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The effect of outer antenna complexes on the photochemical trapping rate in barley thylakoid Photosystem II

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Abstract

We have investigated the previous suggestions in the literature that the outer antenna of Photosystem II of barley does not influence the effective photosystem primary photochemical trapping rate. It is shown by steady state fluorescence measurements at the F_0 fluorescence level of wild type and the chlorina f2 mutant, using the chlorophyll *b* fluorescence as a marker, that the outer antenna is thermally equilibrated with the core pigments, at room temperature, under conditions of photochemical trapping. This is in contrast with the conclusions of the earlier studies in which it was suggested that energy was transferred rapidly and irreversibly from the outer antenna to the Photosystem II core. Furthermore, the effective trapping time, determined by single photon counting, time-resolved measurements, was shown to increase from 0.17±0.017 ns in the chlorina Photosystem II core to a value within the range 0.42±0.036–0.47±0.044 ns for the wild-type Photosystem II with the outer antenna system. This 2.5–2.8-fold increase in the effective trapping time is, however, significantly less than that expected for a thermalised system. The data can be explained in terms of the outer antenna increasing the primary charge separation rate by about 50%.
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Keywords: Antenna effect; Chlorina f2; Photosystem II; Thylakoid; Trapping time

1. Introduction

Plant Photosystem II contains a number of antenna-pigment binding proteins that are coupled by energy transfer to a reaction centre complex (D1/D2/cytb559) where the excited reaction centre chlorophylls (P680, or possibly a reaction centre accessory chlorophyll [1,2]) chemically reduce a primary pheophytin acceptor. It is in this step that the very high oxidising potential of the PSII reaction centre is created (E^0=+0.82 V), sufficient to allow water oxidation. While it is generally agreed that the intrinsic charge separation process is on a picosecond time scale, there is considerable debate as to its exact value, with suggestions falling in the range 0.4–20 ps (e.g., Refs. [3–9]). On the other hand, the overall photochemistry rate, i.e., the effective rate of charge separation (k_{cs}^{eff}) for the entire photosystem, is much slower, being of the order of (300–400 ps)$^{-1}$ [10,11]. This is due to the nature of the antenna, which is both large and complex. The Photosystem II antenna consists of the core antenna complexes (CP43, CP47), tightly coupled to the RC complex, and the four outer antenna complexes (LHCII, CP24, CP26, CP29). While the core complexes bind only chl *a*, the outer antenna complexes bind both chls *a* and *b*. Apart from this difference in the chlorophyll *a*/*b* content, all complexes are nearly isoenergetic from the point of view of the different chlorophyll forms [12,13]. The antenna size is typically that of 200–250 chlorophylls, with the core complexes binding about 40–50 of these according to pigment stoichiometric data (e.g., Ref. [14]) and 35–36 plus 2 pheophytins according to recent crystallographic data [15–17]. It is therefore evident, from the over two orders of

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magnitude of difference with respect to the RC chlorophylls, that it is the antenna that dominates the effective overall rate of charge separation (k_{cs}^{eff}).

Over the years, there has been much discussion of this antenna effect on the effective photosystem photochemistry rate. Early work, chiefly by Butler and colleagues (e.g., [18]), considered PSII diffusion limited with the k_{cs}^{eff} being dominated by the slow diffusion of excited states from the antenna to the RC, where fast and irreversible trapping occurred. The excited state diffusion time from the antenna was subsequently demonstrated to be a linear function of both the number of antenna pigments and the interpigment hopping rate [19] in the assumption of isoenergetic pigment sites. Subsequently, this "diffusion limited" idea was greatly modified by Holzwarth et al. [20], who developed the opposite view, based largely on picosecond fluorescence decay measurements, that excitation diffusion within the antenna was extremely fast, leading to thermal equilibration between the antenna and P680 within about 10–15 ps. In this view, known as trap limited, excitation energy then migrates between the trap and the antenna many times before finally undergoing charge separation. Also, in this case, the $(k_{cs}^{eff})^{-1}$ is expected to be approximately linear with the antenna size, scaling with the large number of degenerate energy levels present in the antenna with respect to the RC. Thus, the "antenna effect" of Butler et al. is largely a kinetic one, while that envisaged by Holzwarth et al. depends mostly on the antenna degeneracy. However, it should be mentioned that in both model views the excited states pass most of the 300–400 ps of the $(k_{cs}^{eff})^{-1}$ in the antenna. More recently, both these extreme views have been somewhat melded together with recognition of some kinetic rate-limiting processes in the antenna [21] and in particular associated with transfer from the core antenna to the RC complex [6,22]. This latter situation, based on the crystallographic structure of cyanobacterial PSII core [15], is known as the transfer-to-trap limited model. At present, nothing is known of transfer rates between the antenna complexes that may also constitute kinetic "bottlenecks".

While it is evident, from the very brief overview given above, that very different views have been presented on the "antenna effect", it should be mentioned that very little experimental evidence is available on just how antenna size and properties influence k_{cs}^{eff}. The recent, detailed, model studies [6,22] on the PSII core, using interchlorophyll distance and dipole orientation parameters to calculate Förster transfer rates and RC trapping of excited states, are a step in this direction. However, it must be pointed out that in these studies the chlorophyll band shapes, Stokes shifts and energy levels of the single pigment sites, none of which are obtainable from the crystallographic structure, are arbitrarily assumed, which leads to considerable uncertainty concerning some of the detailed conclusions. At any rate, from the data presented in these papers, it is not possible to understand the impact of antenna size and properties on the k_{cs}^{eff}. The most clear-cut attempt at experimentally investigating the "antenna effect" in PSII was performed in two papers that were simultaneously published in 1996 [23,24] and subsequently by one of these groups in 2000 [25]. In these papers, the time-resolved fluorescence decay kinetics were determined for the chlorina barley mutant lacking the chlorophyll *a*/*b* complexes of the outer antenna, i.e., essentially "core" complexes, under photochemically active conditions and compared with those of the wild type containing the full PSII antenna complement. Both groups, using different lifetime fluorescence techniques, came to the same conclusion, that when the antenna size was changed by an approximately 4–5-fold factor, the value of k_{cs}^{eff} for PSII was essentially unchanged. This rather surprising result cannot be reconciled with any of the three main models described above, nor with the theoretical expectations of Montroll [26], Pearlstein [27], Kudzmauskas et al. [19], Jean et al. [28], and in fact both authors introduced the ad hoc assumption that energy transfer from the outer antenna to the core is fast and irreversible. This assumption clearly implies that the excited state distribution in the PSII antenna is not thermally equilibrated, with subequilibration population densities in the outer antenna. While it is not easy to envisage the physical basis for such an effect, particularly as the outer and inner antenna complexes are approximately isoenergetic [12,13,29] and are thus expected to be readily thermalised, this suggestion was not further investigated and hence has never been either proven or disproven. There are, however, several criticisms that can be raised concerning the experiments in these two studies. Firstly, in the 1996 papers, even though the chlorina thylakoids contain PSI as well as PSII cores, neither group identified a PSI lifetime component. This may reflect the fact that measurements were performed at one wavelength only and hence a global analysis of the data was not performed. This objection was subsequently remedied [25]. Secondly, presumably due to lack of instrumental resolution, a "fast" (30–60 ps) was not resolved, while it has been demonstrated [22,30] that in isolated PSII core preparations the dominant decay is in this time range. However, it is not possible to exclude, a priori, their suggestion as LHCII, the major component of the external antenna, has been shown over the years to perform rather unpredictably under illumination, displaying reversible fluorescence quenching, circular dichroism changes and disaggregation [31–33]. Thus, a configuration change involving LHCII, impeding the back transfer of energy, could in fact be entertained.

