Biochimica et Biophysica Acta, 1100 (1992) 198–206

BBABIO 43602

The influence of the double reduction of Q_A on the fluorescence decay kinetics of Photosystem II

F.J.E. van Mieghem [a,b], G.F.W. Searle [b], A.W. Rutherford [a] and T.J. Schaafsma [b]

[a] Section de Bioénergétique, Département de Biologie Cellulaire et Moléculaire, CE Saclay, Gif-sur-Yvette (France) and [b] Department of Molecular Physics, Agricultural University, Wageningen (Netherlands)

(Received 30 October 1991)

Key words: EPR; Radical pair state; Plastoquinone; Photosynthesis; Photosystem II preparation; Chlorophyll

The acceptor Q_A of PS II was doubly reduced by treatment of PS II-enriched membranes (200–300 chlorophylls per PS II-reaction centre) with dithionite and benzyl viologen. After double reduction of Q_A, two major differences appeared in the fluorescence decay kinetics (at 4°C), as compared to the situation with all Q_A singly reduced: (1) a dominant fast phase (lifetime approx. 200 ps) was observed, similar to that in samples with Q_A oxidised; (2) a slow phase with a lifetime of approx. 7 ns was observed, which disappeared upon reoxidation of the sample. The fluorescence yield was approximately half of that of samples with singly reduced Q_A. The fast phase is interpreted as being indicative of a high efficiency of charge separation due to the absence of a negatively charged Q_A. This is explained by the double protonation of doubly reduced Q_A giving rise to the electrically neutral quinol. Similar observations were made in a core complex preparation (60–80 chlorophylls per reaction centre). This preparation involves a detergent solubilisation step and data from both EPR and fluorescence indicated that it was more susceptible to double reduction of Q_A by dithionite (as compared to PS II membranes). The possibility that this is a general phenomenon in detergent solubilised PS II preparations is discussed.

Introduction

The reaction centre of PS II is generally considered to be similar in all green plants, algae and cyanobacteria and also to be closely related to the reaction centre of photosynthetic purple bacteria (see Ref. 1 for a review). It is connected to an antenna system that captures the light and transfers the energy to the primary electron donor P, which is located in the reaction centre and consists of one or more chlorophyll *a* molecules. Electron transfer can then occur from P to a nearby pheophytin *a*, the primary acceptor Phe. Thus, the primary radical pair is formed. This charge separation is subsequently stabilised by electron transfer to Q_A, a plastoquinone (see Refs. 2 and 3 for reviews on electron transfer processes in PS II).

It is now generally accepted that prereducing Q_A to its singly reduced semiquinone form decreases the yield of charge separation (see e.g. Refs. 4–9). However, there is some disagreement with regard to what extent this occurs. The lower yield of charge separation in reaction centres with Q_A^- present, compared to that of reaction centres with Q_A oxidised, has been explained by a net repulsive electrostatic interaction between Q_A^- and the primary radical pair [6,10].

When the primary radical pair P^+Phe^- is formed, and if it lives long enough to allow dephasing of the electron spins, charge recombination to the molecular triplet state of P can occur [11]. These conditions can be reached when Q_A is absent [12] or doubly reduced [9]. When Q_A is singly reduced, no triplet state can be detected [9]. As the most straightforward explanation for this observation, it was suggested in Ref. 9 that the yield of the primary radical pair in PS II reaction centres with Q_A^- present, is very low, due to the electrostatic effect, as proposed in Refs. 6 and 10. Double reduction of Q_A, followed by a double protonation neutralises the negative charge at the site of Q_A and the formation of the primary radical pair is no longer electrostatically inhibited [9]. The triplet yield is high, because the lifetime of the primary radical pair is sufficiently long for triplet formation.

Abbreviations: Chl, chlorophyll; EDTA, ethylenediaminetetraacetate; Mes, morpholinoethanesulphonic acid; Mops, 4-morpholinepropanesulphonic acid; P, the primary electron donor in Photosystem II; Phe, pheophytin; PS II, Photosystem II; Q_A, the first quinone electron acceptor.

Correspondence: F.J.E. van Mieghem, Section de Bioénergétique, Département de Biologie Cellulaire et Moléculaire, CE Saclay, 91191 Gif-sur-Yvette Cedex, France.

In this work we test the hypothesis that the double reduction of Q_A modulates the yield of the primary radical pair and hence the yield of the triplet. For this, we looked at the rate of charge separation, using picosecond fluorescence measurements. It was suggested earlier that the double reduction of Q_A may be responsible for fluorescence quenching in PS II [9,13].

We used two types of PS II preparations from spinach, differing in their antenna size. The reduction of the antenna size is usually achieved by detergent treatment, which may affect the intactness of the acceptor side [9].

A preliminary report of these results has appeared elsewhere [14].

Materials and Methods

PS II-enriched thylakoid membrane fragments were prepared from spinach as described earlier [15] using the modifications in Ref. 16. PS II core complexes were prepared according to Ref. 17. In order to obtain reaction centres with Q_A doubly reduced, membrane fragments were maintained in the dark for several hours in the presence of approximately 40 mM sodium dithionite and 100 μM benzyl viologen (sodium dithionite was added from a 10-fold concentrated stock solution, also containing 400 mM Mops at pH 7.0) [9]. In addition, 50 mM Mops (pH 7.0), 300 mM sucrose, 200 mM sodium formate, 10 mM NaCl, 5 mM $MgCl_2$ and 1 mM EDTA were present during the incubation. In experiments in which Q_A was reoxidised, the membranes were washed with buffer in order to remove dithionite and benzyl viologen and the sample was then suspended in a buffer containing 5 mM ferricyanide which was subsequently removed by washing.

