption change decays with typical kinetics of O2-evolving PS II. As the intensity of the laser flash increases, a spike appears in the transient absorption signal, which becomes increasingly dominant as the intensity increases. A similar spike has been observed in inside-out thylakoids [1] and PS II enriched thylakoid membranes [2]. The origin of these components is unclear. It has been suggested to be due to antenna 1Chl* or possibly 3Chl* that decays rapidly by quenching by nearby Car or due to the charge stabilization of the radical pair P680+I- in the RC [3].
Fig. 5.1 (a) Flash-induced 830 nm absorption changes of BBYs suspended at 90 μM Chl in the presence of 1 mM K₃Fe(CN)₆ and excited by a flash energy fluxes: (i) 0.06, (ii) 0.17, (iii) 0.44, (iv) 1.39, (v) 4.52 and (vi) 6.0 mJ cm⁻². (b) Parameters from fitting a sum of 3 exponential decays to the transient absorption traces. Amplitudes (aᵢ) of 4-5 ns (squares), 20 and 200 ns (triangles), and greater than 500 ns (diamonds) decay periods.
These results were further analysed by fitting a sum of three exponential decays to the decay side of absorption signals. The amplitudes of the multiphasic kinetics are shown in Fig 5.1 (b). Traces were taken of the same BBY sample. The amplitudes at 4 mJ cm\(^{-2}\) indicate the extent of irreversible photodamage that occurs after exposure to high flash intensities.

The sum of amplitudes of the decays with lifetimes of approximately 40 ns and 200 ns represents the extent of formation of active PS II with forward electron conduction from the Tyr-161 residue to P680\(^{+}\). At low flash energies, these components dominate and they asymptotically approach an upper limit as the total flash energy increases.

The amplitudes of the components with lifetimes of 4 ns and >500 ns continue to increase beyond the saturation intensity. At the highest pulse energy, the extent of the components with lifetimes of >500 nanoseconds are approximately half that of the combined amplitudes of the components with 20 and 200 nanosecond lifetimes, whereas the amplitude of the 4-5 ns component extends to twice this value.

The fast component could be due to a combination of \(^{1}\text{Chl}\bullet\) decay or charge stabilization of the radical pair P680\(^{+}\)-I\(^{-}\). The slow component could be due to P680\(^{+}\)-QA\(^{-}\) charge recombination in RCs with inactive WOCs or perhaps to other processes such as spontaneous \(^{3}\text{Chl}\bullet\) decay.

If the 20-200 ns and >500 ns components are due to P680\(^{+}\) decay, the ratio of antenna Chl to P680 can be calculated from the absorption change of saturated PS II. The absorption change due to P680\(^{+}\) is, from the Beer-Lambert relation,

\[
\Delta A_{P680} = d\Delta \epsilon(P680^{+} / P680)[P680^{+}]
\]  

(5.1)

where \(d\) is the path length through the suspension, \(\Delta \epsilon(P680^{+}/P680)\) is the
difference extinction coefficient for P680$^+$ and [P680$^+$] is the P680$^+$ concentration.

Knowledge of the total Chl concentration allows the ratio of Chl to P680$^+$ to be calculated from equation (5.1). Using a difference extinction coefficient of 7,000 M$^{-1}$ cm$^{-1}$ [4], the value of 250 obtained is in agreement with published values for BBYs [5]. However, the value obtained here is based on the assumption that the >500 ns component is due solely to P680$^+$ and does not contain any contribution from 3Chl*. The increase of this component at increasing flash energies indicates that there may be a contribution to this component from 3Chl*.

5.2 Transient absorption of free Chl

In order to investigate whether the origin of the spike could be due to free Chl, flash-induced transient 830 nm absorption changes of free chlorophyll suspended in SMM buffer was measured. The absorption change for increasing flash energies is shown in Fig. 5.2 (a). One component closely follows the shape of the flash with a FWHM of 5.5 nanoseconds. After the flash, the $\Delta A_{830}$ signal decays almost completely but begins to increase again, reaching a peak about one tenth in amplitude and 150 nanoseconds after the first absorption peak with a lifetime of at least 800 ns.

The component with the short lifetime is assigned to 1Chl* on the basis of the lifetime of Chl in ethanolic solution [6]. The assignment of the slowly decaying component to 3Chl* is not inconsistent with its lifetime in water of 0.5 - 1 ms [4].
Fig. 5.2 (a) Absorbance changes at 830 nm of chlorophyll suspended in TMM buffer for a range of flash energies and (b) the amplitudes ($a_i$) of the fast (hollow) and slow (solid) components as a function of flash energy flux (b).

