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Reaction centre photochemistry in cyanide-treated photosystem II

Y. Deligiannakis [1], A.W. Rutherford *

Section de Bioénergetique, (URA CNRS 2096) Département de Biologie Cellulaire et Moleculaire, CEA Saclay, 91911 Gif-sur-Yvette, France

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Abstract

EPR was used to study the triplet state of chlorophyll generated by radical pair recombination in the photosystem II (PSII) reaction centre. The spin state of the non-haem Fe^{2+} was varied using the CN^--binding method (Y. Sanakis, V. Petrouleas, B.A. Diner, Biochemistry 33 (1994) 9922–9928) and the redox state of the quinone acceptor (Q_A) was changed from semi-reduced to fully reduced (F.J.E. van Mieghem, W. Nitschke, P. Mathis, A.W. Rutherford, Biochim. Biophys. Acta 977 (1989) 207–214). It was found that the triplet was not detectable using continuous wave EPR when Q_A^- was present irrespective of the spin-state of the Fe^{2+}. It was also found that the triplet state became detectable by EPR when the semiquinone was removed (by reduction to the quinol) and that the triplet observed was not influenced by the spin state of the Fe^{2+}. Since it is known from earlier work that the EPR detection of the triplet reflects a change in the triplet lifetime, it is concluded that the redox state of the quinone determines the triplet lifetime (at least in terms of its detectability by continuous wave EPR) and that the magnetic state of the iron, (through the weakly exchange-coupled Q_A^- Fe^{2+} complex) is not a determining factor. In addition, we looked for polarisation transfer from the radical pair to Q_A^- in PSII where the Fe^{2+} was low spin. Such polarisation is seen in bacterial reaction centres under comparable conditions. In PSII, however, we were unable to find evidence for such polarisation of the semiquinone. It is suggested that both the short triplet lifetime in the presence of Q_A^- and the lack of polarised Q_A^- might be explained in terms of the electron transfer mechanism for triplet quenching involving the semiquinone which was proposed previously (F.J.E. van Mieghem, K. Brettel, B. Hillmann, A. Kamlowski, A.W. Rutherford, E. Schlodder, Biochemistry 34 (1995) 4798–4813). It is suggested that this mechanism may occur in PSII (but not in purple bacterial reaction centres) due the triplet-bearing chlorophyll being adjacent to the pheophytin at low temperature as suggested from structural studies (F.J.E. van Mieghem, K. Satoh, A.W. Rutherford, Biochim. Biophys. Acta 1058 (1992) 379–385).

Keywords: Photosynthesis; Triplet state; EPR; P680; Reaction centre; Photoinhibition

1. Introduction

In the Photosystem II (PSII) reaction centre of oxygenic photosynthetic organisms, absorption of a photon leads to a charge separation between the primary electron donor (P680) and a pheophytin α molecule (Ph) acting as primary electron acceptor [1]. In centres where the subsequent electron acceptor, a quinone molecule (Q_A), is reduced or absent, spin

Abbreviations: PSII, photosystem II; Ph, pheophytin; P680, the photooxidisable chlorophyll in PSII; Q_A and Q_B, the plastoquinones acting as electron acceptors; EDTA, ethylenediaminetetraaceticacid; Tris, tris(hydroxymethyl)aminomethane; $Y_D^{\bullet}$, the stable tyrosyl radical

* Corresponding author. Fax: +33 (1) 6908-8717.

[1] Present address: The Institute of Material Science, NCSR 'Democritos', 15310 Aghia Paraskevi Attikis, Greece.

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dephasing occurs in the $P680^+$ Ph^- radical pair, forming the triplet state $^3P680$, upon charge recombination [2–4]. In the presence of a magnetic field, the $^3P680$ is characterised by an EPR signal with a characteristic polarisation pattern AEEAAE typical for its formation by radical-pair recombination [2] and is similar to that found in the reaction centre of purple bacteria [5,6].

