Biochimica et Biophysica Acta, 935 (1988) 41–52
Elsevier

BBA 42818

Time-resolved chlorophyll fluorescence studies on pigment-protein complexes from photosynthetic membranes

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(Received 2 March 1988)

Key words: Chlorophyll fluorescence; Excitation transfer; Photosystem I; Photon counting; Pigment-protein complex; Photosystem II

Room-temperature single-photon timing measurements at low excitation energies have been carried out on Photosystem II (PS II) pigment-protein complexes containing 40–70 chlorophylls per reaction centre (RC) as a function of PS II trap closure, Chl fluorescence emission spectra and detergent concentration. The lifetime and amplitude values obtained, using a four exponential model, have been compared with the values exhibited by the isolated light harvesting Chl *a*/*b* complex of PS II (LHC II) and a Photosystem I (PS I) complex containing 100 Chl/RC. The results suggest that the two rapid decays seen in the PS II preparations arise from the 47 kDa ($\tau = 54$ ps) and 43 kDa ($\tau = 180$ ps) Chl containing polypeptides but that in vivo these two proteins give rise to a single variable decay. The differences are discussed in terms of a perturbation in excitation energy migration by detergent molecules, although the presence of high detergent levels does not alter the kinetic and amplitude characteristics of these decays. The two long-lived decays seen in the PS II-core complexes have the same lifetimes and characteristics with respect to detergent addition as the isolated LHC II, suggesting that they arise from LHC II contamination. Unlike the two fast decays, the two long-lived components are extremely sensitive to detergent concentration. The requirement of two decay components in LHC II suggests the presence of a heterogeneous population. Isolated PS I also gives rise to four decay components with 99% of the excitation energy located in pigment-proteins which exhibit lifetimes of 110 ps and 27 ps. The lifetime and amplitude values have been used to calculate estimations for the single-step transfer time between interacting Chl molecules, the number of visits required before trapping takes place and the quantum yield of photochemistry.

Introduction

The primary processes of photosynthesis are characterised by the efficient absorption of light energy, by a complex pigment-protein system located in the lipid matrix of the thylakoid membrane, and its rapid transfer to the reaction centres (RC) where charge stabilisation and separation occur. During exciton migration in the antenna, there is a finite probability that it will decay either by non-radiative processes or by fluorescence be-

Abbreviations: Chl, chlorophyll; PS, Photosystem; LHC, light harvesting Chl *a*/*b* complex; PS, Photosystem; RC, reaction centre; Q_A, primary stable electron acceptor of PS II; F_M, maximum Chl fluorescence; F_0, minimum Chl fluorescence; A_{exp}, preexponential factor (amplitude); Mes, 4-morpholine-ethanesulphonic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; OGP, octylglucopyranoside; *JT*, transfer time between chlorophylls; *NV*, number of visits before trapping; *FPT*, first passage time; *QY*, quantum yield.

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fore trapping can take place. However, photosynthesis has been shown to be highly efficient [1] and such energy losses constitute only minor deactivation pathways. Nevertheless, chlorophyll (Chl) fluorescence has become an important and useful tool in trying to understand energy-transfer processes [2–4], pigment orientation [5] and protein organisation [6]. One method by which energy transfer is currently being investigated is by the analysis of Chl fluorescence decay kinetics using single photon counting coupled with low-intensity picosecond laser excitation, to stop quenching by singlet–singlet annihilation [7]. The form of the overall decay, exhibited by intact photosynthetic organisms containing LHC II, is multi-exponential and can usually be statistically well-defined by three kinetic components [8,9] with lifetimes in the order of 50 ps, 200–850 ps and 450–2000 ps according to the state of the primary stable electron acceptor of PS II (Q_A) [10]. More recent studies suggest that this is an oversimplistic model [3,11] and that the overall decay could contain, at least, five individual decay components [3,12]. This apparent complexity is not surprising, since the photosynthetic light-capturing system is also complex, containing two types of reaction centre-core antenna complex (PS I and PS II) each associated with their own peripheral antenna systems (LHC I and LHC II) [13]. The situation is further complicated by the reported structural heterogeneity of PS II into α and β centres [14] and two pools of LHC II differing in polypeptide composition [15].

For a mechanistic description of the overall decay in terms of energy transfer and trapping processes it is necessary to assign each decay component to a functional constituent of the light-harvesting system. This requires the simplification of the 'intact' membrane, due to its complexity. One approach is the use of well-characterised mutants lacking one or more of the pigment-containing complexes [3,16–19]. The results suggest that each of the 'original' three decays have multiple origins [16,17] and that the overall decay exhibited by 'intact' thylakoid membranes is the sum of five different components [3,19]. Our studies [19] suggest that the two long-lived decays (F_M is approx. 2 and 1 ns) arise from LHC II, while PS I produces two rapid decays (less than 200 ps) and the PS II-core-complex a rapid variable decay (40–250 ps). A second approach is to isolate either membranes enriched in certain complexes [20] or the individual pigment-protein-complexes [21–25]. However, this method could lead to complications due to artifacts brought about by the isolation procedures or from the detergents required to produce such preparations. It has already been reported that the average lifetime [26] and the individual component lifetimes of LHC II [21,24] are affected by the presence of different detergent concentrations as well as the type of detergent used [21,26]. Furthermore, the various in vivo deactivation pathways might be perturbed by the isolation of the individual complexes. A third approach is to use treatments known to alter the organisation of the various pigment-protein complexes, like the manipulation of salt levels [27–29], bringing about protein phosphorylation [29,30] or state transitions [29]. This has recently led us to confirm the LHC II origins of the two long-lived decays [29].