Core complexes were also treated with sodium dithionite and benzyl viologen, but this was done in a buffer containing 50 mM Mes (pH 6.0), 400 mM sucrose, 200 mM sodium formate, 10 mM NaCl, 5 mM $CaCl_2$ and 1 mM EDTA.

Incubations with dithionite and benzyl viologen were done in EPR tubes in order to enable the monitoring of the triplet [11] and formate enhanced $Q_A^- Fe^{2+}$ [18] EPR signals during the treatment (see also Ref. 9). The incubations were ended after all Q_A had been doubly reduced (i.e., the triplet EPR signal had reached its maximal level and the $Q_A^- Fe^{2+}$ signal had decreased beyond detection [9]). The samples were stored under argon and at 77 K in the dark until use.

EPR spectra were recorded using a Bruker 200 X-band spectrometer fitted with an Oxford Instruments cryostat and temperature control system. In order to record spectra during illumination, an 800 W tungsten projector was used. The light was filtered through 2 cm water and three Calflex (Balzers) heat filters.

For fluorescence kinetics measurements, samples were diluted in near darkness into fluorescence cuvettes in an argon flushed buffer (4°C), containing 50 mM Mes (pH 6.0), 400 mM sucrose, 10 mM NaCl and 5 mM $CaCl_2$. After dilution, the cuvettes were sealed and kept in the dark until use. Samples with Q_A doubly reduced were diluted in buffer, to which 4 mM sodium dithionite had already been added. Thus, a possible partial reoxidation of doubly reduced Q_A was avoided. Q_A was singly reduced by the addition of 4 mM dithionite to a sample that had not been treated with dithionite and benzyl viologen. The addition was made shortly before the measurement. For measurements under conditions of oxidised Q_A, the sample was in the presence of 0.05 mM ferricyanide and the light intensity of the exciting light was diminished to a level at which the contributions of decay components longer than one nanosecond were minimal. In particularly in the case of oxidised core complexes, the lifetimes of the decay components increased considerably with the intensity of the excitation light.

Fluorescence kinetic measurements were performed in 10 × 10 mm anaerobic cuvettes at a concentration of 1–10 μg Chl/ml. During the measurements, the samples were stirred and maintained at 4°C. The experimental conditions were as described previously [19]. The excitation source was a mode locked Ar ion laser, wavelength 457.8 nm, the pulse frequency was 596 kHz and the pulse energy was approximately 2 pJ/cm^2. The time resolution of the multi-channel analyser was 10.2 ps/channel. The pulse width after detection was 200 ps full width at half maximum. To check for a wavelength dependence of the fluorescence decay kinetics, the detection wavelength was set at 679, 693 or 707 nm using Balzers B-40 interference filters. No significant wavelength dependence was observed. Therefore, subsequent measurements were carried out using a broadband (Balzers K70) filter.

The fluorescence decay of a reference compound (Rose Bengal in methanol, lifetime 0.55 ns) fluorescing at the same wavelengths as chlorophyll was determined before and after the measurement of the fluorescence decay in a PS II sample. The averaged reference was then used to generate a laser pulse shape using a deconvolution programme employing Fourier transformation. The deconvolution parameters were varied and were considered optimal when the resulting pulse shape showed minimal oscillations.

The fluorescence decay kinetics were analysed with the Global Analysis programme of Beechem, Gratton and Mantulin (Globals Unlimited, Urbana, USA, 1990). The input file for this programme consisted of the sample data file and the associated deconvoluted pulse file. The data was weighted with the square root of the number of counts per channel and fitted to a sum of up to 6 discrete components. The criterion for the good-

TABLE I

Analysis of the fluorescence decay data (summary from several experiments) of PS II membranes, before and after dithionite/benzyl viologen treatment of membranes and of reoxidised membranes

Fluorescence decay components are given in ns and the relative amplitude is given as a percentage in brackets; estimated errors are given. Values for the relative fluorescence yields are given in arbitrary units (see Materials and Methods for procedure).

Sample	Additions	Fluorescence decay components				Rel. yield
Before dithionite/benzyl viologen treatment	A 0.05 mM ferricyanide	0.14 ± 0.03 (73 ± 10)	0.33 ± 0.04 (27 ± 5)	3.2 ± 1 (0.04 ± 0.02)		19
	B 4 mM dithionite	0.60 ± 0.1 (25 ± 10)	1.4 ± 0.3 (68 ± 10)	3.3 ± 1 (7 ± 3)		133
Dithionite/benzyl viologen treated	C 4 mM dithionite	0.22 ± 0.04 (69 ± 10)	0.60 ± 0.1 (18 ± 4)	2.0 ± 0.5 (8 ± 2)	7.1 ± 2 (5 ± 1)	77
Reoxidised after dithionite/benzyl viologen treatment	D 0.05 mM ferricyanide	0.11 ± 0.03 (64 ± 10)	0.35 ± 0.05 (35 ± 7)	1.1 ± 0.3 (1 ± 0.3)	5.6 ± 2 (0.03 ± 0.01)	21

ness of the fit was the χ^2 value. The quality of the fits was also judged from a plot of the distribution of weighted residuals (experimental minus calculated counts per channel). In some cases, short components (0.1–40 ps) were found, with lifetimes and relative amplitudes that were not reproducible. The relative amplitudes and lifetimes of the remaining components were reproducible within the errors given in Tables I and II. Therefore, only the latter components are considered in our discussion of the fluorescence decay kinetics. Values for the relative fluorescence yield were calculated by $\Sigma a_i \tau_i$ (a_i is the relative amplitude of component i in percent and τ_i its lifetime in ns). The values for the relative fluorescence yield (Tables I and II), corresponded with those obtained from fluorescence measurements under continuous illumination (Table III). Thus, the values for the relative yield, calculated from the decay kinetics can be taken as being proportional to the fluorescence yield.