In contrast to the bacterial case, in PSII $^3P680$ triplet cannot be observed with standard low temperature EPR when the Q_A is pre-reduced to the semiquinone form [3]. Van Mieghem et al. [3] demonstrated that the triplet was detectable by continuous wave EPR only after double reduction of the Q_A. Subsequently evidence was obtained indicating that this was due to the triplet state having a short lifetime in the presence of Q_A^- but a longer lifetime (making it detectable by continuous wave EPR), when the quinone was doubly reduced [4].

One of the possible reasons proposed to explain this effect was that it resulted from a magnetic effect of the spin of the non-haem iron (i.e. Fe^{2+} $S=2$) on the life-time of the $^3P680$ triplet [3,4] in PSII. Suggestions that second-reduction of Q_A^- may result in loss of the paramagnetic non-haem iron [7] have been shown to be unlikely, at least in our conditions, since the iron is clearly present as a potent relaxer of the Ph^- in PSII where the Q_A is doubly reduced [8]. Nevertheless, it is possible that the presence of the magnetically coupled state resulting from Q_A^- interacting with the non-haem ferrous ion (Fe^{2+} $S=2$) may influence the triplet lifetime. In the bacterial reaction centre, the fast-relaxing spin system of the Q_A^- Fe^{2+} ($S=2$) has marked influences on the pheophytin radical [9,10] and on the triplet state of the primary electron donor [11].

Recently it was demonstrated that treatment of the PSII reaction centre with high concentrations of KCN at pH 8 converts the acceptor-side non-haem Fe^{2+} ($S=2$) to its diamagnetic low-spin ($S=0$) state [12]. In the present work, we have investigated the influence of the spin state of the iron and the redox state of quinone on the P680 triplet detectable by continuous wave EPR and the influence of radical pair formation on the semiquinone in the presence of the low spin iron.

2. Materials and methods

Photosystem II membranes were isolated from market spinach as described previously [13] with the modifications described in [14]. Mn depletion was performed by incubating these PSII membranes (0.4 mg chlorophyll/ml) in 0.8 M Tris-HCl (pH 8.0), 5 mM Na-EDTA for 30 min at 0°C under room light. The Mn-depleted membranes were pelleted and washed once in a buffer containing 60 mM HEPES (pH 8.1), 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$ and resuspended in the same buffer at 4 mg chlorophyll/ml final concentration. In these PSII membranes, the non-haem iron was converted to its low-spin state by incubating the Mn-depleted PSII membranes with 350 mM KCN at pH 8.0 for 3.5 h at 5°C, according to [12]. As a control, Tris-washed PSII membranes, were incubated under similar conditions without the KCN. In both types of PSII membranes, the Ph^- radical was induced by a procedure which involved incubation of the membranes under reducing conditions ($E_h = -420 \pm 20$ mV) for 60 min at 5°C, in the dark followed by illumination at 15°C for 12 min [3,8]. No redox mediators were used. The redox potential was adjusted by sodium dithionite under anaerobic conditions and was measured in the sample as described in [8]. Quantitation of the formation of the Q_A^- and the Ph^- radicals was done by comparison with the double integral of the continuous wave EPR spectrum of the stable tyrosine radical, $Y_D^{\bullet}$, in untreated PSII according to [15]. Oriented PSII membranes were prepared on mylar strips as described in [16].

Continuous-wave EPR spectra were recorded at liquid helium temperatures with a Bruker ER 300 X-band spectrometer equipped with an Oxford Instruments cryostat. The sample temperature was adjusted by using an ITC5 temperature controller. The microwave frequency and the magnetic field were measured with a microwave frequency counter HP 5350B and a Bruker ER035M NMR gaussmeter, respectively.

EPR spectra of the triplet-state $^3P680$ were recorded under continuous illumination in the cavity at liquid helium temperatures. Illumination was performed by 800 W white-light filtered through 4 cm of water and two Calflex IR filters.