In this present work we describe Chl fluorescence decay measurements carried out on isolated PS II-core complexes containing between 40 and 70 Chl/RC, LHC II and PS II in order to reduce the complexity and allow the assignment of individual decays to the constituent parts of the light-harvesting system. For the three types of PS II-core-complex investigated, the effect of PS II trap closure and detergent concentration on the lifetimes and amplitudes derived from a free-running four-exponential model are presented. These results are compared with the kinetic parameters obtained from LHC II, in different aggregation states brought about by modifying the detergent concentrations, and PS I. Finally, the equations of Pearlstein [31], which model excitation migration and trapping, are combined with the lifetime and amplitude data and the antenna sizes to calculate estimates for the single-step transfer time between chlorophylls, the number of visits to the reaction centres before trapping and the quantum yield of photochemistry.

Materials and Methods

The three types of PS II-core complexes were prepared by sucrose density gradient centrifugation; from spinach chloroplasts using dodecyl maltoside as in Ref. 32 which yielded highly active

oxygen-evolving complexes (PS II-70), from thylakoids of a double mutant of *Chlamydomonas reinhardtii* lacking PS I and ATPase as in Ref. 33 (PS II-50) or from pea chloroplasts using Triton X-100 as in Ref. 34 which produced complexes requiring the addition of PS II electron donors to carry out PS II electron-transfer reactions (PS II-40). The PS I complex was isolated using Triton X-100 as described in Ref. 35. LHC II was isolated, in an aggregated form, by density gradient centrifugation as in Ref. 36. LHC II, in a monomeric form, was isolated by electrophoretic separation as in Ref. 37 or by the addition of digitonin (1%, v/v) and octylglucopyranoside (OGP, 4% v/v) as described in Ref. 38.

For experimentation the PS II complexes, PS II-70 and PS II-40, were resuspended at 5 μg Chl/ml in 20 mM Mes (pH 6.5), 300 mM sucrose, 5 mM $CaCl_2$ and 15 mM NaCl whereas PS II-50 was diluted to 5 μg Chl/ml in 50 mM Hepes (pH 7.5). LHC II (aggregated) and PS I were resuspended at 20 μg Chl/ml in 50 mM Hepes (pH 7.5) and 5 mM $MgCl_2$.

All Chl fluorescence decay measurements were carried out and analysed using the apparatus and criteria already described elsewhere [10,11]. Fluorescence was measured at 90° to the excitation beam in a 2 × 2 mm cuvette. The decays were accumulated until 8000 counts at the maximum and deconvoluted by means of a least-square program using the Marquardt search algorithm for non-linear parameters. Time-resolved emission spectra were calculated using the method in Ref. 11. All measurements were carried out on stationary samples. Different degrees of PS II trap opening were generated as follows; for PS II-70 by the addition of different concentrations of atrazine or by the addition of different concentrations of potassium ferricyanide (5–50 μM), for PS II-40 and PS II-50 by the addition of 100 μM potassium ferricyanide. The effect of detergent concentration on the kinetic parameters exhibited by PS II-70 and PS II-50 were carried out by the addition of dodecyl-β-D-maltoside (0–1 mM) to the resuspension media.

Results

Fig. 1 shows the relationship between total fluorescence yield and average lifetime as PS II reaction centres are closed to photochemistry in a PS II-core complex preparation containing 70 Chl/RC (PS II-70). The trend observed in Fig. 1 has been previously reported for the 'intact' photosynthetic system [10].

Fig. 2A shows the evolution of the lifetimes of the four exponential decay components, required to fit the overall decay of PS II-70 complexes, as PS II reaction centres are closed (as indicated by the average lifetime, see Fig. 1). It can be seen that PS II-70 produces three variable components and a rapid, constant lifetime decay. Unlike the 'intact' system a long-lived decay is seen at F_0. Fig. 2B shows the changes in the relative fluorescence yield of each component depicted in Fig. 2A, as a function of average lifetime. The fast, constant lifetime decay, between F_0 and F_M, can be seen to give rise to a constant yield, whereas the yields of the three variable lifetime decays parallel approximately the observed modifications in their respective lifetimes. From Table I it can be seen by the changes in amplitude of the four components between F_0 and F_m, that the energy transfer between individual antenna complexes is not the

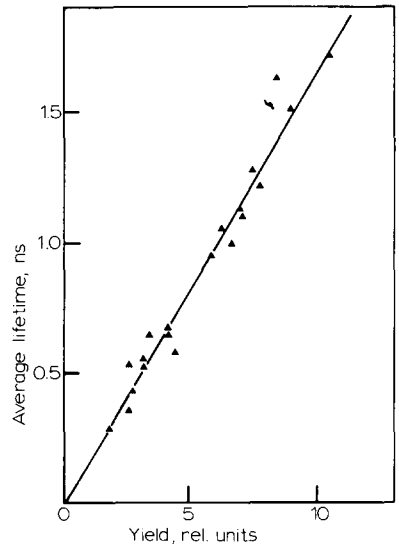

Fig. 1. The average fluorescence lifetime of spinach PS II-core complexes (PS II-70) as a function of total chlorophyll fluorescence yield (YIELD). The different fluorescence yields were obtained by varying the number of closed PS II reaction centres as described in the Materials and Methods. The straight line is the best fit through the datum points following correlation and least-squares regression analysis ($r = 0.98$).