In order to examine this situation, we have initially addressed the question of whether the outer antenna is thermally equilibrated with the core in wild-type PSII, as is usually assumed but which has never been unambiguously demonstrated. This point is essential, as the conclusions of Refs. [23–25] can be correct only if the excited state population in the outer antenna is significantly below the equilibrium population. From steady state measurements of the chlorophyll *b* emission of the chlorina mutant and its wild type, we conclude that the outer antenna is in fact

substantially equilibrated with the core antenna. Moreover, single photon counting fluorescence decay experiments, using both the chlorina mutant of barley and its wild type and analysing data globally between 670 and 740 nm, demonstrated that the effective trapping time is in fact sensitive to the presence of the outer antenna complexes.

2. Materials and methods

Thylakoids were prepared from freshly harvested barley leaves of wild type or chlorina f2 mutant [34] as previously described [35]. While the chlorophyll *a*/*b* ratios were close to 3.0 for the wild-type leaves, they were formally about 40 in the mutant thylakoids when measured according to Porra et al. [36]. This mutant is characterised by the absence of chlorophyll *b* and the principle light-harvesting complex of PSII, LHCII, which accounts for about 80% of the external antenna [12]. Reduced amounts of the polypeptides of some of the three minor outer antenna complexes of PSII have been detected though it is unclear whether they bind chlorophyll. In our calculations, we have allowed for the chlorina core to bind 40 or 50 chlorophylls. The former number represents the situation in the assumption that no minor outer antenna complex remains bound to the chlorina core, while the second number allows for the binding of 1–2 complexes of the minor outer antenna. In addition, it should be mentioned that the content of the LHCI polypeptides is reduced, with Lhca4 being absent [37]. It is not clear whether the remaining LHCI polypeptides bind chlorophyll or not. Thylakoids were prepared and experiments performed at Mg ion concentrations, respectively, of 5 mM for wild type and 20 mM for the chlorina mutant. These concentrations were chosen as they yielded thylakoids with the highest F_v/F_m ratio and, therefore, presumably, the highest amount of membrane stacking. The F_v/F_m ratio was in the range 0.73–0.78 for the wild type and 0.65–0.68 for the chlorina mutant. Fluorescence measurements were performed at a chlorophyll concentration of 4 μg/ml in the presence of Tricine 30 mM, pH 8, sucrose 100 mM, NaCl 5mM, the previously stated Mg ion concentrations and 50 μM methyl viologen, which, together with the low light intensities used, maintained the fluorescence level close to F_0.

Time-resolved fluorescence decay measurements for thylakoids, maintained near the F_0 fluorescence level in the presence of an added electron acceptor (methyl viologen), were performed using the time-correlated, single-photon counting technique (TCSPC) with excitation at 644 nm. This will have led to almost equal absorption by chl *a* and chl *b* in the wild type. The excitation source was a Pulsed Diode Laser (PicoQuant GmbH, Berlin Germany), peaking at 644 nm, operating at a repetition rate of 40 MHz and an intensity of 1.3 mW/m^2 through a controller PicoQuant PDL 800-B. The emission was passed through a polariser set at the magic angle and then through a monochromator (Jasco CT-10, Japan) and detected by a microchannel plate photomultiplier tube (Hamamatsu R3809U-51, Japan). The overall system prompt response was ~80 ps (FWHM), which, after deconvolution with the instrumental response function, yielded a time resolution of 10–20 ps. The emission decays were usually recorded at 10-nm intervals between 670 and 740 and analysed globally with an algorithm that fits the measured decays by iterative numerical reconvolution of the instrumental response function and the weighted sum of exponentials decay model function. The fit was performed using the Frontline Systems Premium Solver Plus (Frontline Systems, Inc. Incline Village, NV, USA) as minimiser. The goodness of the fit of each measurement was determined by the χ^2 value and inspection of the residuals plot.

Steady state fluorescence emission spectra of thylakoids were measured using an EG&G OMAIII (model 1460) with an intensified diode array (model 1420) mounted on a spectrograph (Jobin-Yvon HR320) with a 150 groove mm^{-1} grating. The wavelength scale was calibrated using a spectral line calibration source (Cathodeon). The fluorescence was maintained close to the F_0 level by addition of an electron acceptor (methyl viologen) and the use of low-excitation (440 nm) light intensity.

3. Results and discussion

3.1. Excitation equilibration in the outer antenna

We start out by examining whether the excited state population is thermally equilibrated in the outer antenna of PSII. In the suggestion of Briantais et al. [23] and Gilmore et al. [24], it is expected to be significantly underpopulated with respect to the equilibrium value. As the fluorescence emission spectra of all PSII chlorophyll–protein complexes are very similar at room temperature [13], it is not possible to identify the emission band of a particular PSII complex, either by steady state or time resolved techniques. However, the presence of chlorophyll *b* is a characteristic of the outer antenna complexes, whereas it is absent in the core. Thus, it may be used as a marker signal for the outer antenna. The fluorescence of chlorophyll b is extremely weak due to the large energy difference (~2.7 kT at room temperature) though we have previously demonstrated that it may be detected, in both isolated chlorophyll *a*/*b* complexes [13,38] and also in barley thylakoids [38]. Gaussian decomposition, together with the Stepanov relation, relating the absorption and emission spectra under conditions of thermal equilibration, indicate that its emission band is present near 650–653 nm with the small (2–3 nm) Stokes shift characteristic of the weak electron-phonon coupling of antenna chlorophylls bound to pigment protein complexes (e.g., Ref. [39] and references therein). In Fig. 1, we compare the steady state emission spectra of thylakoids from the chlorina barley mutant with those measured for the wild type. Both spectra