For fluorescence measurements under continuous illumination, a home-built apparatus was used. The onset of the exciting light (expanded He-Ne laserlight, 633 nm) was accomplished by the opening of an electrical shutter (opening time faster than 1 ms). Detection was around 687 nm (monochromator) using a photomultiplier (S20) and an analogue-to-digital conversion card.

Results

PS II-enriched membranes

PS II membranes were pretreated with dithionite and benzyl viologen as described in Materials and Methods, in order to obtain reaction centres with Q_A doubly reduced [9]. This was checked by EPR at liquid helium temperature by measuring the decrease of the $Q_A^- Fe^{2+}$ signal and the increase of the light-inducible reaction centre triplet signal. The triplet signal was small at the start of the treatment and a large $Q_A^- Fe^{2+}$ signal was observed (Fig. 1a). The incubations were ended after the triplet EPR signal had reached its maximal level and the $Q_A^- Fe^{2+}$ signal had decreased

TABLE II

Analysis of the fluorescence decay data (summary from several experiments) of PS II core complexes, before and after dithionite/benzyl viologen treatment

Fluorescence decay components are given in ns and the relative amplitude is given as a percentage in brackets; estimated errors are given. Values for the relative fluorescence yield are given in arbitrary units (see Materials and Methods for procedure).

Sample	Additions	Fluorescence decay components				Rel. yield
Before dithionite/benzyl viologen treatment	A 0.05 mM ferricyanide	0.06 ± 0.02 (75 ± 10)	0.25 ± 0.04 (22 ± 5)	0.68 ± 0.1 (3 ± 1)	3.1 ± 1 (0.2 ± 0.1)	13
	B 4 mM dithionite	0.1 ± 0.03 (32 ± 6)	0.51 ± 0.1 (36 ± 7)	1.5 ± 0.4 (26 ± 5)	4.9 ± 1 (6 ± 2)	90
Dithionite/benzyl viologen treated	C 4 mM dithionite	0.075 ± 0.02 (50 ± 10)	0.32 ± 0.04 (32 ± 6)	1.3 ± 0.3 (13 ± 3)	7.1 ± 2 (5 ± 1)	66
	D 0.05 mM ferricyanide	0.1 ± 0.03 (65 ± 10)	0.37 ± 0.05 (30 ± 6)	1.2 ± 0.3 (4 ± 1)	5.9 ± 2 (1 ± 0.3)	28

TABLE III

Values for the fluorescence yield, measured under continuous illumination in PS II membranes and PS II core complexes

The sample conditions were similar to those used in the fluorescence decay measurements. The fluorescence yield is given in arbitrary units.

Sample	Additions	Fluorescence yield	
		membranes	core complexes
Before dithionite/benzyl viologen treatment	A[a] 0.05 mM ferricyanide	18	26
	B 4 mM dithionite	149	146
Dithionite/benzyl viologen treated	C 4 mM dithionite	73	72
	D[a] 0.05 mM ferricyanide	27	46

[a] Values are those measured at the onset of the illumination.

beyond detection (Fig 1b, see also Ref 9). Samples pretreated in this way are referred to as 'dithionite/benzyl viologen-treated samples' throughout the text. They were kept under reducing conditions unless otherwise mentioned. For the fluorescence decay measurements, we also prepared PS II membranes in the two other redox states of Q_A: with Q_A oxidised and

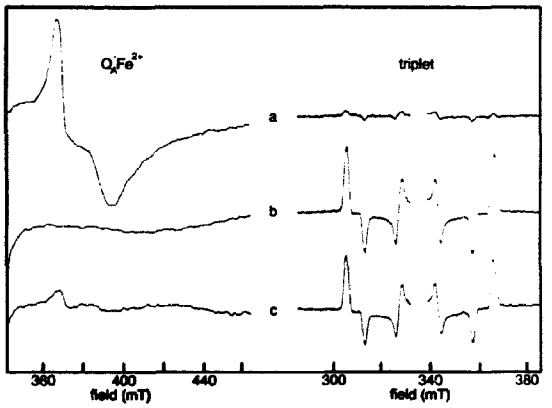

Fig. 1. $Q_A^- Fe^{2+}$ and reaction centre triplet signals from PS II-enriched membranes (4 mg Chl/ml) after different redox treatments; average of two experiments. (a) Spectra recorded 15 min after the addition of 40 mM dithionite; (b) after approximately 4 h incubation in the presence of 40 mM dithionite and 0.1 mM benzyl viologen in the dark at 20°C; (c) after subsequentially washing the sample in buffer, 5 mM ferricyanide, buffer(2×); 15 min before freezing the sample, 40 mM dithionite was added. EPR conditions for the $Q_A^- Fe^{2+}$ spectra: temperature, 4.7 K; microwave power, 32 mW; microwave frequency, 9.44 GHz; modulation amplitude, 25 G. The triplet spectra are difference spectra (light on minus light off); the EPR conditions are the same as for $Q_A^- Fe^{2+}$ except that the microwave power was 63 μW.