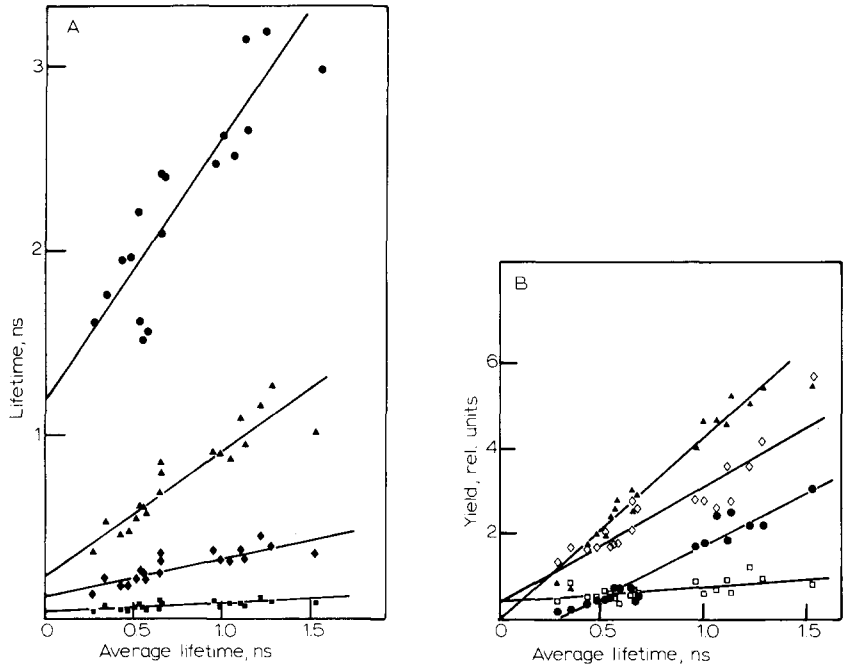

Fig. 2. The effect of PS II trap closure, as monitored by the average fluorescence lifetime, on (A) the lifetime and (B) the yield of the four kinetic components exhibited by PS II-70. The same symbol shape represents the same decay component in (A) and (B). The lines are the best fit after correlation and least-squares regression analyses.

same when PS II reaction centres are open and when they are closed. A striking observation from Table I is that at F_0 nearly all of the excitation energy is located in the complexes which give rise to the two rapid decays.

Fig. 3 shows the room temperature Chl fluorescence emission spectra for each of the four decays exhibited by PS II-70 complexes at F_M. Each decay appears to exhibit slightly different emission maxima between 679 nm (long-lived decays) and 682 nm (rapid decays). The absence of any red-shifted (686–695 nm) fast decay confirms the absence of any PS I-associated complexes (see Ref. 12).

TABLE I

LIFETIMES (ps) AND AMPLITUDES (A_{exp} (%)) OF THE CHLOROPHYLL FLUORESCENCE DECAY COMPONENTS EXHIBITED BY VARIOUS TYPES OF PS II-CORE COMPLEX PREPARATIONS

Origin	Chl/RC	Condition		Component values				τ_{mean}
Spinach	50–70	F_M	τ	3008	1104	393	99	1196
(PS II-70)			A_{exp}	3	20	41	36	
		F_0	τ	1581	434	190	56	315
			A_{exp}	0.4	14.5	34	51	
C. reinhardtii	50	F_M	τ	3567	841	199	38	1910
(PS II-50)			A_{exp}	4.5	16.5	27	52	
		F_0	τ	2882	616	150	35	864
			A_{exp}	1	8	25	86	
Pea	40	F_M	τ	2870	694	164	30	742
(PS II-40)			A_{exp}	0.8	6.5	32	61	
		F_0	τ	2420	597	208	57	464
			A_{exp}	0.5	6	39	55	

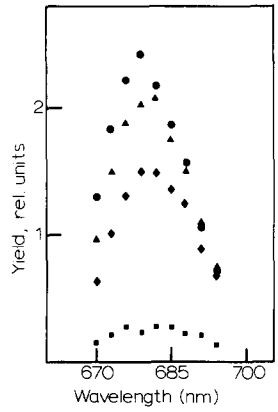

Fig. 3. The time-resolved emission spectra of the four individual decay components exhibited by PS II-70 complexes at F_M. The component lifetimes are: ●, 3.071 ns; ▲, 0.878 ns; ◆, 252 ps; ■, 27 ps.

It has been previously reported, for isolated LHC II, that the presence of detergents can drastically alter Chl fluorescence lifetimes and yields [21,26]. Therefore, the effect of detergent (as used in the isolation procedure) concentration on the lifetimes and yields of the individual decay components was examined. The increase in the total Chl fluorescence yield, brought about by the increase in detergent level, was seen to be linear with the logarithm of the detergent concentration over the range used (not shown). Fig. 4A gives the lifetimes of the four decay components as a function of total Chl fluorescence yield; which was modified by the addition of dodecyl maltoside (0–1 mM). It can be seen that the lifetimes of the two, long-lived decays are preferentially altered by the addition of detergent, leading to a large increase in their lifetimes (3–6 ns and 1–3 ns). The two rapid decays are less affected by detergent addition. The effect of detergent concentration on the yields of the four components is depicted in Fig. 4B. It can be seen that, as for the lifetimes of the two rapid decays, their respective yields are not affected. However, the addition of detergent brings about a large increase in the yields of the two slower components; the yield of the 1–3 ns decay being greatly augmented with respect to the modification in its lifetime.