The amplitudes these components have been plotted as a function of flash energy. The increase in absorption of these components with increasing
The non-linear behaviour of the fast component indicates bimolecular annihilation processes occur under these conditions.

The existence of a rapidly decaying component in the absorption kinetics of free Chl suspended in water is consistent with the suggestion that the spike in the absorption transients of BBYs is due to free Chl, but could also be due to bound Chl.

5.3 Effects of flash energy on highly scattering BBYs

In order to investigate whether the spike is due to free Chl, the BBYs were washed before flash-induced measurements were made. Washing removed the excess detergent and any free Chl that may be present after the isolation of BBYs. This was achieved by centrifuging the BBY sample at 40,000 g for 15 minutes, discarding the supernatant and resuspending the pellet in fresh TMM buffer.

The resulting absorption changes, shown in Fig. 5.3, have characteristics of unwashed BBYs as well as new components with apparently negative absorptions. The peak absorption of these samples is only about 20% of the unwashed BBYs because they were suspended at much lower concentrations than those of the unwashed BBYs. This was necessary because at higher concentrations, the washed BBYs fall out of suspension and form aggregates which scatter the 830 nm probe beam, significantly reducing the signal to noise ratio.
Fig. 5.3 Flash-induced 830 nm absorption change of washed BBYs in the presence of 1 mM K₃Fe(CN)₆ and excited by a range of total flash energies (a) i) 0.07, ii) 0.20, iii) 0.37 and iv) 0.78 mJ cm⁻² and (b) v) 2.62, vi) 3.55, vii) 4.23, viii) 4.83 ix) 5.74 mJ cm⁻². The extent of photodamage is indicated by trace x) 0.48 mJ cm⁻², which was measured after exposure to high-energy flashes.
The origin of the component with the negative absorption needs to be understood before a proper quantitative analysis of the results can be made. However, a semi-quantitative analysis reveals a delay of 20 - 30 nanoseconds in the formation of the peak ΔA_{830} signal at low flash energies, which disappears at higher the flash energies. As the flash energy increases, a spike appears and at the highest flash energy, the amplitude of the spike is comparable to that of the 4-5 ns component in the unwashed BBYs.

The appearance of a spike at high flash intensities in the washed BBYs, suggests that it is due to antenna Chl rather than free Chl. However, the spike could also be due to the decay of the radical pair P_{680}^{+I} to the charge stabilized state P_{680}^{+QA}. In order to investigate this possibility, flash-induced absorption changes of PS II cores were made. If the 4-5 ns component is due primarily to antenna singlet Chl*, the spike should be relatively smaller, because the ratio of antenna Chl to P_{680} is smaller in PS II cores than in BBYs. On the other hand, if this component is due to radical pair formation, then this component should be approximately the same size of that in BBYs.

### 5.4 Effects of flash energy on PS II core complexes

The flash-induced absorption changes of PS II core complexes, shown in Fig. 5.4 (a), are similar to those of BBYs except that the spike at high intensities, is not as prominent as in BBYs. A component with a lifetime of approx. 7 ns, in PS II core complexes has also been reported. This component is S-state dependent and has been assigned to the fast decay of P_{680}^{+} [7].

The amplitudes of a sum of three exponential decays fitted to the decay side of each trace Fig. 5.4(b) show the extent of formation of excited
states of PS II. The sum of amplitudes of the decays with lifetimes of approx.
Fig. 5.4 (a) Flash-induced 830 nm absorption changes of PS II core complexes suspended at a concentration of 100 μM Chl in the presence of 2 mM K₃Fe(CN)₆. (b) The amplitudes of components with lifetimes of 4-5 ns (squares), 40 and 200 ns (triangles), and with lifetimes greater than 500 ns (circles), show the extent of formation of excited states as a function of actinic energy density.
40 ns and 200 ns represent the extent of formation of active PS II with forward electron conduction from the Tyr-161 residue to P680+. At low flash energies, these components dominate and asymptotically approach an upper limit as the total flash energy increases. This plot can be characterised by the flash energy density at which the sum of these amplitudes reach 50% (0.168 mJ cm$^{-2}$) and 95% (1.5 mJ cm$^{-2}$) of the saturation level.

The amplitudes of the components with lifetimes of 5 and >500 nanoseconds continues to increase beyond this level of energy density. At the highest pulse energy, the extent of the components with lifetimes of 5 and >500 nanoseconds are approximately half that of the combined amplitudes of the components with 20 and 200 nanosecond lifetimes.