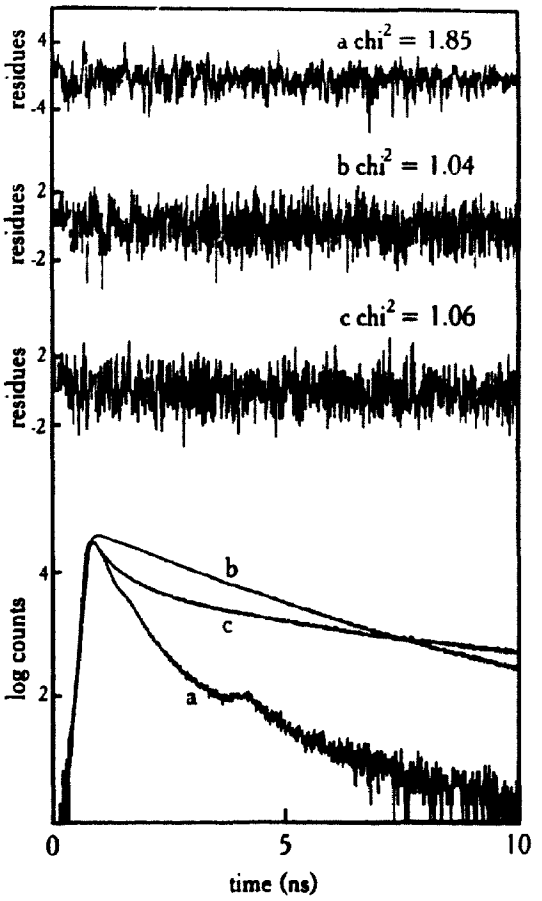

Fig. 2. Fluorescence decay (normalised) in PS II-enriched membranes. Weighted residual plots are also shown (upper traces). (a) 0.05 mM ferricyanide present in the measuring cuvette; (b) 4 mM dithionite present; (c) sample, treated as described in the legend to Fig. 1b; the dithionite concentration in the cuvette was 4 mM. The number of counts in the peak channel was: (a) 29 000; (b) 41 000; (c) 22 000.

with Q_A singly reduced (see Materials and Methods for details on the experimental conditions).

Typical fluorescence decay curves for the three types of samples are shown in Fig. 2 for PS II membranes. Comparing traces A (Q_A oxidised) and B (Q_A singly reduced) reveals that upon reducing Q_A with one electron, the fluorescence lifetime increases drastically. This is characteristic for PS II (see Refs. 20 and 21 for reviews on fluorescence of PS II). When Q_A is doubly reduced (trace C), there is an initial fast decay, similar to that in oxidised samples. However, there is also a very slow component present, slower than that of samples with singly reduced Q_A.

To obtain more quantitative information on the fluorescence decay kinetics, we fitted the decay curves to a multi-exponential decay function (see Materials

and Methods for details on the fitting procedure). The results are shown in Table I (A, B and C). Fitting of the curves yielded three (Q_A oxidised and singly reduced) or four (Q_A doubly reduced) components. As shown in the weighted residual plots of Fig 2, the fitted curves agree well with the experimental curves. In samples with oxidised Q_A, relatively high values for χ^2 were found, due to a lower signal to noise ratio. The lower signal to noise ratio was caused by the relatively low fluorescence yield and also the low excitation intensity used for samples with oxidised Q_A (see Materials and Methods). The results of the analyses of the decay kinetics were reproducable within the errors given in Table I.

The data of Table IA, B, C confirm the qualitative impressions from Fig. 2. When Q_A is oxidised, the kinetics are dominated by a fast component of approx. 140 ps. Singly reducing Q_A results in an increased fluorescence yield. This is caused by the lengthening of the fluorescence lifetime of the components and a shift in the distibution to slower components, of which a component with a lifetime of approx. 1.4 ns is dominant. These observations are in agreement with reports in the literature on similar preparations [4,8,22].

In samples with Q_A doubly reduced, the fluorescence yield is also higher than that in samples with Q_A oxidised, but it is only half that of samples with Q_A singly reduced (see also Table IIIB, C). There is a short component of 220 ps with high relative amplitude, which is similar to the 140 ps component found when Q_A is oxidised. A similar component with high relative amplitude together with an irreversible decrease in fluorescence yield has been observed previously after strong illumination in the presence of dithionite [4] (see also Ref. 23). It has been shown recently that such conditions indeed generate reaction centres with Q_A doubly reduced [9]. Finally, there is an unusually long component of 7.1 ns present when Q_A is doubly reduced, which is absent when Q_A is in the semiquinone form. Since the time window used was 10 ns, the value for the slow component is an approximate value. However, it is clearly slower than that in samples with singly reduced Q_A (see Fig. 2a,b). Slow (25–35 ns) fluorescence decay components, similar to the lifetime of the primary radical pair, have been observed before, in D1/D2/cyt *b*-559 particles which contain no Q_A [24–26].