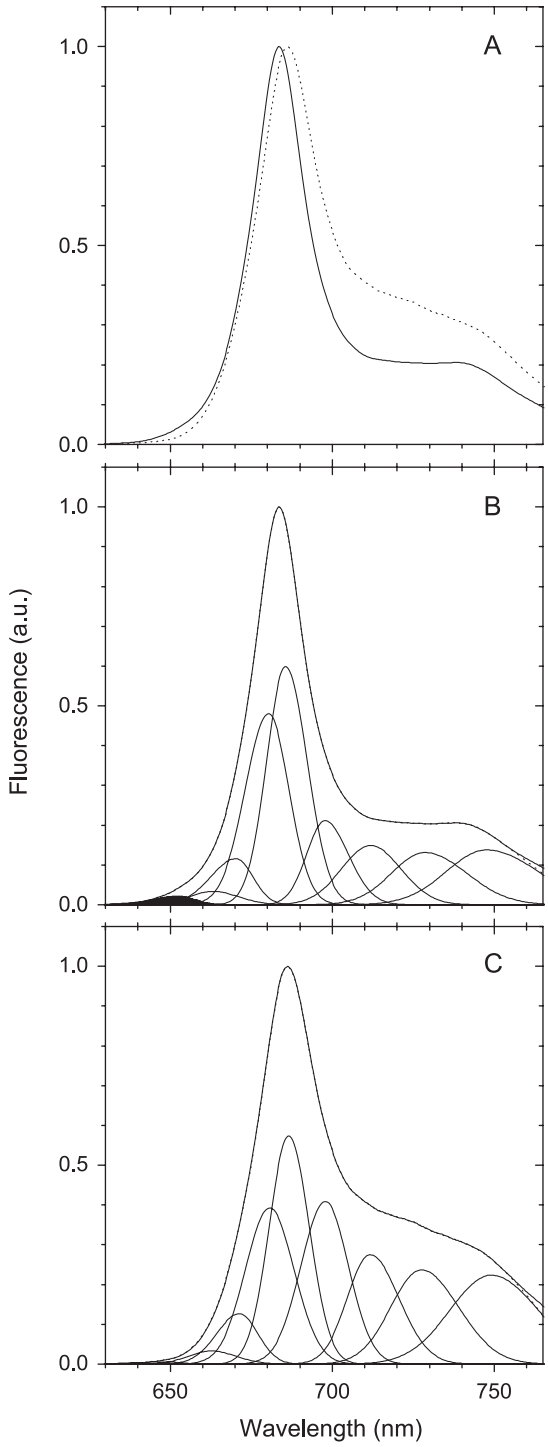

Fig. 1. Steady state fluorescence measurements of barley thylakoids at the F_0 level of fluorescence. (A) Solid curve, wild type; dotted curve, chlorina mutant. (B) Gaussian subband fit for the wild-type measurement. The 650 nm subband, corresponding to chlorophyll *b* emission is indicate in black. (C) Gaussian subband fit for the chlorina mutant. Spectra were accumulated over a large number of measurements with the accumulated counts at the peak value of the wild type being 302,000 and the chlorina mutant 281,000. All spectra are presented on a scale that is normalised to these peak values.

were measured under conditions of open PSII traps (F_0) with excitation into the Soret band of chlorophyll a (440 nm). Under these conditions, chlorophyll *b* can only be populated by energy transfer from the excited chlorophyll *a*. It can be readily seen that the mutant spectrum is red shifted at the peak wavelength and has a more intense long wavelength emission. Both these differences are, at least in part, due to the greater contribution of PSI and its associated red shifted chlorophyll forms to the chlorina spectrum. Of particular interest is the small difference in the short wavelength tail, near 650 nm, where the wild-type emission is significantly more intense than that of the chlorophyll-*b*-less mutant, after the spectra are normalised each to its maximal value. We stress that spectra were accumulated from a very large number of samples measured from four different thylakoid preparations in the optical multichannel measuring device in order to accumulate over 300,000 counts at the emission maximum and around 8500 counts at 650 nm in the wild-type spectrum and a similar number at the peak position with chlorina thylakoids. In accordance with our above-discussed earlier studies [13,38], this difference is attributed to the weak chlorophyll *b* emission, peaking near 650 nm, present in the wild-type spectrum and absent in the mutant spectrum (Fig. 1A). This chl *b* emission can be described by a Gaussian subband near 650 nm, which is present only in the wild-type emission spectrum (Fig. 1B,C). This subband carries about 1.3% of the total Q_y oscillator strength, when the redmost subbands, representing vibrational transitions, are excluded. This value can readily be compared with an approximation for the equilibrium value determined by the standard Boltzmann equation:

$$N_b/N_a = (g_b/g_a)\mathrm{e}^{-\Delta E/kT}$$

where $N_{b,a}$ are the equilibrium populations of excited states in chlorophylls *b* and *a*; $g_{a,b}$ are the absorption weighting factors of chlorophylls *a* and *b*. These are approximated from the known chlorophyll *a*/*b* ratio of 2.5–2.6 and considering that the Q_y oscillator strength of chlorophyll *b* is about 0.7 that of chlorophyll *a* in solvents [40]; ΔE is the energy gap between chlorophylls *b* and *a*, which is taken as 2.75 kT, where kT is the molecular thermal energy. In this way, one estimates that chlorophyll *b*, present in the outer antenna, is expected to have an equilibrium population of about 1.2%, rather close to the fluorescence, Gaussian subband approximation of 1.3%. These considerations demonstrate that the excited state population of chlorophyll *b* of the outer antenna is not underpopulated with respect to its equilibrium value. Thus, it would seem correct to conclude that rapid and irreversible energy transfer from the outer antenna complexes to the core does not occur.