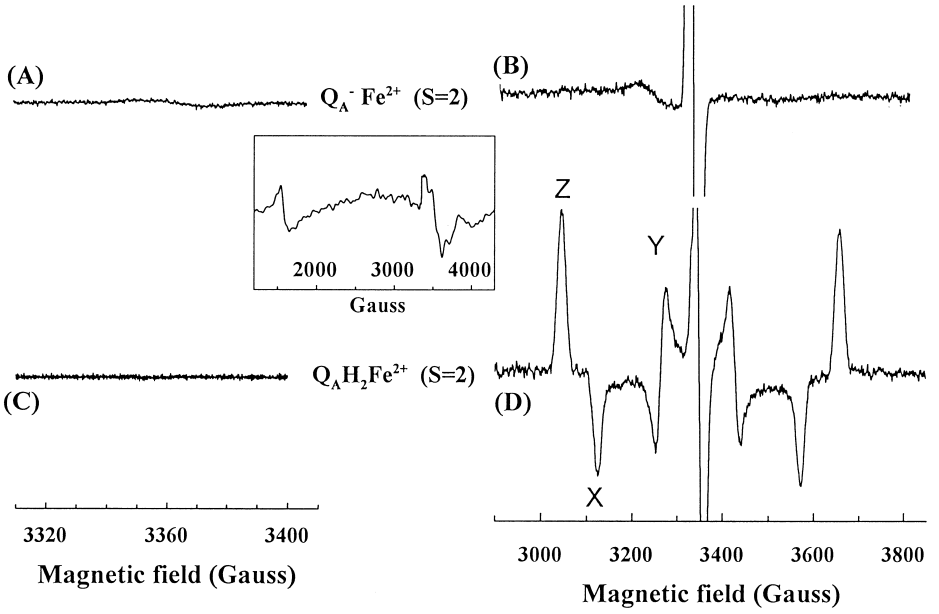

Fig. 1. Relationship between the spin polarised continuous wave EPR spectrum of ^{3}P680 and the redox state of the primary acceptor, Q_A, in PSII. Samples are Tris-washed PSII membranes poised to $E_h = -120$ mV, pH 8.2, 30% glycerol. (A) No illumination: state Q_A^- Fe^{2+} ($S=2$), under conditions favouring the detection of the uncoupled Q_A^- radical at around $g=2$ region, i.e. low-power (25 μW) and at 15 K. The inset is the same sample as in (A), but recorded under conditions which favour the detection of Q_A^- Fe^{2+} ($S=2$), i.e. at 32 mW of microwave power and 4.5 K. (B) Same state as in (A) but under continuous illumination at 4 K. EPR conditions: microwave power, 64 μW, modulation amplitude 10 G; modulation frequency, 100 kHz. (C) Sample poised at −400 mV, after illumination for 6 min at 4°C followed by incubation in the dark for 30 min: state Q_A H_2 Fe^{2+} ($S=2$), conditions as per (A) $g=2$ region. (D) same as in (C) under continuous illumination at 4 K, other conditions as in (B).

3. Results

The low-temperature EPR signal for a Mn-depleted PSII sample reduced with dithionite is shown in Fig. 1A. The EPR signal at $g=1.9$ which is characteristic of the iron-semiquinone Q_A^- Fe^{2+} ($S=2$) state [17] is resolved at high microwave power (see inset in Fig. 1). In the same sample at low microwave powers, no signal is resolved in the $g=2$ region, Fig. 1A. In contrast, in the CN-treated PSII a strong narrow signal ($g=2.0045$ and $\Delta H=9.4$ G) is resolved, see Fig. 2A. As shown by [12], treatment of PSII with high concentrations of CN^- converts the high-spin iron ($S=2$) into the low-spin form ($S=0$) and therefore eliminates its magnetic interaction with the semiquinone anion radical. Accordingly, the radical in Fig. 2A is assigned to the semiquinone radical, Q_A^-, of the acceptor side of PSII [12,18].

Fig. 1B shows that in the state Q_A^- Fe^{2+} ($S=2$) no ^{3}P680 signal is detected under continuous illumination at liquid helium temperatures. This result agrees with the earlier studies in which it was shown that the ^{3}P680 signal (shown Fig. 1D) is detected only after double reduction of the Q_A and detrapping of the reduced pheophytin, [3]. Fig. 2B shows that in the state Q_A^- Fe^{2+} ($S=0$), i.e. in the CN-treated sample, no ^{3}P680 is detected in the presence of Q_A^-. The same result was obtained when we repeated this measurement at a range of illumination intensities, microwave powers and temperatures.

