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Are the trapping dynamics in Photosystem II sensitive to Q_A redox potential?

L.M.C. Barter [a], M.J. Schilstra [a], J. Barber [b], J.R. Durrant [a], D.R. Klug [a,*]

[a] *Department of Chemistry, Imperial College, London SW7 2AY, UK*
[b] *Department of Biochemistry, Imperial College, London SW7 2AY, UK*

This paper is dedicated to George Porter, fondly known to us as “Prof”. We hope that you enjoy reading this paper, which like much of our work, has been inspired by your contributions to the field. Many thanks for all your support and encouragement over the years, and for introducing us to the photochemistry of photosynthesis in particular.

Abstract

Removal of the manganese complex from photosystem II (PSII) causes a 145 meV shift in the redox potential of the plastoquinone Q_A [Biochim. Biophys. Acta 1229 (1995) 193; Photosynthetica 27 (1–2) (1992) 89; Biochim. Biophys. Acta 1229 (1995) 202]. Time resolved single photon counting has been used to monitor the time dependent concentrations of the singlet states in intact and manganese depleted BBY-type core particles studied with Q_A in a fully open state. Surprisingly, no difference is observed between the experimental results obtained from the intact and manganese depleted samples. We demonstrate that this could be indicative of a very deep primary radical pair trap formed in PSII core particles with Q_A in a fully open state. Alternatively, static inhomogeneity or a possible time-dependence of the Q_A redox potential via relaxations such as proton motions should be considered. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Trapping dynamics; Photosystem II; Redox potential

1. Introduction

Photosystem II (PSII) is one of the photosynthetic complexes present in oxygenic photosynthetic organisms, and is unique because of its ability to create a high enough oxidising potential that enables the oxidation of water [4]. PSII is a multi-pigment protein complex [5,6] that contains approximately 250 molecules of chlorophyll, whose function is to absorb photons of light and transfer the energy to the reaction centre. The PSII reaction centre comprises the D1 and D2 proteins, as well as the α and β subunits of cytochrome *b*559. These proteins support six chlorophyll *a* molecules, two pheophytin *a* molecules, two β carotenes, the plastoquinones Q_A and Q_B, the redox active tyrosines Y_Z and Y_D, and one atom of non-heme iron.

Following either direct absorption of a photon of light by the reaction centre, or via energy transfer from the antenna, a multimer comprising four chlorophyll *a* and two pheophytin *a* molecules becomes excited [7]. Charge separation reactions then proceed from this delocalised excited state and result in the formation of the primary radical pair $P_{680}{}^{+}Phe^{-}$ [8]. Secondary charge transfer reactions to the plastoquinones (Q_A and Q_B) may then occur, resulting in $P_{680}{}^{+}Q_A{}^{-}$ and subsequently $P_{680}{}^{+}Q_B{}^{-}$ formation. The highly oxidising cation $P_{680}{}^{+}$ is reduced by Y_Z, a tyrosine at the D1-161 position, in a multiphasic reaction that occurs over a time scale of 20 ns to 30 ms. Y_Z^{+} is in turn, reduced by electrons that have been extracted from water, via the oxygen evolving complex. (For a more detailed discussion, see [4,9]). For reviews on the redox reactions occurring in PSII, the reader is also referred to [4,9–13], and references within.

It has been demonstrated that the loss of water splitting activity, by either the removal of calcium or manganese from the water splitting complex, shifts the midpoint potential of Q_A from a low potential form (−80 mV) to a high potential form (+65 mV) [1,2]. Interestingly, both the high and low potential forms have also been observed in cells from the green algae *Scenedesmus obliquus*. The high potential form of Q_A has been observed in cells grown under dark conditions, where the manganese cluster is not able to be photoactivated, whereas, the low potential form is observed in samples grown in the light [3].

The work discussed in this paper, presents a comparison between the trapping dynamics observed in intact BBY-type PSII core particles with those occurring in particles where the water splitting activity has been inhibited. The technique of time resolved single photon counting was used to monitor

* Corresponding author. Fax: +44-171-594-5806.
E-mail address: d.klug@ic.ac.uk (D.R. Klug).

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the time dependent absolute singlet state populations in all of the samples investigated. This study investigates the sensitivity of the trapping dynamics in PSII to the redox potential of Q_A.

A recent study of PSII core particles, investigated with Q_A in a fully closed state [14] suggested that the slow trapping of excitation energy via the formation of a shallow primary radical pair state $P_{680}{}^{+}Phe^{-}$, that has been observed in a large number of studies made on isolated reaction centres ([15–27]) is indeed a genuine feature of the bioenergetics of PSII [14]. The results reported here may also be discussed in light of work that investigates energy transfer and trapping in core particles with Q_A in a fully open state (to be published shortly). By making a comparison between the dynamics of energy transfer and trapping observed in intact and manganese depleted BBY preparations, the shift in the redox potential of Q_A that results from the removal of the manganese complex, is being employed as a tool to probe the effect on the trapping dynamics in PSII that would result from a change in Q_A redox potential.

2. Experimental details

2.1. Sample preparation

Oxygen evolving PSII membrane fragments (BBYs) [28] were prepared as described previously [29]. For the spectroscopy presented here the samples were resuspended in 0.3 M sucrose, 25 mM MES, 10 mM $MgCl_2$, 10 mM NaCl and 5 mM $CaCl_2$ at pH 6.5.