Similar experiments to those depicted in Figs. 1–4 have also been carried out on the PS II-core-complexes of *Chlamydomonas reinhardtii* which contain 50 Chl/RC (PS II-50). Fig. 5A shows the evolution of the lifetimes of the four decay components, required to best fit the overall decay, as

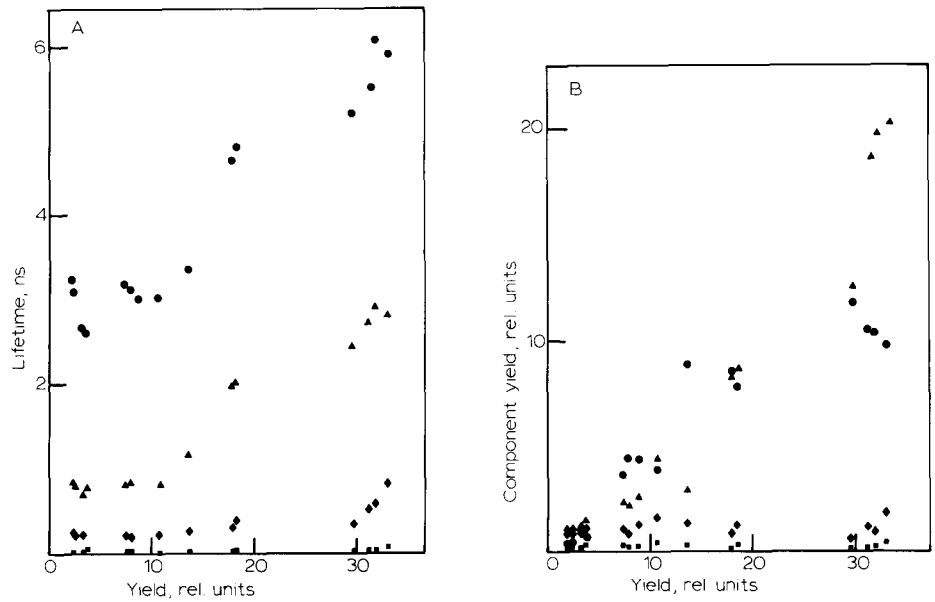

Fig. 4. The effect of detergent concentration (0–1 mM (0.051%)), as monitored by total chlorophyll fluorescence yield (YIELD), on (A) the lifetime and (B) the yield of the four kinetic decay components exhibited by PS II-70 complexes at F_M. The same symbol shape represents the same decay component in (A) and (B).

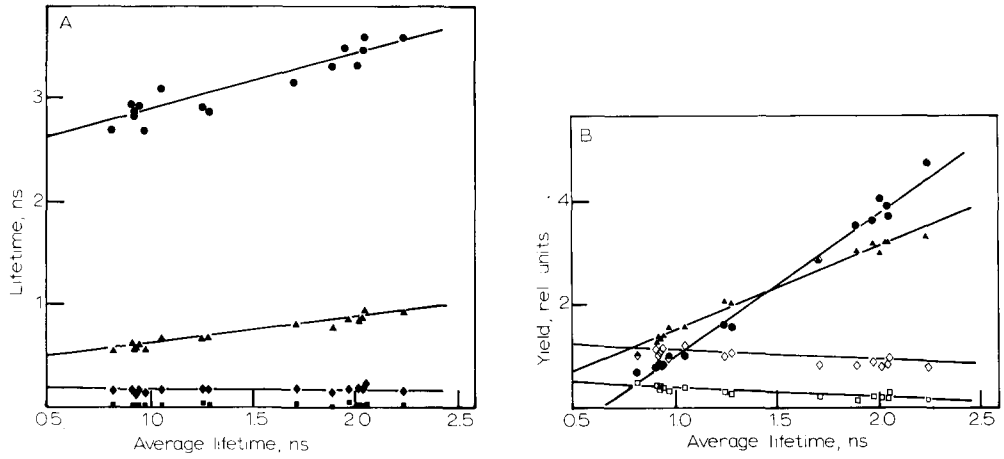

Fig. 5. The effect of PS II trap closure, as monitored by average lifetime, on (A) the lifetime and (B) the yield of the four kinetic components exhibited by *C. reinhardtii* PS II-core complexes (PS II-50). The shape of the symbols in (A) and (B) correspond to the lifetime and yield of the same individual component. The lines are the best fit through the datum points following correlation and least-squared regression analyses.

PS II reaction centres were closed (as indicated by average lifetime). These preparations also exhibited a linear relationship between fluorescence yield and average lifetime (not shown). These PS II-core complexes show only a very small degree of lifetime variability between F_0 and F_M. However, the yields of the two long-lived decays increase by a factor of between 2 and 4, as seen in Fig. 5B which shows the respective modifications in component yields for PS II-50. It can also be seen from Fig. 5B that the yields of the two rapid decays decrease slightly. The lifetimes and amplitudes of the four individual decay components at F_0 and F_M are given in Table I, where it can be seen that at F_0, as for PS II-70, 91% of the excitation energy is located in the chlorophylls associated with the two fast components.