In contrast, the ^{3}P680 signal is easily detected in the CN-treated PSII where the Q_A is double reduced, Fig. 2D. Under these conditions, the polarisation pattern is AEEAAE and the zero-field-splitting parameters are $D=286\times10^{-4}$ cm^{-1} and $E=41\times10^{-4}$ cm^{-1} which is similar to that in the control sample in which the iron is in the high spin form (Fig. 1D and see [2]). The intensities of the triplet EPR signals detected in the control and the CN-treated PSII are comparable within the experimental error. We have previously demonstrated that the yield of the triplet under these conditions in control samples is 50% [4],

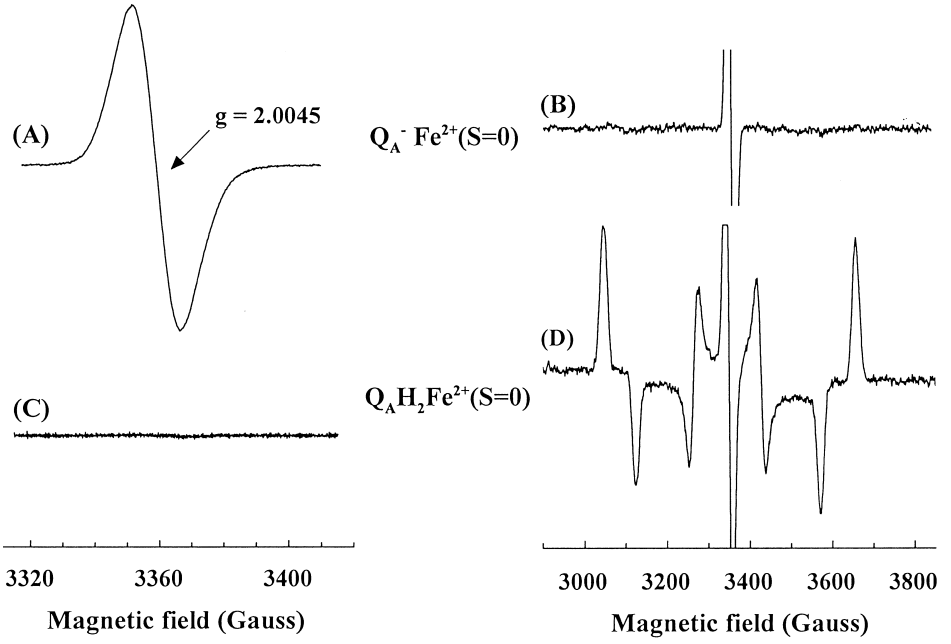

Fig. 2. Relationship between the spin polarised continuous wave EPR spectrum of ^{3}P680 and the redox state of the primary acceptor in PSII when the iron is in its low spin state. Samples are Tris-washed PSII membranes treated with 340 mM KCN for 4 h, poised to $E_h = -120$ mV, pH 8.2, 30% glycerol. (A) No illumination: state Q_A^- Fe^{2+} ($S=0$), low-power (25 μW) $g=2$ region 15 K. (B) Same state as in (A) under continuous illumination at 4 K. EPR conditions are the same as in Fig. 1B. (C) Sample poised at −400 mV, after illumination for 6 min at 4°C followed by incubation in the dark for 30 min: state Q_A H_2 Fe^{2+} ($S=0$), low-power $g=2$ region 15 K. (D) same as in (C) under continuous illumination at 4 K, other conditions as in Fig. 1B.

therefore we can conclude that the triplet yield in the CN-treated sample is comparable to this.