If the 4-5 ns component was due to recombination of the radical pair P680$I^-$, this component should be relatively larger in PS II cores because there are relatively more RCs in cores than BBYs. Since the spike is smaller in cores than BBYs, the assertion that the spike is due to antenna Chl, is strengthened. The suggestion that this component is due to PS I contamination, is also weakened by the observation that low-temperature fluorescence measurements show a greater proportion of PS I contamination in cores than in BBYs.

5.5 Applying the kinetic model of PS II

Transient absorption kinetics of BBYs and PS II cores have been analyzed by fitting multi-exponential decays to the decay side of these transients. The non-linear increase in both the amplitude of the <5 ns and >500 ns components suggests they may both have more than one cause. It was concluded that the spike appearing at high flash energies is mainly due
to antenna $^1\text{Chl}^\ast$. However, the origin of the $>500$ ns component remains unclear. Flash-induced decay kinetics of Chl suspensions also showed a non-linear response to flash-energy and it was suggested that this may be due to bimolecular annihilation.

Simulations of BBY kinetics

The kinetic model described in Chapter 4 was used to simulate the observed changes in the concentration of components in BBYs as a function of the intensity of the flash excitation. The results of the predictions of the 830 nm absorption changes of BBYs simulate the results for BBYs excited by the same flash intensities that were used during flash excitation of BBYs.

The absorption change at 830 nm is the sum of absorption changes due to $P680^+$, as described in equation (5.1) and contributions by the following

$$\Delta A_{\text{Chl}} = d\Delta \varepsilon (^1\text{Chl}^\ast / \text{Chl})[^1\text{Chl}^\ast]$$  \hspace{1cm} (5.2)

$$\Delta A_{P680^+} = d\Delta \varepsilon (P680^+ I^+ / P680 I)[P680^+ / I^-]$$  \hspace{1cm} (5.3)

$$\Delta A_{3\text{Chl}} = d\Delta \varepsilon (^3\text{Chl}^\ast / \text{Chl})[^3\text{Chl}^\ast]$$  \hspace{1cm} (5.4)

where, $\Delta \varepsilon (^1\text{Chl}^\ast / \text{Chl})$, $\Delta \varepsilon (P680^+ I^+ / P680 I)$ and $\Delta \varepsilon (^3\text{Chl}^\ast / \text{Chl})$ are the molar difference extinction coefficients at 830 nm for the formation of $\text{Chl}^\ast$, $P680^+ I^+$ and $^3\text{Chl}^\ast$ respectively and square brackets represent concentration of the species.

The flash-induced transient absorption of BBYs at selected flash energies and the fitted traces are shown in Fig. 5.5. These traces were fitted by solving equations (4.20) to (4.34), using the appropriate boundary conditions of equation (4.22) and the parameters shown in table (5.1).
Fig 5.5 Flash-induced 830 nm absorption of BBYs fitted using the kinetic model of PS II. As with the experimental results, changes in the fitted kinetics were obtained by varying only the total flash energy ($E_{\text{flash}}$). The experimental and simulated absorption changes due to $^1\text{Chl}^*$, $\text{P680}^+\text{I}^-$, $\text{P680}^+$ and $^3\text{Chl}^*$ for the following values of $E_{\text{flash}}$: 0.06 mJ cm$^{-2}$ (red), 0.17 mJ cm$^{-2}$ (green), 0.44 mJ cm$^{-2}$ (blue), 1.39 mJ cm$^{-2}$ (cyan), 4.52 mJ cm$^{-2}$ (magenta) and 6.0 mJ cm$^{-2}$ (gold).
Table 5.1 Parameters used to fit the transient absorption kinetics of BBYs.