In order to check whether the appearance of the 7.1 ns component was reversible, dithionite/benzyl viologen-treated samples were reoxidised by washing with ferricyanide (see Materials and Methods). Table ID summarises the fluorescence decay kinetics of the reoxidised samples. The decay kinetics and the relative fluorescence yield were similar to those of samples that had not been treated with dithionite and benzyl viologen (Table IA). The amplitudes of decay components of 1 ns or longer were small (approx. 1%). Generally, the kinetics were more similar to those of oxidised samples not treated with dithionite and benzyl viologen (Table IA). This indicates that the double reduction of Q_A is reversible when the sample is reoxidised and that slow components, observed in samples with dithionite present are not due to the presence of disconnected antenna.

EPR measurements were also carried out on reoxidised samples, in order to test whether the state $Q_A^-Fe^{2+}$ could be made again in its normal form [18] by re-adding dithionite. We found that only in a small proportion of the sample could this state be formed (in approximately 10%, comparing Fig. 1a and c). At the same time, there was a significant reaction-centre triplet signal detectable (Fig. 1c), although it was smaller than in samples with all the Q_A doubly reduced. This indicates that in most of the reaction centres in this preparation, Q_A is immediately doubly reduced upon dithionite addition. This is probably due to an irreversible conformational change that in turn results in a destabilised Q_A^- state.

PS II core complexes

The experiments described above were repeated with a different type of PS II preparation, which is designated 'core complex'. In this preparation there is a solubilisation step involved, for which the detergent octylglucopyranoside was used [17]. The core complexes are devoid of the major PS II light-harvesting protein, LHC II, and have approx. 70 chlorophylls per reaction centre [17]. The dithionite/benzyl viologen treatment for the double reduction of Q_A was similar to that described for PS II membranes. However, we noted that in this preparation, the yield of the reaction centre triplet was already significant at the beginning of the treatment (approx. 30% of the maximum obtainable level, data not shown). This suggests that in part of the reaction centres double reduction is essentially instantaneous when dithionite is added.

The results of the fluorescence lifetime measurements on the PS II core complexes are shown in Fig. 3. The traces show a number of features which are similar to those indicated for PS II membranes (Fig. 2): trace C (Q_A doubly reduced) shows a fast initial decay, as does trace A (Q_A oxidised) and there is a slow component in trace C that seems slower than that in trace B (Q_A singly reduced). However, the difference between the traces before and after dithionite/benzyl viologen treatment (Fig. 3b,c) is less pronounced than for the case of membranes (Fig. 2b,c).

The decay curves of core complexes were fitted using the same procedure as for membranes (Table IIA,B,C). The results for samples with Q_A oxidised and Q_A singly reduced agree with recent data obtained from a similar preparation [27]. A more quantitative

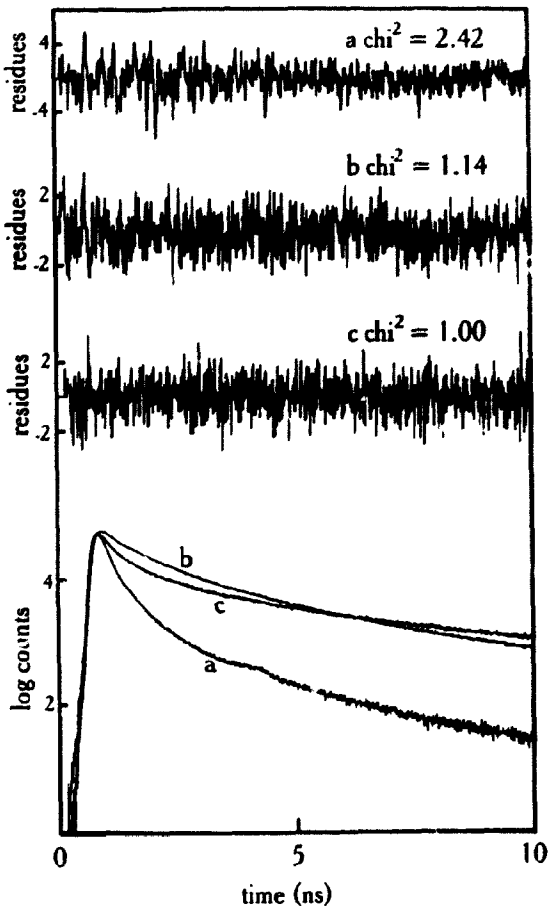

Fig. 3. Fluorescence decay (normalised) in PS II core complexes. Weighted residual plots are also shown (upper traces). Sample conditions were as described in the legend to Fig. 2. The number of counts in the peak channel was: (a) 61 000; (b) 50 000; (c) 16 000.

comparison between the results from membranes and core complexes can be made when Table I (A,B,C) and Table II (A,B,C) are compared. This reveals one major difference: the presence of a fast component (100 ps) in core complexes with dithionite added in order to singly reduce Q_A (Table IIB), compared to membranes, in which the fastest component has a lifetime of 600 ps under these conditions (Table IB). In core complexes, fast components (of the order of 100 ps) are present both before (Table IIB) and after (Table IIC) dithionite/benzyl viologen treatment. This accounts for the fact that in core complexes, the traces before (Fig. 3b) and after (Fig. 3c) dithionite/benzyl viologen treatment are more alike than they are in membrane samples.

Dithionite/benzyl viologen-treated core complex samples were tested for the reversibility of the presence of slow components by measuring them also in the presence of 0.05 mM ferricyanide. The slow components (1.3 ns and 7.1 ns) observed under reducing conditions decreased considerably in relative amplitude in the presence of ferricyanide.