3.1. Temperature dependence of the triplet

The temperature dependence of the intensity of the ^{3}P680 EPR signal was examined by recording a spectrum at a series of temperatures. For this study, a series of similar samples was prepared with or without CN; at every temperature a different sample was used and a spectrum was recorded in a single (∼80 s) scan in order to minimise the effects from pheophytin anion photoaccumulated at this temperature [19]. In both the control and the CN-treated sample, the maximum intensity for all the EPR peaks was detected at the lowest temperatures, the amplitudes of the *X*, *Y* and *Z* peaks at ∼15 K decreased by about 50% compared to that at 4 K. For both types of sample, the spectra remained symmetrical and there was no sign-reversal of the peaks over the range 4.2–30 K. Fig. 3 shows the normalised intensity for the *X*, *Y* and *Z* peaks recorded over the temperatures 4.2–30 K with a microwave power of 64 μW. It is seen that within the experimental error the temperature dependence for the three peaks is the same in the control (triangles) and the CN-treated (circles) PSII.

3.2. Orientation dependence of the ^{3}P680

The orientation dependence of the ^{3}P680 EPR signal was examined in PSII membranes oriented on mylar strips as in [16]. It was found that the orientation of the ^{3}P680 peaks is similar in both the control and the CN-treated PSII (data not shown).

3.3. The Q_A^- Fe ($S=0$) signal illuminated at low temperature

We looked for polarisation of the Q_A^- signal in the presence of low-spin iron when given continuous illumination at liquid helium temperatures. Light-induced variations in the intensity of the EPR signal of Q_A^- were observed in the CN-treated PSII (data not shown); however, the observed intensity changes matched the variation expected from the illumina-

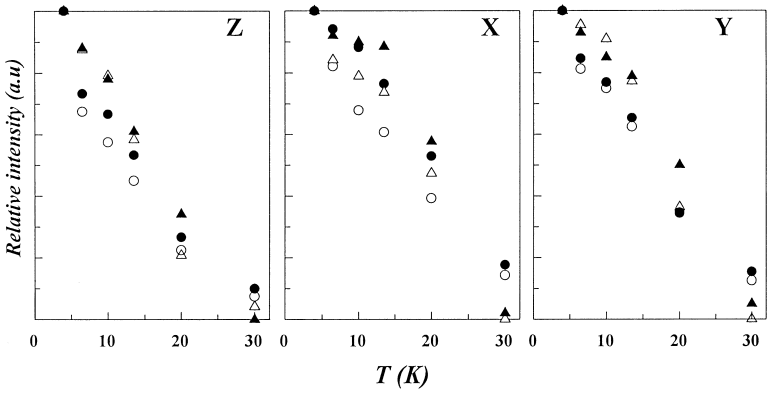

Fig. 3. Temperature dependence of the relative amplitudes of the *X*, *Y* and *Z* peaks, respectively of the spin-polarised EPR signal of ^{3}P680 in Tris-washed PSII in the state Q_A H_2 Fe^{2+} ($S=2$) (triangles) and in the state Q_A H_2 Fe^{2+} ($S=0$) (circles), i.e. after CN^- treatment. Data were obtained using samples at two different values of pH: solid symbols, pH 8.2; open symbols, pH 6.5. The intensity of each peak is normalised to the amplitude recorded in a single scan at the lowest temperature under continuous illumination. EPR conditions: 64 μW, modulation amplitude 10 G; modulation frequency, 100 kHz.

tion-induced temperature changes leading to changes in the amplitude of Q_A^- following Curie Law. Thus we conclude that no spin polarisation of the Q_A^- is detectable by steady state EPR in the CN-treated PSII. This is in agreement with the conclusion in [20]. Long illumination at low temperature led to only small increase in the size of the radical-type EPR signal and this was attributed to the trapping of the pheophytin anion (see [19]). No polarisation effects were detected. In contrast, Hoff and Proskuryakov [20] reported the generation of an EPR signal after illumination at 15 K which was five times bigger than the Q_A^- signal. Furthermore, they reported that this signal decreased under illumination and this was attributed to polarisation transfer from the $P680^+$ Ph^- radical pair to the stable, unidentified radical. We propose a possible explanation for this discrepancy in the discussion.