The effect of detergent addition to the PS II-50 complexes, as for the changes brought about by PS II trap closure, were not identical to the PS II-70 complexes. Fig. 6A shows the effect of detergent addition (as monitored by total Chl fluorescence yield) on the component lifetimes. It can be seen that only the two long-lived decays had lifetimes which were affected by detergent addition. Fig. 6B gives the respective changes in component yields by the increase in detergent concentration. The yields of the three fastest decays appear to follow the slight changes in their respective lifetimes. However, the yield of the longest-lived decay increases in a disproportionate manner with respect to its lifetime.

Table I gives the lifetimes and amplitude values for the four kinetic-decay components required to best fit the overall decay exhibited by PS II-core

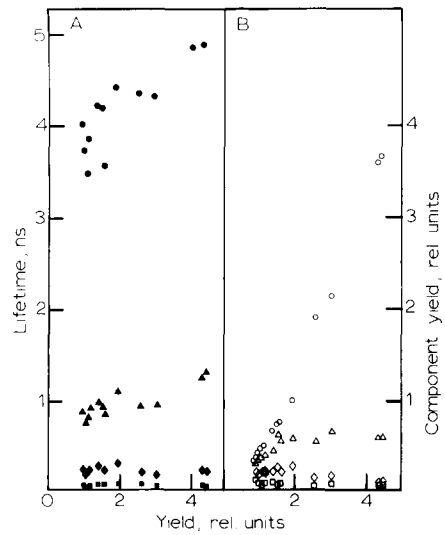

Fig. 6. The effect of detergent concentration (0–0.6 mM), as monitored by total chlorophyll fluorescence yield (YIELD), on (A) the lifetime and (B) the yield of the four decay components of PS II-50 complexes at F_M. The same symbol shape represents the same decay component in (A) and (B).

complexes from pea containing only 40 Chl/RC. It is evident that at F_0, nearly all of the excitation energy is located on the pigments associated with the two fast decays. A situation similar to that seen for PS II II-70 and PS II-50.

The three PS II-core complex preparations still contain a small contamination from LHC II (see Ref. 32). Therefore it is probable that one or more of the decay components are associated with LHC II. Table II gives the lifetime and amplitude values for the two kinetic components necessary to best fit the overall decays exhibited by LHC II in different states of aggregation. It can be seen that 'aggregated' LHC II produces two, relatively fast decays whereas monomeric LHC II (gel or in solution) leads to the presence of two long-lived decays. It is interesting to note that the ratio of the longer-lived decay with respect to the faster component is always between 2 and 3. Furthermore, the addition of detergent to 'aggregated' LHC II not only increases the lifetime values, but selectively increase the amplitude of the slowest decay component (as seen in Fig. 5B for the longest decay of PS II-50). Fig. 7 shows the effect of a known fluorescence quencher, dinitrobenzene, on the lifetimes exhibited by 'aggregated' LHC II. This treatment has already been used to study the origins of the decay components in *Chlamydomonas reinhardtii* mutants [19,39]. It can be seen that successive additions of the quencher brings about a similar decrease in the lifetimes of the two decays as a function of total Chl fluorescence yield. Such a trend is also observed in 'intact' systems for the two long-lived components, which also differ in lifetime values by a factor of 2 [39].

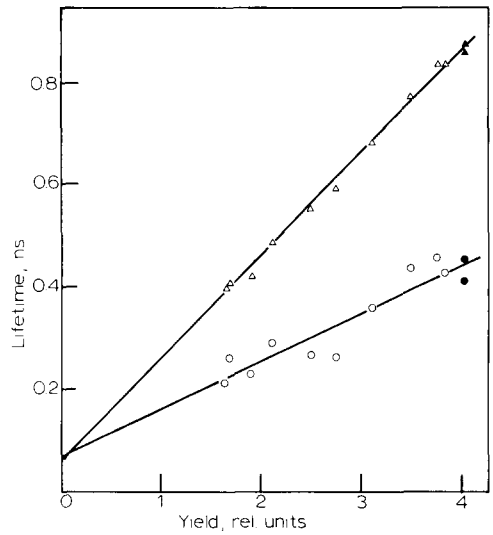

Fig. 7. Variations in component lifetime as a function of total chlorophyll fluorescence yield (YIELD), in aggregated LHC II, brought about by the successive additions of dinitrobenzene (open symbols) and the absence of dinitrobenzene (closed symbols).