3.2. Influence of outer antenna on the effective photochemical trapping time of PSII

In order to directly determine the effect of the outer antenna on the effective photochemical trapping time, $(k_{\mathrm{cs}}^{\mathrm{eff}})^{-1}$, we have measured the fluorescence decay of thylakoids of the chlorina mutant and those of its wild type

between 670 and 740 nm, under photochemically active conditions (F_0), and analysed the data globally, as described in Materials and methods, to obtain the set of amplitudes and lifetimes. The aim is to identify those lifetime components associated with PSII in order to calculate $(k_{cs}^{eff})^{-1}$. This is achieved, as we have previously demonstrated [41], by determination of the so-called "average lifetime" value ($\tau_{av}=\sum A_i\tau_i/\sum A_i$) that can be represented by $(k_{cs}^{eff}+k_t)^{-1}$, where k_t are the trivial relaxation processes associated with each chlorophyll. In order to clarify the exact meaning of this parameter, we consider a simple two state system $A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} B \xrightarrow{k_2}$, where A represents the initially populated and coupled chlorophyll system and B the primary donor. In this case, the rate process k_2 is thus the primary photochemistry, where k_2 represents the effective rate constant for any number of reversibly relaxing primary radical pair states. We do not enter into any of the details of these processes, which are discussed in Refs. [1,22]. From the well-established analytical solutions for the eigenvalues and eigenvectors of this two-level system, one readily demonstrates that $\tau_{av}^{-1}\equiv k_1k_2/(k_{-1}+k_2)$ [42]. The overall rate of depopulation of this system is given by $k_2[B]_t$, where $[B]_t$ is the concentration of B at any time. For the steady state, $[B]_{ss}=k_1k_2[A]_{ss}/(k_{-1}+k_2)=\tau_{av}^{-1}[A]_{ss}$. Thus, it is clear that τ_{av}^{-1} can be considered to be the effective rate constant for depopulation of the A level. In the present study, this parameter will be referred to as the effective photosystem decay rate constant, k_{cs}^{eff*}, as it represents the decay of excited states in the whole photosystem, where $k_{cs}^{eff*}=k_{cs}^{eff}+k_t$. It is generally assumed that $k_t \ll k_{cs}^{eff}$. We wish to emphasise that $(k_{cs}^{eff*})^{-1}$ is not given by the "mean lifetime" value ($\tau_m=\sum A_i\tau_i^2/\sum A_i\tau_i$), which is the real "centre of gravity" of the lifetime distribution. This point is important, as we will show below that Gilmore et al. [24,25] used the τ_m and thus greatly overestimated the photochemically derived decay time for the chlorina mutant.

It is well established that five exponential DAS components are necessary to adequately describe the fluorescence decay in wild-type material under photochemically active conditions (e.g., Refs. [10,43]) and while the three-component description of Briantais et al. [23] may have been adequate from a purely numerical point of view, for the chlorina mutant, as pointed out above, a PSI component was missing and must have been mixed in with the PSII decay components. The same applies to the lifetime distribution analysis of Ref. [24]. We have therefore studied the fluorescence decay of this mutant in detail. We started out by using three decay components, following Ref. [23]. While the lifetime components obtained are not very different from those presented by these workers Table 1 of Ref. ([23]), it can be seen that the plot of the residuals (Fig. 2) is unsatisfactory and the χ^2 value is high. On the other hand, when four exponentials are used, the errors are greatly improved (Fig. 2) and the fit may be considered satisfactory from a purely numerical point of view. However, in the four-component description a DAS component

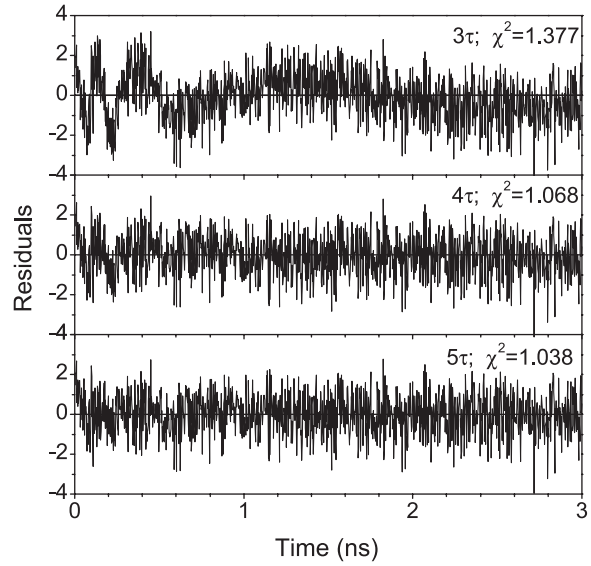

Fig. 2. Comparison of the residuals for the fluorescence decay of thylakoids of the chlorina barley mutant that was described but the sum of 3, 4 or 5 exponential processes. The χ^2 values are 1.377, 1.068 and 1.038, respectively.

corresponding to PSI is absent (data not shown), i.e., a component with pronounced long wavelength amplitude values and a lifetime in the 80–100-ps interval, characteristic of this photosystem (e.g., Refs. [41,44]). When the fit was performed with five exponential components, this problem associated with PSI was resolved. From the point of view of the fit residuals, there is little difference between the four- and five-component description. However, the five-component decomposition has a distinct PSI decay, as judged by its DAS band shape, of lifetime close to 80 ps. We therefore conclude that five exponential decays are required to satisfactorily describe the fluorescence decay of chlorina thylakoids under photochemically competent conditions. That this yields a good description of the measurement is shown in Fig. 3A, where the lifetime fit, convolved with the instrumental response, is presented along with the instrument response itself and the residuals plot of the fit. The DAS description for measurements performed on a large number of different thylakoid preparations is presented in Fig. 4 with lifetime components near 500, 200, 80, 30 ps and a low-amplitude nanosecond component.

The band shapes of the 534±18, 186±19 and 33±5 ps components are very similar and are thus all attributed to PSII decay components. The presence of a PSI transfer component, seen for the decay of wild-type thylakoids (Fig. 6), is not evident. Despite this, we have checked out whether the 33-ps decay, of dominant amplitude, for the chlorina thylakoids, may in fact be a mixed PSII–PSI decay. This was achieved by purification of a PSII core complex binding CP43, CP47 and the D1D27cytb559 complex [45]. A typical decay pattern for this purified PSII core at 680 nm is 564 ps (15.4), 142 ps (32.4), 38 ps (52.2), where the numbers in brackets are the relative amplitudes that sum to