4. Discussion

4.1. On the mechanism of fast P680 triplet decay

In this report, we have characterised some of the EPR properties of PSII reaction centre in the presence of high concentrations of CN^-, i.e. when the non-haem iron is converted to the low spin form. This allowed a study of the effect of the magnetic state of the non-haem iron on P680 triplet formation in the presence and absence of the semiquinone Q_A^-. The triplet signal was not detectable when the semiquinone was present irrespective of the spin state of the non-haem iron. When the quinone was doubly reduced, the triplet was detectable, also irrespective of the spin state of the iron. Thus the results demonstrate that the magnetic state of the non-haem iron, and thus the presence of Q_A^- Fe^{2+} ($S=2$), does not determine whether the P680 triplet EPR signal is detectable by continuous wave EPR. Since it is likely that the detectability of the ^{3}P680 is determined by the lifetime of the triplet state itself (see [4]), then we conclude that the paramagnetic state of the iron (and hence Q_A^- Fe^{2+} ($S=2$)) is not responsible for the unusually short lifetime of the triplet. Clearly it is the redox state of the semiquinone itself which directly correlates with the short lifetime of the triplet (n.b. the assay used here, i.e. the steady state detectability of the triplet EPR signal, only reports on the very marked change in the triplet lifetime characterised earlier [4], therefore we cannot determine whether minor change in the triplet kinetics occur in samples containing Q_A^- Fe^{2+} ($S=0$) compared to those with Q_A^- Fe^{2+} ($S=2$). Our interest in this work is with the marked change in lifetime which correlates with detectability of the triplet EPR signal).

What is responsible for the short lifetime of the triplet when the semiquinone is present? In what follows we shall briefly treat the suggestions that have been made in the literature to explain the unexpected behaviour of the triplet state in PSII.

4.1.1. An electrostatic effect

When it was first demonstrated that the triplet was formed only after the quinone was double-reduced or absent, it was suggested that an electrostatic effect of the semiquinone could have resulted in a decreased yield and lifetime of the $P680^+$ Ph^- radical pair thus greatly decreasing triplet formation [3]. When experiments were done to test this [4], it was found that this is probably the case but only at high temperatures, while at low temperatures, it does not occur. The experiments showed that although the semiquinone does indeed decrease the lifetime of the radical pair at low temperature, this has virtually no influence on the radical pair yield nor on the triplet yield [4]. Instead it was found that in the presence of the semiquinone, the triplet decays more than two orders of magnitude more rapidly than in its absence [4]. Although it is possible that the charge on the semiquinone could influence the triplet lifetime, it seems unlikely that a coulombic effect, which has only a minor influence on radical pair recombination, should have such a major effect on the triplet lifetime. It thus seems unlikely that the charge on the semiquinone anion is responsible for the short triplet lifetime.

4.1.2. Quenching by carotenoid

The most obvious potential triplet quenching mechanism is one involving carotenoid and it is possible to imagine some structural event associated with quinone double reduction [3,4] which leads to an uncoupling of the carotenoid from the chlorophyll. However, it was shown experimentally that a role for triplet quenching by carotenoid was highly unlikely [4,21].

4.1.3. A magnetic effect

The possibility that the non-haem iron, mediated by the Q_A^- Fe^{2+} ($S=2$), determines the triplet lifetime [3,4] is ruled out by the present work. Furthermore, a magnetic effect of the uncoupled semiquinone radical on the triplet lifetime is considered highly unlikely. Indeed, we have found that, when the iron is low spin, there is no detectable magnetic effect of the semiquinone on the neighbouring Ph^- (Deligiannakis and Rutherford, unpublished data).

4.1.4. An electron transfer quenching mechanism

It has been suggested [4] that the following electron transfer mechanism could be responsible for the rapid triplet quenching in PSII:

$$^3P680\ Ph\ Q_A^- \Leftrightarrow P680\ ^3Ph\ Q_A^- \Rightarrow$$

$$P680\ Ph^-\ Q_A \Rightarrow P680\ Ph\ Q_A^-$$

The idea is that the unusual triplet decay occurs in PSII due to an electron transfer from the semiquinone (Q_A^-) to the pheophytin triplet (^3Ph), forming the Ph anion which reduces the semiquinone in the normal way [4]. This requires that the triplet Ph is formed as an intermediate or possibly as a virtual intermediate in a super-exchange mechanism. Some thermodynamic aspects of this model have recently been considered [21]. There is currently no direct evidence for such a mechanism but it does have at least one redeeming feature: namely, that it has not yet been ruled out. It is seems worth looking for evidence of Ph triplet formation in samples in which the quinone is doubly reduced (or in samples in which the quinone is removed). Possibly relevant in this respect is the report in D1/D2 cytochrome *b*559 reaction centres of PSII of a small pheophytin triplet [22] although this state did not seem to be related to charge recombination.