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Symbol</th>
<th>Quantity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molar extinction coefficient of Chl at 532 nm</td>
<td>$\varepsilon$</td>
<td>864 M$^{-1}$ cm$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Refractive index of water</td>
<td>$r$</td>
<td>1.33</td>
<td></td>
</tr>
<tr>
<td>Central wavelength of flash</td>
<td>$\lambda$</td>
<td>5.32 x 10$^{-5}$ cm</td>
<td></td>
</tr>
<tr>
<td>Total energy flux of flash</td>
<td>$E_{\text{flash}}$</td>
<td>6.0 mJ cm$^{-2}$</td>
<td></td>
</tr>
<tr>
<td>Temporal centre of flash</td>
<td>$t_0$</td>
<td>37.0 ns</td>
<td></td>
</tr>
<tr>
<td>Temporal width of flash</td>
<td>$W_t$</td>
<td>4.0 ns</td>
<td></td>
</tr>
<tr>
<td>Molar diff. extinction coeff. [1Chl*/Chl]</td>
<td>$\Delta \varepsilon^{(1}\text{Chl}*/\text{Chl})$</td>
<td>4,000 M$^{-1}$ cm</td>
<td>[8]</td>
</tr>
<tr>
<td>Molar diff. extinction coeff. [2Chl*/Chl]</td>
<td>$\Delta \varepsilon^{(2}\text{Chl}*/\text{Chl})$</td>
<td>3,800 M$^{-1}$ cm</td>
<td>[8]</td>
</tr>
<tr>
<td>Molar diff. extinction coeff. [P680*I-/P680I]</td>
<td>$\Delta \varepsilon^{(P680^+I-/P680I)}$</td>
<td>13,000 M$^{-1}$ cm$^{-1}$</td>
<td>[8]</td>
</tr>
<tr>
<td>Molar diff. extinction coeff. [P680*/P680]</td>
<td>$\Delta \varepsilon^{(P680^+/P680)}$</td>
<td>7,000 M$^{-1}$ cm$^{-1}$</td>
<td>[8]</td>
</tr>
<tr>
<td>Pathlength of 830 nm beam through cuvette</td>
<td>$d$</td>
<td>4 cm</td>
<td></td>
</tr>
<tr>
<td>Rate constant for <em>invitro</em> 3Chl decay</td>
<td>$k_r$</td>
<td>(5.0 x 10$^5$ ns)$^{-1}$</td>
<td>[9]</td>
</tr>
<tr>
<td>Rate constant for spontaneous decay of $^1$Chl</td>
<td>$A$</td>
<td>0.33 ns$^{-1}$</td>
<td></td>
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<td>Rate constant for exciton trapping in RCs with oxidised QA</td>
<td>$k_{2ox}$</td>
<td>10 ns$^{-1}$/C/a</td>
<td>[10] (estimated)</td>
</tr>
<tr>
<td>Rate constant for radical pair recombination in RCs with oxidised QA</td>
<td>$k_{-2ox}$</td>
<td>0.5 ns$^{-1}$</td>
<td>[10]</td>
</tr>
<tr>
<td>Rate constant for exciton trapping of RCs with reduced QA</td>
<td>$k_{2red}$</td>
<td>1.65 ns$^{-1}$/C/a</td>
<td>[10] (estimated)</td>
</tr>
<tr>
<td>Rate constant for radical pair recombination in RCs with reduced QA</td>
<td>$k_{-2red}$</td>
<td>1.5 ns$^{-1}$</td>
<td>[10]</td>
</tr>
<tr>
<td>Rate constant for charge stabilization</td>
<td>$k_3$</td>
<td>3.33 ns$^{-1}$</td>
<td>[10]</td>
</tr>
<tr>
<td>Rate constants for P680$^+$ reduction by Tyr-Z</td>
<td>$k_{40} = k_{41}$</td>
<td>(3.0 ns)$^{-1}$</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td>$k_{42} = k_{43}$</td>
<td>(120.0 ns)$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Rate constants for Y-161$^+$ reduction by Mn oxidation</td>
<td>$k_{50}$</td>
<td>(3.0 x 10$^4$ ns)$^{-1}$</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td>$k_{51}$</td>
<td>(1.0 x 10$^5$ ns)$^{-1}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$k_{52}$</td>
<td>(3.5 x 10$^8$ ns)$^{-1}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$k_{53}$</td>
<td>(1.5 x 10$^6$ ns)$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Rate constant for oxidation of QA$^-$ by Fe(CN)$_6^{3-}$</td>
<td>$k_G$</td>
<td>(9.5 x 10$^5$ ns)$^{-1}$</td>
<td>[10, 13]</td>
</tr>
<tr>
<td>Rate constant for decay by charge recombination</td>
<td>$k_{GQ}$</td>
<td>(1.8 x 10$^5$ ns)$^{-1}$</td>
<td>[14]</td>
</tr>
</tbody>
</table>
The experimental traces were fitted iteratively until a good fit to all the experimental absorption kinetics was obtained by varying only $E_{\text{flash}}$. In modelling the results, the degeneracies of the energy levels, $g_1$ and $g_2$ were assumed equal. The number of Chl/RC in BBYs, $N_{\text{pig}} = 270$ and the concentration of BBYs was $C = 80 \ \mu$M. The proportion of RCs that form the charge stabilized pair but with inactive WOCs $p^i = 0.1$. The absorption cross section $\sigma_{12} = 7.8 \times 10^{-18} \ \text{cm}^2$. The coefficient of singlet-singlet annihilation $\gamma_{ss} = 4.4 \ \mu\text{M}^{-1} \ \text{ns}^{-1}$. The rate constant for intersystem crossing $k_{\text{isc}} = 0.05 \ \text{ns}^{-1}$. The coefficients of exciton trapping $k_{2\text{ox}}$ and $k_{2\text{red}}$ were estimated by dividing the first order trapping rate constant by $C$ and a dimensionless parameter $a = 0.03$.