Discussion

PS-II membranes

Fluorescence measurements with short time resolution have been used extensively to study the primary photochemical reactions in PS II. A large variety of preparations has been used and the fluorescence kinetics have been found to be significantly dependent on the type of preparation and the organism used and probably also on the measuring system used (see Refs. 20 and 21 for recent reviews). Our results on samples with Q_A singly reduced and Q_A oxidised are in agreement with previous work on similar preparations (e.g. Refs. 4, 8 and 22. They are also in agreement with results from intact chloroplasts and green algae (see Refs. 20 and 21), except that we did not have contributions from PS I. We will focus in the following on the doubly reduced redox state of Q_A, which has not been investigated before using picosecond fluorescence measurements, and compare its fluorescence decay kinetics with those of the oxidised and singly reduced states of Q_A.

We will analyse our results, assuming that the fluorescence decay kinetics of PS II are trap-limited (see e.g. Refs. 6 and 28, also Ref. 29, and Refs. 20 and 21 for comparisons with other concepts). In line with those studies, we assume that the excitation energy equilibrates over the antenna system, including P-680, within a few picoseconds (faster than the time resolution of our measuring system). The lifetime and amplitude of the fastest decay component thus reflect the trapping of the equilibrated excited state into the primary radical pair state. The lifetime and the amplitude of the fastest decay component are to a large extent responsible for the initial part of the fluorescence decay curve. Therefore, we assume that the initial decay observed in the fluorescence decay curve (Fig. 2), reflects the formation of the primary radical pair.

Comparing different redox states of Q_A in the same kind of PS II preparation will reveal influences of the Q_A state on primary photochemistry. When the traces A (Q_A oxidised) and B (Q_A singly reduced) of Fig. 2 are compared, a much faster initial decay is observed in the oxidised situation. Similar observations have been explained previously by a decreased yield of charge separation when Q_A is reduced, assuming trap-limited fluorescence decay kinetics [6,28]. A net repulsive electrostatic interaction between Q_A^- and the radical pair was thought responsible for this reduced yield [6,10].

Comparing traces B and C of Fig. 1 reveals that the initial decay in trace C (Q_A doubly reduced) is faster than that in trace B (Q_A singly reduced). Assuming trap-limited decay kinetics, this indicates a higher rate of charge separation (resulting in a higher yield) when Q_A is doubly reduced, compared to reaction centres with Q_A singly reduced. This was proposed before in a study on the triplet yield in relation to the redox state of Q_A [9]. In the latter study, a negligible triplet yield was found in reaction centres with Q_A singly reduced at liquid helium temperature and a drastic increase in triplet yield (close to 100%) was observed when Q_A was subsequently doubly reduced [9]. In a purely electrostatic model in which charges at the Q_A site directly influence the yield of the primary radical pair [6,10], the high rate of charge separation in the doubly reduced state implies that the negative charge at the Q_A site is neutralized. To explain this, we suggest that Q_A undergoes protonation upon double reduction forming the quinol, as was suggested before [9].

If double reduction of Q_A involves a protonation resulting in the electrically neutral quinol, the electrostatic repulsion between Q_A and the radical pair is absent and the yield of charge separation is probably similar to that of reaction centres with Q_A oxidised. This seems indeed the case from Table I: the lifetime and the relative amplitude of the fastest decay component are similar when comparing samples with Q_A oxidised (140 ps, 73%, Table IA) and Q_A doubly reduced (220 ps, 69%, Table IC).

It should be pointed out that the data might also be explained by conformational changes triggered by the Q_A redox state [9]. The electrostatic model discussed above and models invoking conformational changes are not necessarily mutually exclusive.

Apart from the similarities in the fastest decay component of oxidised and doubly reduced reaction centres mentioned above, the other decay components are different from each other. The most obvious difference is the presence of a component of approx. 7 ns with significant amplitude in samples with Q_A doubly reduced, whereas components longer than 1 ns are negligible in samples with Q_A oxidised. We stress that decay components in the range of 1–10 ns always disappeared upon reoxidation of samples (see Tables ID and IID). Thus, they are not the result of disconnected antenna. It is more likely that the presence of the 7 ns component in samples with doubly reduced Q_A is related to the longer lived radical pair in these samples. In the oxidised state, the second component observed has a lifetime of 330 ps, which is in the range of values reported for the lifetime of the primary radical pair under those conditions [10,30–32].