In the bacterial reaction centre, the triplet lifetime does not show huge changes in the triplet lifetime depending on the state of the quinone. However, the triplet lifetime does become slightly longer (by a factor of 7.5 at room temperature) when the quinone is double reduced or is removed compared to when the semiquinone is present [35]. This has remained unexplained but it is worth considering that the electron transfer mechanism proposed to exist in PSII may also be responsible for this much less marked effect in bacterial reaction centres. The much smaller effect in the bacterial reaction centre may be rationalised in terms of: (a) the energetics of chlorophyll and pheophytin in PSII compared to bacteriochlorophyll and bacteriopheophytin; (b) the triplet of P in the bacterial reaction centre being shared over a pair of bacteriochlorophylls (and is thus at a lower energy) in bacteria, while in PSII, at least at low temperature, it is localised on a single chlorophyll molecule; or (c) the monomeric chlorophyll bearing the triplet at low temperature in PSII

being the likely counterpart of the monomeric bacteriochlorophyll in bacterial reaction centre [16,23]. This chlorophyll molecule is expected to be adjacent to the pheophytin and thus triplet transfer to the pheophytin will be favoured.

The last of these options is attractive not least because it relates the two most striking properties of the triplet of PSII which differentiate it from that of the purple bacterial reaction centre: namely its very short life-time in the presence of semiquinone [4] (resulting in its non-detection using conventional EPR [3]) and the unexpected orientation of the triplet bearing chlorophyll [16,24].

4.2. Electron spin polarisation (ESP) of $^3P680$ and Q_A^- in PSII with and without high spin Fe^{2+}

In the bacterial reaction centre the iron influences the polarisation of the reaction centre triplet through a coupling mediated by the Q_A^- Fe^{2+} ($S=2$) [11,29]. Using the standard instrumentation, in the present study we were unable to detect the triplet in the presence of Q_A^- Fe^{2+} ($S=2$). However, given the potential structural differences in PSII compared to the bacterial reaction centre, especially those concerning P680, it seemed worth checking whether the iron might influence the triplet polarisation in the absence of the semiquinone. We found that within the time resolution of our experimental set-up, the magnetic state of the iron has no effect on the observed ESP pattern and the temperature dependence of the ^{3}P680. To test the influence of the Q_A^- Fe^{2+} ($S=2$) on the triplet polarisation, higher time resolution (ns to μs) would be required.

In addition, we were unable to detect polarisation of the static Q_A^- signal through polarisation transfer from the radical pair, in agreement with an earlier report [20] (n.b. we were able to detect very large polarisation effects in Zn-containing reaction centres of *Rhodobacter sphaeroides*, essentially as described by Gast and Hoff [30] (not shown)). We conclude, in agreement with [20], that polarisation transfer of the static Q_A^- radical does not occur in PSII. This conclusion can made much more confidently from the present work since we know radical pair formation is taking place and giving rise to triplet formation (see also [4] for radical pair yields under comparable conditions).