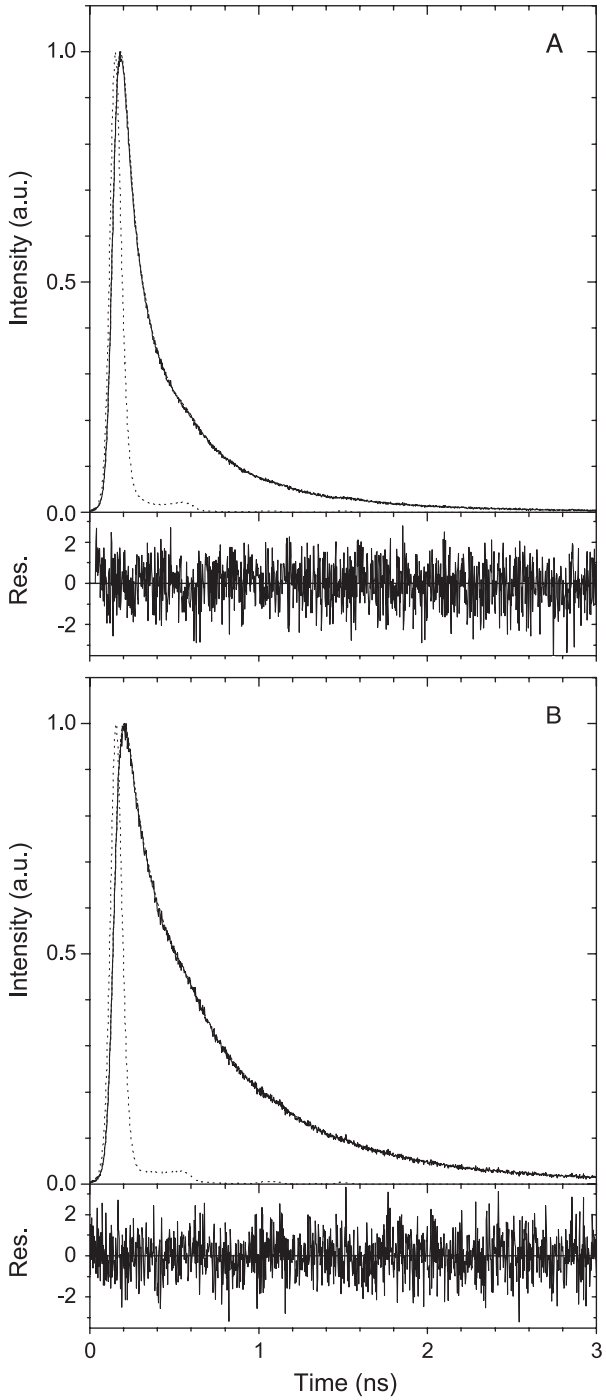

Fig. 3. Typical fluorescence decay measurement of chlorina barley mutant thylakoids (A) and wild-type thylakoids (B) at the F_0 level of fluorescence (noisy lines) together with the five exponential components fits (smooth lines). The instrument response is also shown (dotted line) and the fit residuals.

100 (manuscript in preparation). It is therefore evident that the 30–40-ps decay component, of dominant amplitude, is a characteristic of the PSII core. The low-amplitude nanosecond component seems to be associated with a small fraction of closed centres as it is sensitive to the laser light intensity, increasing with increasing intensity (data not shown). Thus, the approximately 500, 200 and 30 ps DAS components have therefore been used to calculate the mean $(k_{cs}^{eff*})^{-1}$ values for the chlorina core over the wavelength range 670–740 nm (Fig. 5).

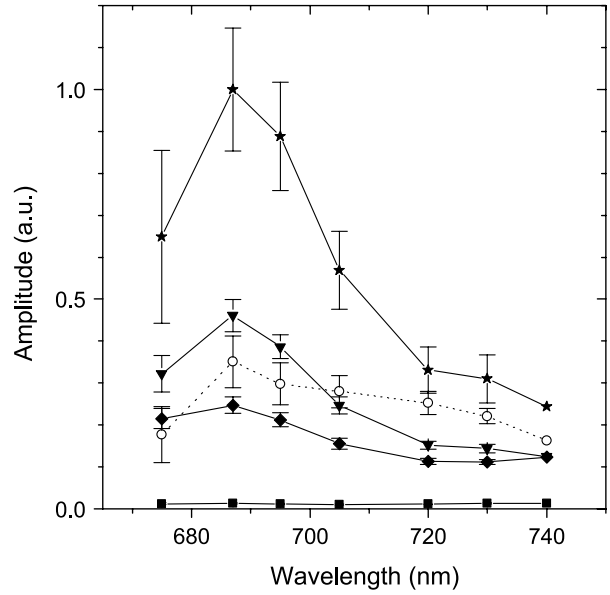

Fig. 4. Decay associated spectra for the chlorina f2 barley mutant measured near the F_0 level. Stars, 33±5 ps; triangles, 186±19 ps; diamonds, 534±18 ps; circles, 78±18 ps; squares, 2.05±0.32 ns. Errors are the standard lifetime deviations deriving from a large number of different thylakoid preparations. The error bars on the decay-associated spectra are the intersample amplitude standard deviations deriving from a large number of measurements made on different thylakoid preparations.

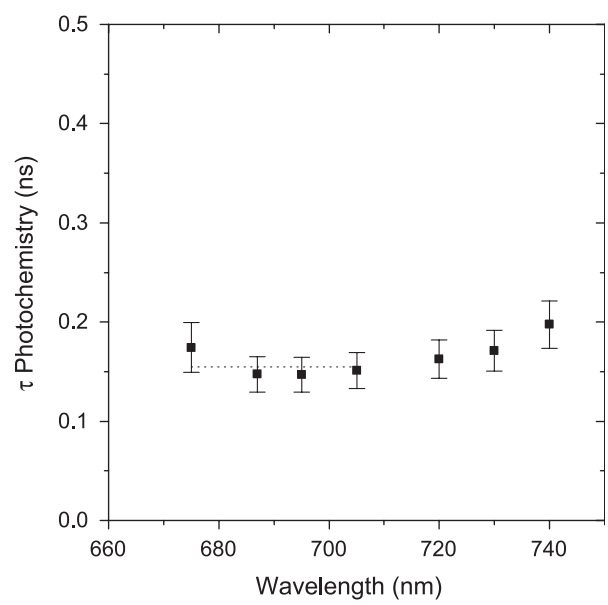

Fig. 5. The effective trapping time, $(k_{cs}^{eff*})^{-1}$, for the photosystem II core of the chlorina barley mutant thylakoids. Data have been calculated from the decay-associated spectra of Fig. 4, as described in the text, using the 33, 186 and 534 ps components. Errors are the convolutions of the intersample errors with those associated with the lifetime spread. The dashed line indicates the mean value for the four indicated wavelengths.