The organisation of the PS I and PS II-core complexes are believed to be very similar in that each reaction centre is associated with 40–50 chlorophylls [13,14] and therefore we have investigated the lifetime characteristics of isolated PS I. Table II gives the lifetime and amplitude values of the four decay components required to fit the overall fluorescence decay of PS I. It is seen that 99% of the excitation energy is located in the pigment-protein complexes which give rise to the two rapid decays (27 and 110 ps). Fig. 8 shows the room-temperature emission spectra of the four kinetic components. The rapid (110 ps) decay produces

TABLE II

LIFETIMES (ps) AND AMPLITUDES (A_{exp} (%)) OF THE CHLOROPHYLL FLUORESCENCE DECAY COMPONENTS EXHIBITED BY ISOLATED LHC II AND PS I

Complex	Condition		Component values				τ_{mean}
LHC II	aggregated	τ	886	419			816
		A_{exp}	73	27			
	monomeric	τ	3460	988			3118
	(gel)	A_{exp}	64	36			
	monomeric	τ	4200	1700			4019
	(detergent)	A_{exp}	90	10			
PS I	solution	τ	2760	550	110	27	677
		A_{exp}	0.4	0.6	22	77	

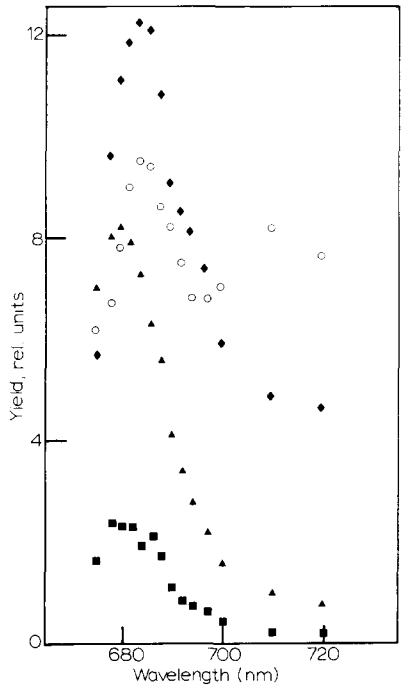

Fig. 8. The time-resolved emission spectra of the four individual decay components exhibited by isolated PS I complexes. The lifetime of each component is: ○, 110 ps, ◆, 27 ps; ▲, 550 ps; ■, 2.76 ns.

two maxima at 685 nm and 710 nm whereas the 27 ps decay has its maximum at 685 nm. The two longer-lived decays both have their maxima at 680 nm.

An estimate of the single-step transfer time between communicating Chl molecules (JT) and the number of revisits to a reaction centre before trapping takes place (NV) in the PS II-core-complexes and PS I have been obtained using the data in Tables I and II and the theoretical treatments of Pearlstein [31]. The calculations begin with the expression for the lifetime of the excitation (presumed to be exponential) [31].

$$\tau_{ex} = \left(1 + \frac{Fd}{Ft}\right)(N-1)kp^{-1} + (q \cdot Ft)^{-1} - (q \cdot Fa)^{-1} \cdot \frac{(N-1)^2}{N} + \alpha \cdot N \cdot Fa^{-1} \quad (1)$$

where Ft and Fd are the average Förster rate constants (forward and reverse) for excitation transfer between antenna pigments and the reaction centre, Fa is the average Förster rate constant for transfer between neighbouring Chls, N is the antenna size and kp is the rate constant for photochemistry (or in certain cases the rate constant for transfer to another antenna complex). The parameter q is the coordination number of the lattice ($q = 4$ for a square lattice [40]) and α is a numerical constant ($\alpha = 0.429$ for a square lattice [40]).

Eqn. 1 contains only two parameters which are accessible from our data: τ_{ex} and N; however, spectral and kinetic data are available to make reasonable estimations of kp^{-1} (2.8 ps for the reaction centre of *Rhodobacter sphaeroides* [41]), Fd/Ft and Ft/Fa. For the estimations presented in Table III we calculate Fd/Ft, which is the Boltzmann factor between the first excited singlet states of the trap (at 680 nm for PS II and 700 nm for PS I) and the antenna molecules (at an average of 672 nm for PS II-cores (see Ref. 43) and 680 nm for PS I (see Ref. 44)). Using the fact that the

TABLE III

EXCITATION ENERGY TRANSFER AND QUANTUM YIELD OF PHOTOCHEMISTRY FOR DIFFERENT PIGMENT-PROTEIN COMPLEXES

τ_{ex} = experimental lifetimes; N_{Chl} = antenna size; JT = transfer time between chlorophylls; NV = number of visits before trapping; QY = quantum yield; τ_{calc} = lifetime calculated from Eqn. 4; kp = rate constant for photochemistry or complex–complex transfer; k_{ant} = deactivation rate constant for antenna.

Complex	τ_{ex} (ps)	N_{Chl}	JT (fs)	NV	QY	τ_{calc} (ps)	$1/kp$ (ps)	$1/k_{ant}$ (ps)
PS II-core	54	24	816	2	0.79	52	2.5	250
PS II-core	180	16	1470	5	0.55	180	20	400
PS I	27	77	206	1	0.85	34	0.5	250

reaction centre chlorophylls form a dimer we utilise an Ft/Fa ratio of 1.57 as in Refs. 3 and 42.

Putting these parameters and the fluorescence data into Eqn. 1, the single-step transfer time, $(q \cdot Fa)^{-1}$, is calculated. It should be noted that the number of chlorophylls associated with each decay component is estimated from the amplitude value and the known number of Chl/RC (where $N = A_{exp} \times$ number of Chl/RC). This procedure gives 40 Chl/RC for the PS II core and 77 Chl/RC for PS I, which is in close agreement with the published data [13,14,44]. This procedure gives various JT values for each component (Table III). For example, the rapid PS II-core complex decay leads to a JT of 816 fs, whereas a JT of 206 fs is calculated for the rapid PS I decay; however, it is necessary to have a smaller kp^{-1} value for the PS I.