In [20], an unidentified radical, five times bigger than that of the semiquinone was formed upon low temperature illumination. Here, however, we observed the photo-accumulation of a radical attributable to the pheophytin anion from a fraction of centres in agreement with earlier observation of Ph^- photo-accumulation at low temperature [19]. In [20], the unidentified signal underwent a marked decrease while under illumination. In our studies, however, we were unable to detect any changes attributable to spin polarisation of the radicals. In the context of the present results and the current understanding of the structure of PSII, we can attempt to rationalise some of the previous observations. It seems possible that in [20], the biochemical treatment used to disrupt the iron site, led to loss of the quinone and/or that the pre-illumination treatment used to form the Q_A^- resulted in its double reduction. If so, the Q_A^- signal reported in [20] would represent a small fraction of the centres and thus the 5-fold increase in radical signals upon illumination at low temperature could be due to trapping of Ph^- anion. The light-induced decrease of this signal remains unexplained and indeed is difficult to explain in terms of polarisation phenomena in the context of the proposed explanation. An obvious explanation is that heating of the sample occurred during illumination leading to a decrease in signal amplitude and such an effect might be expected under conditions where the microwave power was non-saturating. Although arguments against a temperature effect were given in the original paper, their weight and the improvements in our understanding of PSII suggest that perhaps this explanation should be reconsidered. In any case, the present study, in which we have a quantitative measure of the radicals and good biochemical control of the preparation, shows no evidence for polarisation transfer, and at least as far as the Q_A^- is concerned, we are in agreement with the earlier work [20].

In the bacterial reaction centre, according to the model of [30,31], the observed polarisation of Q_A^- arises from transfer of spin polarisation from the photo-reduced BPh^- to pre-reduced Q_A^-. The intensity of the light-induced polarised Q_A^- signal is a function of: (1) the light flux; (2) the microwave-induced transition rate which is controlled by the microwave power; (3) the spin lattice relaxation

(T_1) of Q_A^- (the removal of the high-spin Fe^{2+} is required); (4) the transfer probability which can be correlated to the exchange interaction; and (5) the net spin polarisation induced on BPh^-. The absence of the polarisation signals in PSII may be due to one or more of these factors. The light intensity and the microwave power can and were varied over a wide range with no evidence for polarised Q_A^- being obtained. The T_1 of the Q_A^- in PSII-treated with CN has been measured [32] and is similar to the T_1 of Q_A^- in iron-depleted reaction centres from *R. sphaeroides* [33]. As for the exchange interaction, in bacteria $J_{Bpheo-Q_A^-}$ is estimated to be 2–5 Gauss [34,29], while in PSII, we know: (1) that $J <$ (linewidth) since no broadening is resolved when Ph^- and Q_A^- are present simultaneously (not shown); and (2) that the (B)Ph^- interaction with the Q_A^- Fe^{2+} is comparable in both kinds of reaction centre judging by the spilt EPR signals induced in both cases (see [18]). Lastly, for the spin polarisation on Ph^-, we lack information but there is little reason to expect a significant difference between the two systems at this level. In conclusion, based on our understanding of the polarisation transfer effect and the current knowledge of the two reaction centres, it would be reasonable to expect polarisation transfer to occur, and yet it does not.

A possible explanation for the lack of detectable polarisation transfer to the quinone is provided in the context of the abnormally rapid triplet lifetime seen under these conditions. The electron transfer mechanism for triplet decay involving the semiquinone [4], which is described above, could also be responsible for the loss of polarisation on the semiquinone since electron polarisation would be lost due to the electron transfer to and from the pheophytin.

4.3. Potential physiological significance

The rapid quenching of the charge recombination triplet in PSII probably represents a significant protective mechanism, in that whenever the $P680^+$ Ph^- radical pair is formed in the presence of Q_A^- (and under normal circumstances the radical pair yield is relatively small: see [4,25,26]), the triplet state formed will be rapidly quenched thereby diminishing the likelihood of a reaction with oxygen. In the context of the electron transfer mechanism for triplet quenching as described above [4], such a mechanism will not work on the triplet formed by charge recombination from the $P680^+$ Ph Q_A^- radical pair since the triplet is predicted to be present in the absence of Q_A^-. The triplet formed under these circumstances is likely to be long-lived and thus to be more likely to react with oxygen to form singlet oxygen. Relatively high yields of damage have indeed been reported from $P680^+$ Ph $Q_A^-(Q_B^-)$ charge recombination [27]. It has been suggested that the reaction centre is protected against such damage, under some (physiologically relevant) conditions, by changes in the redox potential of Q_A, which modulate the yield of $P680^+$ Ph^- Q_A and thereby the yield of long-lived $^3P680$ [28].

Acknowledgements

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