Before proceeding further, it is appropriate to discuss the errors associated with these measurements. It is customary in studies on fluorescence lifetime experiments, to present data, and the associated statistics, for single sample measurements. While this approach is above criticism for well-defined chemical samples, it may not be the case for biological preparations that are well known to display variability. In the present study, we have therefore performed a relatively large number of measurements on different preparations of the same sample type, each of which was analysed according to the standard global procedure, and the intersample errors on both lifetime and amplitude were determined for the fits displaying the lowest χ^2 values. The amplitude errors are the error bars associated with each DAS data point in Fig. 4 and for all, except the shortest lifetime component, are usually about ±12% of the mean value. In the case of the approximately 30-ps component, the errors fall within a ±20% interval. The lifetime errors are indicated in the figure legend. We believe that this provides a correct representation of the sample parameters. This point is not trivial as it explicitly recognises intersample variability, which should not be ignored when fluorescence decay data are used for model calculations. The importance of obtaining an exact convergence of model calculations to a single sample measurement should not be overestimated.

The intersample errors, discussed above, are not the only source of experimental uncertainty, as the five exponentials lifetime fit presented above for chlorina, and below for wild-type thylakoids, do not represent a completely unique description of the fit minimum in any but the formal statistical sense of the lowest χ^2 value. These minima are, in fact, in both cases, rather broad. From single sample measurements of chlorina we have estimated, accepting descriptions in which the fit residuals, χ^2 and DAS band shapes were almost indistinguishable (range of χ^2 values, 1.038–1.049) from those presented in Fig. 4, which is near the centre of the range of lifetime values, that the fit minimum is approximately 180-ps broad (full width) for the 534±18 ps decay, 60 ps for the 186±19 ps and 20 ps for the 33±5 ps one. This means that within the lifetime intervals of 440–620, 160–220 and 20–40 ps for the three PSII decays we are not really able to distinguish between the different descriptions. However, for the main aim of this study, that of determining values for $(k_{cs}^{eff*})^{-1}$ for PSII, this is, fortunately, not very important, as we show below that this large spread of lifetime solutions has only a quite small effect on the $(k_{cs}^{eff*})^{-1}$ values calculated for PSII. This is because the amplitudes (eigenvectors) and lifetimes (eigenvalues) are cross-correlated.

The $(k_{cs}^{eff*})^{-1}$ values, determined as the mean values of the single experiments, with associated errors are presented in Fig. 5. The $(k_{cs}^{eff*})^{-1}$ values for the 670–700 nm interval, where PSII emission is greatest, are spread over an interval of 0.14±0.019–0.18±0.019 ns, where the errors are the intersample ones. As the intersample spread of the mean values in the 0.14–0.18 interval follows a normal statistical distribution (unpublished data), we can represent the mean value as approximately 0.16±0.019 ns. If these intersample errors are then convoluted with the lifetime spread errors, also demonstrated to follow a normal statistical distribution (unpublished data), we obtain 0.16±0.021 ns. These are the error bars in Fig. 5. As can be seen, the error distribution is almost entirely associated with the intersample variation. The $(k_{cs}^{eff*})^{-1}$ value we obtain seems to be slightly greater than the value of 0.12 ns, which may be calculated from the single-sample measurements of previous experiments with isolated PSII core complexes [22,30], though in these cases we do not know the intersample errors and the spread of the decomposition data. We have purified PSII core particles from maize plants by the procedure of Ghanotakis et al. [45] and find $(k_{cs}^{eff*})^{-1}$ values for different preparations, which fall in the 0.12–0.18 ns range (unpublished data), apparently in agreement with the value of 0.16±0.021 ns for the chlorina core.

In order to determine the influence of the outer antenna on $(k_{cs}^{eff*})^{-1}$, similar experiments to those described above for the chlorina mutant were conducted with samples of wild-type barley thylakoids under conditions of photochemically active centres (F_0 level) and the DAS components determined for measurements performed on a large number of different thylakoid preparations is presented in Fig. 6 with the associated intersample errors. As has been commonly encountered by others (e.g., [10,43]), five exponential decays provide a good description of the fluorescence decay (Fig. 3B). In the present case, the

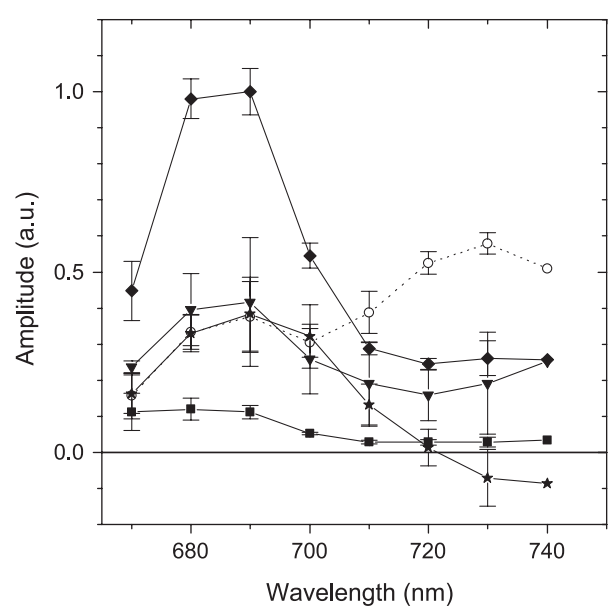

Fig. 6. Decay-associated spectra of wild-type barley thylakoids measured near the F_0 fluorescence level. Stars, 54±3 ps; triangles, 230±8ps; diamonds, 550±30 ps; circles, 115±3 ps; squares, 1.40±0.13 ns. Errors are the standard lifetime deviations deriving from a large number of different thylakoid preparations. The error bars on the decay-associated spectra are the intersample amplitude standard deviations deriving from a large number of measurements made on different thylakoid preparations.