We can also calculate the ratio of lifetime to the first passage time (FPT, the time between excitation and the first visit to the trap). FPT is calculated from Ref. 31 by

$$FPT = \frac{N}{q \cdot Ft} + \left(\alpha - \frac{1}{q}\right)\frac{N}{Fa} \tag{2}$$

If τ_{ex}/FPT (NV in Table III) is 1, then the photochemical event is 'diffusion controlled', whereas if τ_{ex}/FPT is greater than 1 then multiple visits occur and the photochemical event is 'reaction controlled'. It can be seen that for the rapid decay of PS II, two visits to the RC are necessary before trapping. However, for the fast PS I component the photochemical event appears to be strictly diffusion limited.

Furthermore, the estimated jump time can be used, along with the equations described by Van Grondelle [46] for a random walk model in a square lattice with excitation transfer between nearest neighbours, to calculate the quantum yield (QY) and the expected excitation lifetime (τ_{calc}). The quantum yield is calculated by

$$QY = \frac{1}{\frac{Nk_{ant}}{4kh}\left(\frac{\ln N}{\pi} + 0.195 + \frac{4kh}{kp}\right) + 1} \tag{3}$$

where k_{ant} is the rate of deactivation in the antenna and kh is $1/JT$. The value of QY is then used to produce the calculated lifetime (τ_{calc}) by

$$\tau_{calc} = \frac{1 - QY}{k_{ant}} \tag{4}$$

Such an approach was used to verify the calculated jump times so as to have QY values of between 0.7 and 0.8 for the reaction centre components (as seen from fluorescence induction curves in the presence of inhibitors, see Ref. 47) and similar τ_{ex} and τ_{calc} values. If the various criteria were not met, then the spectral and kinetic input parameters to Eqn. 1 were altered.

Discussion

It can be seen from the results presented in this work that the 'simplified' PS II-core complexes give rise to four kinetic decay components, which is one more than in PS II-enriched membranes containing 200 Chl/RC [20]. This suggests that the preparation procedure brings about certain modifications in the organisation and energy-transfer pathways. The fluorescence emission spectra (Fig. 3) suggest that each of the individual decays arise from PS II-associated pigments and that the rapid decays do not originate from contaminating PS I proteins. The results therefore confirm the presence, at F_M, of the fast PS-II-associated decay components (see Refs. 11, 19 and 20).

The PS II-core complexes are known to contain between 40 and 70 Chl/RC [32–34] and therefore are enriched in the antenna pigments close to the reaction centre (40–50 Chl associated with the 47 and 43 kDa polypeptides of PS II (see Refs. 13 and 14)). At F_0, between 80 and 90% of the excitation energy is located in the pigments which produce the two rapid decays, strongly suggesting that they are associated with the core antenna. Indeed, a simple calculation using the amplitude values and the total number of Chl/RC shows that these two decays arise from 40 chlorophylls. However, unlike the PS II-enriched membranes [11] which show a single rapid decay exhibiting a variable lifetime and yield between F_0 and F_M, the PS II-core complexes give rise to a constant 50 ps decay and in PS II-50 and PS II-40 a constant 180 ps decay (whereas in PS II-70 this fast compo-

nent is variable in both lifetime and yield (190–390 ps)). This discrepancy might arise from a modification in the excitation energy-transfer pathways by the additional isolation procedures; perhaps by detergent-protein interactions or from the delipidation of the complexes. Based on the model of Bassi and Simpson [48] for PS II-core complex organisation, we suggest that the excitation transfer between the 43 and 47 kDa polypeptides is altered so that the transfer from the 43 to the 47 kDa protein is inhibited or slowed down, thereby giving rise to two lifetime values, one of which is similar to that found in PS II-enriched membranes at F_M (arising from the 43 kDa protein) and the other similar to that observed at F_0 (from the 47 kDa polypeptide which is in contact with the reaction centre).

The two other decay components seen in the PS II-core complex preparations, have lifetime values which depend on the isolation procedure or thylakoid origin. These two decay account for 10–20% of the total Chl (approx. 10 Chl/RC). The lifetimes of these two minor decay components are very similar to those exhibited by isolated LHC II (see Table II and Refs. 21, 24 and 25). Therefore, we conclude that these two decays arise from contaminating LHC II. If a trimer is the minimum functional LHC II unit [45] and each polypeptide contains 7 Chl [13] then it can be seen from the amplitude values at F_M (when there is no quenching effect from open reaction centres) that the 1 ns decay arises from one LHC II trimer/RC while the 3 ns decay is emitted by 1 trimer/10 RC. In 'intact' thylakoids there are approx. 250 LHC II Chl/RC [14] which gives 12 trimers/RC. It can be seen, therefore, that the PS II-core complex preparations contain very little LHC II with respect to the 'intact' system but that the small quantities which remain are very visible by Chl fluorescence. The requirement for two decay components to best fit the overall decay of isolated LHC II, as well as the proposed in vivo LHC II components, infer that there is indeed a heterogeneous population of LHC II where energy transfer between different populations is not possible.