Comparing samples with Q_A singly and doubly reduced (Table IB,C), it is seen that in samples with Q_A doubly reduced (dithionite/benzyl viologen-treated), four components were found. In the case of singly reduced Q_A, three components were found which is in agreement with earlier work (see Refs. 20 and 21 for reviews). It is possible that the 2 ns component (8%) in dithionite/benzyl viologen-treated samples (Table IC) is due to a small fraction of reaction centres with Q_A still singly reduced but not detectable by EPR, in which case the doubly reduced state may also be characterised by three decay components. The presence of a 7 ns component when Q_A is doubly reduced, compared to 3 ns for the singly reduced state, indicates that the lifetime of the radical pair is longer in the doubly reduced state. However, the relation between the fluorescence decay kinetics and the lifetime of the primary radical pair is complex, and it is not straightforward to determine it from the fluorescence decay. For a homogenous sample, neglecting singlet-triplet mixing, the kinetics of the radical pair are described by an expression in which all of the exponential fluorescence decay components and their relative amplitudes appear (see Refs. 6, 8 and 19, for example). In the most simple model, in which there are forward and reverse reactions between two states (the excited state and the singlet primary radical pair state), only two decay components are present [6]. Here (see also Ref. 22), the situation is more complex because there are at least three decay components in samples with Q_A singly or doubly reduced. The presence of three or more PS II fluorescence decay components in green algae has been described assuming PS II-α/β heterogeneity [33]. Although we used PS II membranes [15] that were prepared from the grana which consist only of PS II-α, we can not rule out that our samples are heterogenous as regards the primary photochemical reactions. Thus, heterogeneity may explain the fact that three or more components are found in PS II membranes. However, in the case of doubly reduced Q_A, the fluorescence decay kinetics are probably influenced by the singlet-triplet mixing of the primary radical pair, the decay of which is then described by a Stochastic Liouville Equation [34]. Therefore, analysing the fluorescence decay kinetics as a sum of discrete, exponential, decay components is probably approximate and a rigorous mathematical analysis is hampered. Also, sample heterogeneity may give rise to a distribution of lifetimes, rather than discrete decay components. This is the case if the values for the rate constants of the primary reactions are gradually distributed around some average value. Nevertheless, there are some other indications, from flash absorption measurements, that the radical pair lives longer when Q_A is doubly reduced, compared to the singly reduced state. (1) Strong illumination in the presence of dithionite (now known to result in double reduction of Q_A [9]), gave an increase in the lifetime of the radical pair up to 15 ns [4]. (2) Comparison of different PS II preparations led to the suggestion that

the the yield of charge separation and also the lifetime of the primary radical pair increase when Q_A is doubly reduced [5,9].

PS II core complexes

Above, using the fluorescence decay curves of PS II membranes (Fig. 2), we have compared the yields of charge separation in samples with different states of the Q_A acceptor system, assuming trap-limited fluorescence decay. The same features as seen in PS II membranes (Fig. 2) are, to a somewhat lesser extent, present in PS II core complexes (Fig. 3): the initial decay of samples with Q_A oxidised and doubly reduced is faster than that of samples with Q_A singly reduced. We will now in some more detail compare the results from PS II membranes and PS II core complexes as shown in the Tables IA,B,C and IIA,B,C.

The core complexes were obtained from the membranes by a detergent solubilisation step, which results in a smaller antenna size (approximately 70 chlorophylls per reaction centre [17]). For trap-limited fluorescence decay, the rate of trapping is dependent on the antenna size [6]: decreasing the antenna size increases the free energy of the excited state relative to the primary radical pair state and hence the rate of trapping increases. This is illustrated for example under oxidised conditions: the fast component in core complexes is shorter (60 ps, Table IIA) compared to membranes (140 ps, Table IA).

There is a more striking difference between core complexes and membranes obvious in samples with Q_A singly reduced by addition of dithionite (Tables IB and IIB). In core complexes there is an additional 100 ps component present which is absent in PS II membranes. Such a component was also found in samples with Q_A doubly reduced (Table IIC). It is thus likely that the 100 ps component found in core complexes in the presence of dithionite reflects a significant percentage of reaction centres in the doubly reduced state. This corresponds to the observation made by EPR, that double reduction of Q_A in core complexes occurs immediately upon dithionite addition in a fraction (approx. 30%) of the reaction centres. The data thus suggest that in core complexes the Q_A site has been modified to the extent that double reduction of Q_A can occur rapidly when dithionite is added. Thus, a sample with part of the Q_A population in the singly reduced state and part in the doubly reduced state is obtained. Accordingly, the primary photochemistry and the fluorescence kinetics are heterogenous.

It was suggested before [9] that the intactness of the Q_A site decreases gradually with the severity of detergent treatments applied (usually in order to purify the reaction centre). This could be the factor (apart from antenna size effects) that was responsible for the differences in the yield of charge separation and the radical pair lifetime, reported for different preparations [5]. In particular, the stability of Q_A^- may decrease, going from intact membrane preparations towards small reaction centre preparations. The Q_A^- state may be less stabilised in these preparations due to detergent-induced conformational changes (see the situation in purple bacteria after removal of Fe^{2+} [35,36]) and an increased accessibility to dithionite. Therefore, we propose that dithionite, added with the aim of singly reducing Q_A, will at least partly doubly reduce Q_A in these preparations. This would give rise to a higher yield of charge separation and probably a longer radical pair lifetime than expected for a sample with all its Q_A singly reduced. Double reduction of Q_A can also occur in membrane preparations, if the sample is illuminated or incubated in the presence of dithionite [9]. The amount of double reduced Q_A is thus dependent on several factors, which are often not well controlled. It may also depend on the type of organism used.

The presence of different amounts of doubly reduced Q_A may explain part of the controversy in the literature on the yield of charge separation and the lifetime of the primary radical pair in reaction centres with Q_A thought to be singly reduced (see e.g. Refs. 5–7 and 10 for measurements on PS II samples with antenna sizes of 60–100 chlorophylls). In fact, in some cases, two phases were resolved in the radical pair decay [5,7]; the slow phase found (10 or more nanoseconds) probably reflects the radical pair lifetime in the fraction of reaction centres with Q_A doubly reduced.