The individual components of the absorption changes are shown in Fig. 5.6 to illustrate the effects of the coefficients on the total absorption at various flash energies. Fig 5.6 (a) shows the changes in the temporal $^1\text{Chl}^*$ concentration. These curves are similar to the flash irradiances, except that the heights of the top traces are reduced because of singlet-singlet annihilation. The peak amplitude of the top two traces, due to excitation by total flash energies of 4.5 and 6 mJ cm$^{-2}$, are significantly different. The coefficient of singlet-singlet annihilation controls the extent of the spike, if the value of this coefficient was higher, there would be less difference between the peak amplitudes of these functions.

Fig. 5.6 (c) shows the transient absorption due to P680$^+$/I$^-$. The simulation shows that further increases in flash energy will not cause much increase in the height of the peak. This is due to this component becoming almost saturated at the highest flash energies. A second peak at high irradiances occurs due to the formation of P680$^+$/I$^-$ in RCs with QA$^-$. The formation of the second peak is not noticeable at low flash energies because $^1\text{Chl}$ will have decayed significantly by the time QA$^-$ has formed.
Fig. 5.6 (d) shows the transient absorption due to P680+QA− and is the sum of contributions from RCs with intact WOCs, illustrated in Fig. 4.2 (a) and RCs with inhibited RCs, illustrated in Fig. 4.2 (b). As with the transient absorption of P680+I−, the top two traces have also reached peak saturation. However, the top four traces meet within 10 ns after the flash. This shows that if the >500 ns component was due only to RCs with impaired electron transfer from the WOC at a fixed proportion, the traces at high energies would coincide along their entire length. Clearly this does not occur, either in the case of BBYs, as shown in Fig 5.1 or PS II cores, as shown in Fig. 5.4. This means that the >500 ns component is not only due to RCs with inhibited electron transfer from the WOC but also has another cause.

The separation of the absorption kinetics can be achieved by altering the decay rates of components with flash energy; however a mechanism that would cause changes in decay rates as the flash energy increased is not known. Alternatively, an additional term can be included, to increase the absorption as a function of flash energy. The new term that gives a satisfying fit to the absorption changes is equation (4.21), which represents the formation of triplet Chl in the antenna. The transient absorption of this term is shown in Fig. 5.6 (b) increases with increasing flash energy, and as shown in Fig. 5.5, causes the total absorption transient to separate at flash energies below the saturation energy.

Exciton annihilation may occur when there is more than one exciton on an isolated antenna complex. This will happen increasingly as the exciton concentration exceeds the RC concentration or if excitons freely transfer between PS II units. Accordingly, the model should predict a greater proportion of exciton annihilation at high 1Chl∗ concentrations and little or no exciton annihilation when the concentration is below the concentration of LHC II. These effects are illustrated in Fig. 5.7.
Fig. 5.6 Predicted changes in the absorption at 830 nm due to the formation of (a) $^1$Chl*, (b) $^3$Chl*, (c) P680$^+$I$^-$ and (d) P680$^+$QA$^-$ due to excitation by a flash with energies of: 0.06 mJ cm$^{-2}$ (red), 0.17 mJ cm$^{-2}$ (green), 0.44 mJ cm$^{-2}$ (blue), 1.39 mJ cm$^{-2}$ (cyan), 4.52 mJ cm$^{-2}$ (magenta) and 6.0 mJ cm$^{-2}$ (gold).

As shown in Fig. 5.7 (a), on excitation by a flash energy flux of 0.06 mJ cm$^{-2}$, the predicted $^1$Chl* concentration was hardly affected when the exciton annihilation coefficient $\gamma_{ss}$ was increased from 0.0 to 4.0 $\mu$M$^{-1}$ ns$^{-1}$. By contrast, on excitation by a flash of 1.39 mJ cm$^{-2}$, the exciton formation is
significantly reduced when the exciton annihilation coefficient $\gamma_{ss}$ is increased from 0.0 to 4.0 $\mu$M$^{-1}$ ns$^{-1}$.