The PS I complex, like the PS II-core complexes, is enriched in two fast decay components (the lifetimes are very similar to those reported in Ref. 22). It is seen from Table II that 99% of the initial excitation energy is found in the two rapid decays and suggests that the energy transfer and photochemical trapping processes are very similar in the two types of photosystem. The fluorescence emission spectra (Fig. 8) shows that the two rapid decays are red-shifted with respect to the fast PS2 decays, although the maximum of the fastest decay is not the same as that found in vivo [11]. This could arise from detergent effects on the fluorescence emission (see Ref. 49) as could the presence of two maxima for the 110 ps decay. The two other decays probably reflect LHC I contamination.

On the whole the results and conclusions agree with the origins proposed for the different decay components from studies using mutants [19,39], organisational modifications [29] and photosynthetic component enrichment [20].

From Fig. 1, where a linear relationship between total fluorescence yield and average lifetime is seen, it is suggested that the antenna systems present in the PS II-70 complex are connected to a number of reaction centres and that there is a certain degree of connectivity between centres. This conclusion is in disagreement with the observations made in Ref. 32, where it was reported that the complexes were isolated in a monomeric form. However, this discrepancy can be explained by the effect of detergent concentration on the aggregation state of proteins. In the lifetime measurements the detergent was diluted out in the resuspension buffer leading to aggregation of the proteins and hence to the possibility of energy transfer. In Refs. 32 and 33 the estimation of complex size was achieved in the presence of 'moderate' detergent concentrations which would have led to micelle formation and non-aggregated protein complexes.

It has been known for a long time that the concentration of detergent can drastically modify Chl fluorescence lifetimes and yields of isolated LHC II [21,24,26]. At low detergent concentrations, where there is no micelle formation, there is the possibility of detergent-protein interactions which can modify Chl–Chl interactions. It has been seen that under such conditions, there is an aggregation of LHC II which leads to the development of a new Chl *b*–Chl *a* interaction and

complex sub-ns decay kinetics [24]. The presence of 'medium' levels of detergent produces numerous average lifetimes (2.3–3.4 ns [26]). It has been suggested that these detergent concentrations can lead to mixed populations of aggregated (1.12 ns) and non-aggregated LHC II (3.5 ns) [24] and delipidation of the protein complexes [26]. Finally, at 'high' detergent concentrations there is a complete inhibition of Chl–Chl interactions leading to lifetimes similar to those seen for Chl in micelles (5.5–6 ns). Our data (Figs. 4 and 6; Table II) show that the addition of detergent only alters the yields and lifetimes of the two long-lived LHC II-associated decay components. This suggests that the contaminating LHC II is not closely connected to the PS II-core antenna and that in the presence of detergent micelles it is possible to disconnect the LHC II. The average lifetime for the 'monomeric' LHC II shown in Table II is in close agreement with that already reported for a monomeric form [26] and is very similar to the LHC II component of Fig. 6A. Perhaps the 5.5–6 ns decay observed at high detergent levels in the PS II-70 complexes arises from free chlorophylls or from the complete inhibition of Chl–Chl interactions in this LHC II complex. The lack of a detergent effect on the two rapid decays infer that they are always to be found in a 'monomeric' form or that in the aggregated form no new Chl interactions are produced which modify their lifetimes and yields.

Using the average, lifetime and amplitude values for the three types of PS II-core complex we have calculated estimations for various excitation energy transfer parameters (Table III). It is important to make clear that the values are only estimations and that the JT lifetimes are highly dependent upon the calculated Boltzmann factor (Fd/Ft), the number of chlorophylls (see Ref. 3) and the experimental lifetimes. However, we have tried to overcome these problems by using parameter values which give good quantum yield estimations and lifetimes as calculated from Eqns. 3 and 4. The JT for the fastest decay components of both PS I and PS II are in the order of 200–800 fs, which is in close agreement with Refs. 3 and 50. It can be seen from the number of visits (NV) that the trapping is approximately 'diffusion limited' in both cases. To obtain a good fit with respect to QY and τ_{calc}, it was necessary to assume that the 180 ps complex was not directly associated with the reaction centre and that the lifetime depended upon the rate of transfer to the protein giving rise to the fastest decay. This could arise because the two proteins are now separated by detergent molecules which modifies the normal Chl–Chl interactions between the two proteins believed to make up the PS II-core antenna (as excitation energy transfer is highly dependent upon the distance and orientation of interacting chlorophylls [46]). We suggest that in vivo this transfer is extremely rapid and efficient which leads to a single lifetime decay component associated with the two polypeptides. On the whole it can be seen that the calculated number of chlorophylls (from the amplitude values) and the experimental lifetimes give rise to reasonable energy-transfer parameters (based on Eqns. 1 and 2) and quantum yields (Eqn. 3) when using the kp and k_{ant} values given in Table III. The kp value used for PS II is in close agreement with the rate constant found experimentally for charge separation in bacterial reaction centres [41], whereas the kp for PS I is larger (although in the same order of magnitude when taking into account the possible parameter variations).

Acknowledgements

M.H. Would like to thank the Royal Society (London, U.K.) for financial support. This work has also been financed by the C.N.R.S. and grant No. SAV 7738 CNRS-CEA. We are grateful to C. DeVitry (PS II-50), L.G. Franzen and S. Styring (PS II-70), A Picaud (PS II-40) and R. Remy (LHC II and PS I) for the different types of preparation. Interesting discussions during this work concerning the origins of fluorescence lifetime components were carried out with Professor P. Joliot and Drs. J. Lavergne and F.-A. Wollman.

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