nanosecond component displays greater amplitudes than in the chlorina mutant, presumably because of the large increase in optical cross section of PSII in the wild type leading to a greater population of PSII with closed centres. It is interesting to note that the PSI component, with a DAS lifetime of 115±3 ps, has more pronounced and somewhat red shifted long wavelength fluorescence amplitudes than that of the chlorina mutant. Similar observations were previously made by Gilmore et al. [25]. As they pointed out, this result is not unexpected, as Knoetzel et al. [37] have shown that lhca4, and its associated long-wavelength emission, is missing in this mutant. It is noteworthy that the difference spectrum band shape of the PSI component for the wild type minus that of the chlorina mutant, after normalisation in the 680–690 region (data not shown), resembles the long wavelength emission form, F735 [46,47]. We wish to add that this PSI DAS for wild-type thylakoids can be almost perfectly overlain with the steady state emission of isolated PSI-LHCI (data not shown; [48]), which lends credence to this DAS description. From the DAS band shapes, it is evident that both the approximately 500-ps and the 200-ps decay components are associated with PSII. The lifetime spread for the longer decay is from 490 to 620 ps and 190 to 260 ps for the shorter decay components, respectively. These data are in quite good agreement with earlier experiments performed by others (e.g., [10,43]). Given the quite broad overlap of the spread of lifetime values with those of the chlorina mutant, it is not possible to determine whether the presence of the outer antenna is accompanied by increases in the lifetimes of the two principle PSII decays, as is expected from compartmental modelling of PSII (data not presented). On the other hand, attribution of the fastest decay, which has a spread of approximately 40–60 ps, to one or other of the photosystems is less clear. The low and negative DAS amplitudes above 730 nm indicate that there is a PSI energy transfer contribution ("bulk" chlorophyll to red forms) and this has been shown to occur in isolated PSI with approximately 20 ps dynamics [44,49]. It also seems probable that there is a PSII contribution to this component, particularly as this DAS decay time increases significantly upon closure of the PSII traps (F_M; data not shown). Thus, we conclude that the 40–60 ps DAS component in wild-type barley is a mixture of both PSI and PSII decays. Of particular interest for the PSII contribution to this decay component is the dramatic decrease in its relative amplitude with respect to chlorina, in which a component of approximately this lifetime is the dominant one. As it is not possible to determine the relative contributions of the two photosystems, this leads to a measure of uncertainty in the values of $(k_{cs}^{eff*})^{-1}$ estimated for PSII. For this reason, two series of $(k_{cs}^{eff*})^{-1}$ values are presented in Fig. 7, each with the spread of decomposition mean values and the associated intersample errors. The mean $(k_{cs}^{eff*})^{-1}$ values for the 670–700 nm interval, where PSII emission is greatest, are spread over ranges of 0.29–0.35 ns and 0.40–0.42, with and without the fast, mixed, PSI–PSII component, respectively. The intersample lifetime errors for the 670–700-nm interval are approximately ±10% of the mean value. Thus, by convolution of the mean distribution with the lifetime spread errors, we obtain values of 0.32±0.036 and 0.41±0.044 ns. These data for PSII in which the outer antenna is present are slightly higher than the single-sample measurements of $(k_{cs}^{eff*})^{-1} \approx 0.30$ ns, which may be calculated from earlier experiments on thylakoids (e.g., Refs. [10,43]). Thus, the presence of the outer antenna of PSII in barley thyakoids increases the measured effective *photosystem* trapping rate $[(k_{cs}^{eff*})^{-1}]$ by a factor that lies between 2.0 and 2.6. As discussed at the beginning of this section, these values are based on the τ_{av} values. For comparison, we now mention the approximate values for τ_m, as this is the parameter used by Gilmore et al. [24,25]. For the chlorina mutant τ_{av}=0.16 and τ_m=0.36. This latter value is similar to that found by Gilmore et al. [25], with the large difference between the two chlorina τ values being due to the high-amplitude 30–40-ps component. On the other hand, as this component is greatly reduced in the wild type, the τ_{av}=0.36 and τ_m=0.42 values are rather close. Thus, on the basis of the τ_m values, we do not see a great difference between the two types of thylakoids. The difference between our results and those of Gilmore et al. [24,25] may therefore be more apparent than real and due to their choice of τ_m. In fact, if one inspects their data closely, e.g., Gilmore et al. [25] Figs. 3 and 4, the initial decay rate is in fact faster in their chlorina measurements than in the wild-type data.

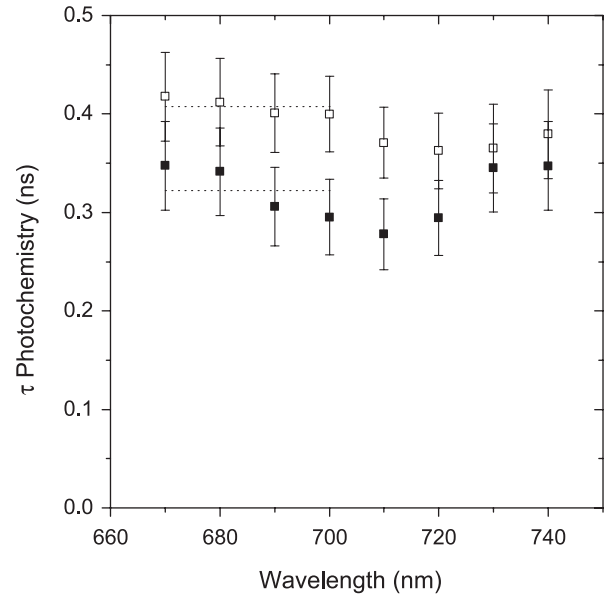

Fig. 7. The effective trapping time, $(k_{cs}^{eff*})^{-1}$, for photosystem II of wild-type barley thylakoids. Data have been calculated from the decay-associated spectra of Fig. 6, as described in the text, using the 54 p, 230 and 550 ps components (lower series) and the 230 and 550 ps components (upper series). Errors are the convolutions of the intersample errors with those associated with the lifetime spread. The dashed lines are the mean values for the four wavelengths indicated.

The above results and discussion concern $(k_{cs}^{eff*})^{-1}$, where $(k_{cs}^{eff*})^{-1}=(k_{cs}^{eff}+k_t)^{-1}$. In order to obtain the exact value of the effective photosystem photochemical rate $(k_{cs}^{eff})^{-1}$, we must correct for the trivial decay processes. The photochemical yield (Φ) of a photosystem is given by $\Phi=(k_{cs}^{eff})\cdot(k_{cs}^{eff}+k_t)^{-1}$. As Φ is expected to be very high for the PSII core (e.g., [50]), for Φ=0.93 we estimate that the $(k_{cs}^{eff})^{-1}$=0.17±0.017 ns. For the wild-type PSII, binding the outer antenna, experimental values for Φ are often reported in the 0.80–0.85 range. Taking Φ=0.83, the values for the two intervals defining the mean experimental values for this parameter are 0.39±0.036 and 0.49±0.044. We therefore conclude that the presence of the outer antenna in barley thylakoids does in fact increase the effective photosystem photochemical trapping time $(k_{cs}^{eff})^{-1}$ by a factor that lies within the range of $(k_{cs}^{eff})_{c+oa}^{-1}/(k_{cs}^{eff})_c^{-1}$=2.3–2.9. It is interesting to compare this range of values with those expected for a perfectly thermalised PSII. This may be done as follows. The rate of primary photochemistry is given by the product of the excited state population of the primary donor (P^*) with the effective intrinsic rate constant for charge separation (k_{cs}), i.e., $k_{cs}^{eff}=P^*k_{cs}$. It should be emphasised that $k_{cs}=k_2$ of the two-state system discussed above and, as such, is an effective constant that includes reversible photochemistry via relaxing radical pair states. For equilibrium conditions, within the pigment system, P^* is approximated by a population weighted Boltzmann term $P^*=\alpha N^{-1}e^{-\Delta E/kT}$, where α is the number of pigments in the primary donor, N is the number of pigments in the antenna, ΔE is the mean energy gap between the antenna and the primary donor chls and kT has its usual meaning. For the rate ratio for photochemistry of the core with respect to the core plus outer antenna, we can therefore write (Eq. (1))