Acknowledgments

We would like to thank A. van Hoek for technical assistance with the time-resolved fluorescence measurements and J.-M. Ducruet for his help with the fluorescence measurements under continuous illumination. We also thank P.J. Booth, K. Brettel, H.J. van Gorkom and W. Leibl for useful discussions and T.A. Roelofs for sending a preprint of unpublished work. F.v.M. is supported by the EEC (SCIENCE Programme) and A.W.R. is supported by the CNRS (URA 1290).

References

1 Rutherford, A.W. (1988) in Light-Energy Transduction in Photosynthesis: Higher Plant and Bacterial Models (Stevens, S.E. and Bryant, D.A., eds.), pp. 163–177. The American Society of Plant Physiologists, Rockville.
2 Van Gorkom, H.J. (1985) Photosynth. Res. 6, 97–112.
3 Hansson, Ö. and Wydrzynski, T. (1990) Photosynth. Res. 23, 131–162.
4 Eckert, H.J., Renger, G., Bernarding, J., Faust, P., Eichler, H.J. and Salk, J. (1987) Biochim. Biophys. Acta 893, 208–218.
5 Hansson, Ö., Duranton, J. and Mathis, P. (1988) Biochim. Biophys. Acta 932, 91–96.

6 Schatz, G.H., Brock, H. and Holzwarth, A.R. (1988) Biophys. J. 54, 397–405.
7 Schlodder, E. and Brettel, K. (1988) Biochim. Biophys. Acta 933, 22–34.
8 Leibl, W., Breton, J., Deprez, J. and Trissl, H.-W. (1989) Photosynth. Res. 22, 257–275.
9 Van Mieghem, F.J.E., Nitschke, W., Mathis, P. and Rutherford, A.W. (1989) Biochim. Biophys. Acta 977, 207–214.
10 Schatz, G.H., Brock, H. and Holzwarth, A.R. (1987) Proc. Natl. Acad. Sci. USA 84, 8414–8418.
11 Rutherford, A.W., Paterson, D.R. and Mullet, J.E. (1981) Biochim. Biophys. Acta 635, 205–214.
12 Okamura, M.Y., Satoh, K., Isaacson, M.A. and Feher, G. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. 1, pp. 379–381, Martinus Nijhoff, Dordrecht.
13 Rutherford, A.W., Zimmermann, J.L. and Mathis, P. (1984) Advances of Photosynthesis Research (Sybesma C., ed.), Vol. 1, pp. 445–448, Martinus Nijhoff, Dordrecht.
14 Van Mieghem, F.J.E., Searle, G.F.W., Rutherford, A.W. and Schaafsma, T.J. (1991) 37th Harden Conference: The Molecular and Structural Basis of Regulation in Photosynthesis, p. 52, The Biochemical Society, London.
15 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234.
16 Ford, R.C. and Evans, M.C.W. (1983) FEBS Lett. 160, 159–164.
17 Ghanotakis, D.F., Demetriou, D.M. and Yocum, C.F. (1987) Biochim. Biophys. Acta 891, 15–21.
18 Vermaas, W.F.J. and Rutherford, A.W. (1984) FEBS Lett. 175, 243–248.
19 Searle, G.F.W., Tamkivi, R., Van Hoek, A. and Schaafsma, T.J. (1988) J. Chem. Soc. Faraday Trans. 2 84, 315–327.
20 Geacintov, N.E. and Breton, J. (1987) CRC Crit. Rev. Plant Sci. 5, 1–44.
21 Holzwarth, A.R. (1991) in The Chlorophylls (Scheer, H., ed.), pp. 1125–1151, CRC Press, Boca Raton.
22 Hodges, M. and Moya, I. (1986) Biochim. Biophys. Acta 849, 193–202.
23 Renger, G., Koike, N., Yuasa, M. and Inoue, Y. (1983) FEBS Lett. 163, 89–93.
24 Mimuro, M., Yamazaki, I., Itoh, S., Tamai, N. and Satoh, K. (1988) Biochim. Biophys. Acta 933, 478–486.
25 Seibert, M., Picorel, R., Rubin, A.B. and Connolly, J.S. (1988) Plant Physiol. 87, 303–306.
26 Crystall, B., Booth, P.J., Klug, D.R., Barber, J. and Porter, G. (1989) FEBS Lett. 249, 75–78.
27 Hodges, M. and Moya, I. (1988) Biochim. Biophys. Acta 935, 41–52.
28 Schatz, G.H. and Holzwarth, A.R. (1986) Photosynth. Res. 10, 309–318.
29 Van Gorkom, H.J. (1986) Bioelectrochemistry and Bioenergetics 16, 77–87.
30 Nuijs, A.M., Van Gorkom, H.J., Plijter, J.J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 848, 167–175.
31 Eckert, H.-J., Wiese, N., Bernarding, J., Eichler, H.-J. and Renger, G. (1988) FEBS Lett. 240, 153–158.
32 Trissl, H.-W. and Leibl, W. (1989) FEBS Lett. 244, 85–88.
33 Lee, C.-H., Roelofs, T.A. and Holzwarth, A.R. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), Vol. I, pp. 387–390, Kluwer Academic Publishers, Dordrecht.
34 Hoff, A.J. (1981) Q. Rev. Biophys. 14, 599–665.
35 Dutton, P.L., Prince, R.C. and Tiede, D.M. (1978) Photochem. Photobiol. 28, 939–949.
36 Debus, R.J., Feher, G. and Okamura, M.Y. (1986) Biochemistry 25, 2276–2287.