As shown in fig. 5.7 (b), the effect of exciton annihilation flows onto P680$^+/I^-$ formation which is observed to follow similar trends to those observed in $^1$Chl. Formation of the secondary peak in RCs with $Q_A^-$ is significantly suppressed by exciton annihilation.

### 5.6 Conclusion

The flash-induced 830 nm absorption changes of BBYs and PS II cores have been measured over a range of flash energies. Multi-exponential fits to the decay kinetics reveal components with expected lifetimes of 5 ns, 20-30 ns, 140-200 ns and >500 ns. The components with 20-30 and 140-200 ns lifetimes are associated with forward electron conduction from the Tyr-161 residue of the D1 polypeptide and saturate at flash energy densities of between 1.5 mJ and 2.0 mJ cm$^{-2}$ at Chl concentrations of 80-100 $\mu$M.

The origin of the 5 ns component, which causes the appearance of a ‘spike’ at high flash energies, was considered from a range of possible causes. These include PS II that also absorb at 830 nm, decay of the radical pair P680$^+I^-$ to form the charge-stabilized state P680$^+Q_A^-$, spontaneous decay of $^1$Chl*, rapid decay of $^3$Chl* by annihilation with Car and contamination from PS I.

The transient absorption of free Chl suspended in an aqueous environment was found to have kinetics similar to the $\Delta A_{830}$ component with a lifetime of 5 ns. This component was assigned to $^1$Chl* on the basis of the rapid decay of the absorption. This result confirms that the spike can be due to free Chl or bound antenna Chl.
Fig. 5.7 Effect of the exciton annihilation coefficient ($\gamma_{ss}$) on the predicted concentration of (a) $^1\text{Chl}^*$ and (b) P680$^+$/I$^-$. The simulations were obtained using the kinetic model shown in Chap. 4. Without exciton annihilation $\gamma_{ss}=0$ (square) and with exciton annihilation $\gamma_{ss}=4.0 \ \mu\text{M}^{-1} \ \text{ns}^{-1}$, (line) for flash energies of 0.06 mJ cm$^{-2}$ (red) and 1.39 mJ cm$^{-2}$ (cyan).
The $\Delta A_{830}$ of washed BBYs appears to have a similar 5 ns component to BBYs. This suggests that the 5 ns lifetime component in BBYs is due to bound Chl rather than free Chl.

The ratio of the extent of the 5 ns lifetime component to the total peak amplitude is greater in BBYs than in PS II cores. This also confirms that Chl is responsible for the spike rather than decay of the radical pair P680$^+$I$^-$, since the ratio of Chl to pheophytin is higher in BBYs than in cores. Alternative sources of the component with a lifetime of >500 were also explored. These were recombination of the charge-stabilized state P680$^+$Q$_A^-$, spontaneous $^3$Chl* decay and P700$^+$ decay due to PS I contamination.

The kinetic model described in Chap. 4 was used to fit the flash-induced absorption kinetics of BBYs over a range of flash energies. The model predicts that kinetics associated with P680$^+$I$^-$ and P680$^+$Q$_A^-$ decay asymptotically approach upper limits with increasing flash energy. A spike that continues to increase in amplitude with increasing flash energy is also predicted by the model.

The value of the absorption cross section that gave a satisfying fit the absorption traces can be used to obtain a value for the relative amount of Chl in the upper level ($C_2/C$) during measurements of the extinction coefficient. Assuming that the degeneracy of the lower and upper energy levels of the antenna Chl are equal, $C_2/C$ in equation (4.12) would be about 0.4, suggesting perhaps that the value obtained for the absorption cross section is not unreasonable.

By examining the components from the flash-induced absorption predictions, it was shown that the spike can be assigned to excited antenna Chl. Furthermore, it was shown that charge stabilization of the radical pair P680$^+$I$^-$ is not responsible for the spike that appears at high flash intensities.
It was also shown that the >500 ns component is not due to charge recombination from QA to P680+ alone, another component is required. The results from the model demonstrated that formation of 3Chl* by intersystem crossing can explain > 500 ms component over the range of flash-energies. The coefficient of intersystem crossing obtained by fitting with the model (0.05) was in good agreement with that obtained from experimental values estimated for spinach CP 47 (0.0835). However, whether the >500 µs component is due to P700+ from PS I contamination or to 3Chl decay remains unknown.