$$\left(k_{cs}^{eff}\right)_c/\left(k_{cs}^{eff}\right)_{c+oa}=\left(P_c^*/P_{c+oa}^*\right)\left(k_{cs,c}/k_{cs,c+oa}\right). \quad (1)$$

In the assumption that the outer antenna does not influence k_{cs}, $k_{cs,\ c}=k_{cs,\ c+oa}$. However, we cannot be sure of this a priori so we distinguish the two k_{cs} parameters.

For equilibrium conditions, Eq. (1) can be rewritten

$$\left(k_{cs}^{eff}\right)_c/\left(k_{cs}^{eff}\right)_{c+oa}=N_c^{-1}/\left[\left(N_c+\left(N_{oa}\cdot e^{-\Delta E/kT}\right)\right]^{-1}\cdot k_{cs,c}/k_{cs,c+oa}, \quad (2)$$

where ΔE is the mean energy gap between the core and the outer antenna and N_c and N_{oa} are the number of pigments in the core and outer antenna, respectively. It was previously shown by a detailed analysis of the distribution of absorption subbands and the steady state fluorescence characteristics of all chlorophyll–protein complexes comprising PSII [12,13] that the core has a mean energy that is slightly (0.29 kT) lower than that of the outer antenna at room temperature. This is due to the presence of chl *b* in the outer antenna and not to the chl *a* spectral forms. It is generally accepted that PSII binds between 200 and 250 chl molecules. In order to calculate the expected outer antenna effect on $(k_{cs}^{eff})^{-1}$, we assume 230 chls for the core plus outer antenna and either 40 or 50 molecules for the core. The value of 40 is close to recent crystallographic structural data [15–17], though 50 is closer to the older biochemical data (e.g., Ref. [14]) and also takes into account the possibility that the chlorina core may bind 1–2 of the minor outer antenna complexes binding pigments. Using the energy gap of 0.29 kT between the core and the outer antenna and these chl numbers, from Eq. (2), we thus obtain values for $N_c^{-1}/[(N_c+(N_{oa}e^{-\Delta E/kT})]^{-1}$, which lie between 3.7 and 4.6, which is significantly higher than the experimental value of $(k_{cs}^{eff})_c/(k_{cs}^{eff})_{c+oa}$=2.3–2.9. For the value of 0.29 kT for the core-outer antenna energy gap, unfortunately we have no idea of the errors, as estimates were made on single samples only, i.e., single chlorophyll–protein complex preparations (as is usually done). However, the $N_c^{-1}/[(N_c+(N_{oa}e^{-\Delta E/kT})]^{-1}$ ratio is not very sensitive to small changes in this value. For example, even in the unlikely case of errors of ±100%, i.e., ΔE=0.29±0.29 kT, the expected values lie in the range 3.7±0.7–4.6±0.8, which remain significantly higher than the experimental values (2.3–2.9). In principle, two possibilities may be invoked to explain this discrepancy. First, the equilibrium assumption may lead to an overestimation of the $N_c^{-1}/[(N_c+(N_{oa}e^{-\Delta E/kT})]^{-1}$ ratio due to an imbalance between the rate of slow energy transfer into the photochemical trapping chls with respect to the rate of primary photochemistry itself and this could, in principle, be greater for the core than for the core plus outer antenna. While this kind of trap induced equilibrium perturbation of the primary donor state is possible, it may be readily shown that in the case of slow energy transfer from CP43, 47 to the reaction centre complex [6], the P^*_c/P^*_{c+oa} ratio is scarcely changed with respect to the equilibrium one. This possibility thus seems unlikely. The second explanation is that the ratio $k_{cs,c}/k_{cs,c+oa}$ is sensitive to the presence of the outer antenna. In terms of this explanation, from Eq. (2), $k_{cs,c}/k_{cs,c+oa}$= 0.62–0.63. These data therefore suggest that the presence of the outer antenna may in fact influence the primary photochemical rate (k_{cs}), and the present data indicate that it may be increased by approximately 50% with respect to that in which only the core antenna is present. As the exact physical nature of primary charge separation is not yet known, we are unable to speculate as to what form such an effect might take, though small changes in reaction centre cofactors configuration upon binding of the bulky outer antenna complexes may occur.

4. Concluding discussion

In this final discussion, we address the general problem of the biological function of the outer antenna of PSII. In the present paper, it is demonstrated that its presence leads to a decrease of the overall rate of primary photosystem photochemistry, k_{cs}^{eff}, from 0.17^{-1} ns^{-1} to values that lie in the range of 0.39^{-1} to 0.49^{-1} ns^{-1}, i.e., by 56–65%. This

decrease in the photochemical rate by almost 2/3 seems, at first sight, to be rather large. However, it is the photochemical efficiency, and not the photochemical rate in itself, which is the important parameter from a biological point of view and this decreases from about 0.90–0.95 for the core to 0.80–0.85 for the complete photosystem, i.e., by a factor of about 10–15%, much less than that for the photochemical rate. This decrease would seem to be quite a small "price to pay" for the large increase in optical cross section of PSII, which increases from about 40 chlorophyll molecules in the core to between 200 and 250 with the outer antenna, i.e., by a 5–6-fold factor. It should be mentioned, however, that for mature leaves the relation between photon absorption and chlorophyll content is markedly nonlinear due to their high chlorophyll content (30–40 μg cm^{-2}) and thus the 5–6-fold increase in PSII cross section associated with the outer antenna will result in a somewhat smaller increases in the photon absorption flux of PSII, which, it is easily shown, is expected to increase by about a 3-fold factor, assuming 200 chlorophylls per PSI and PSII. In addition, the presence of the outer antenna of PSII leads to the optical cross sections of the two photosystems under normal "daylight" conditions being almost equal [51], which is the condition for maximal photosynthetic electron transport efficiency.

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