Two types of manganese depleted preparations were prepared: tris-washed samples involved a ∼10 min incubation on ice, in the dark, in 1 M tris and 500 mM $MgCl_2$ at pH 8.5, followed by a wash in a buffer containing 300 mM sucrose, 25 mM MES, 10 mM $MgCl_2$, 10 mM NaCl and 5 mM $CaCl_2$ at pH 6.5. The NH_2OH-treated samples were incubated on ice and in the dark for ∼10 min in 50 mM NH_2OH, in a buffer containing 300 mM sucrose, 25 mM MES, 10 mM $MgCl_2$, 10 mM NaCl and 5 mM $CaCl_2$ at pH 6.5 followed by a wash in this buffer without the NH_2OH. For optical spectroscopy, both of the samples were resuspended in this buffer (without the NH_2OH).

2.2. Time resolved single photon counting

The time resolved single photon counting apparatus has been described in full detail in [14,30]. A modelocked coherent YAG laser synchronously pumps a DCM dye laser. The cavity dumped output has a repetition rate of 5 MHz and an 8 ps pulse duration. The data were collected with an instrument response measured to have a FWHM of 36 ps using a LUDOX scattering solution.

The samples were excited using an excitation wavelength of 620 nm, and all of the emission was collected to the red of a 640 nm high pass filter.

The experiments carried out on the intact and the manganese depleted BBY-type PSII membranes fragments employed low light intensities in combination with the addition of 2 mM $K_3Fe(CN)_6$ to ensure that the samples were in a fully open state throughout the experiments. The laser beam spot diameter used during all of the experiments was ∼4 mm. The samples were chilled to 4°C and stirred continuously throughout the experiments. The fluorescence decay kinetics did not alter during the 15 min data acquisition time.

Power-dependence studies were performed on both of the samples to ensure that Q_A was in a fully open state throughout the experiments in a similar manner to that described in [14]. Typical laser powers used were 0.5×10^{13} photons s^{-1}.

2.3. Measurement of absolute singlet state populations

Rather than purely focusing on a multiexponential lifetime analysis, the data presented in this paper has allowed the absolute singlet state populations to be monitored during the trapping process (for further details see Barter et al. [14]). In brief, the samples optical density was recorded and changes in laser power and photon number reaching the detection system were also monitored using dye standards. An absolute scale for the singlet state populations was obtained using the isolated reaction centre data as a standard, as discussed previously [14].

3. Results

Time resolved single photon counting has been used to monitor the absolute singlet state populations in intact and manganese depleted BBY-type PSII membrane samples. Results were obtained from two samples that used different chemical treatments (washing with tris or NH_2OH) to remove manganese and thus to inhibit the oxygen evolution. All of the samples were studied in an open state, which was achieved with the combined use of 2 mM $K_3Fe(CN)_6$ and low light intensities. Fig. 1 shows typical singlet state decays obtained from the two different preparations of manganese depleted BBY samples, and makes the comparison with the fluorescence decay obtained from intact BBY-type PSII membranes. It is clear from this figure that neither the kinetics, nor the absolute populations of the singlet states are affected by the inactivation of the manganese complex, or the method of inactivation employed, despite the shift in the redox potential of Q_A that occurs.

Johnson and coworkers demonstrated, using redox titrations of the chlorophyll fluorescence, that the redox potential of Q_A is shifted by approximately 145 meV in manganese depleted samples compared to that observed in intact preparations [1,3]. It is possible to estimate the magnitude of the change in the rate of Q_A reduction that would result from a 145 meV shift in the free energy of the $P_{680}{}^{+}Q_A{}^{-}$ using non-adiabatic electron transfer theory. A simple form is

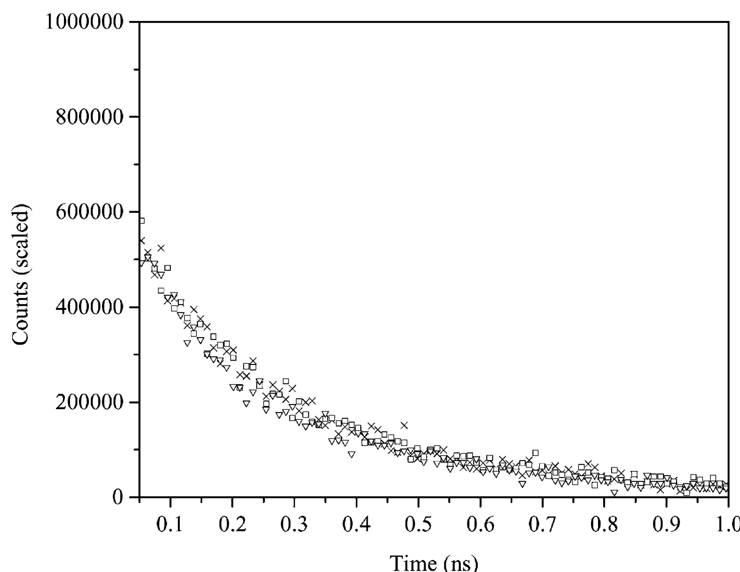

Fig. 1. Fluorescence decays obtained from two different types of manganese depleted BBY preparations; tris-washed (▽) and NH_2OH-washed (×), are compared with a decay obtained from an intact BBY-type PSII membrane sample (□). The compound $K_3Fe(CN)_6$ has been added to all the samples to maintain Q_A in a fully open state.

shown in Eq. (1) [31].

$$\log_{10}k_{et} = 15 - 0.6R - 3.1\frac{(\Delta G + \lambda)^2}{\lambda} \quad (1)$$

where k_{et} is the rate of electron transfer (s^{-1}), R the edge-to-edge distance between the cofactors (Å), ΔG the free energy (eV) and λ the reorganisation energy (eV). The free energy gap (ΔG) between the primary radical pair state $P_{680}{}^{+}Phe^{-}$ and the secondary radical pair $P_{680}{}^{+}Q_A{}^{-}$, in intact BBY-type PSII membranes, was taken as 330 meV [3], and the electron transfer was assumed to be activationless ($\Delta G = -\lambda$). The calculation to estimate the effect of the 145 meV shift in the free energy of $P_{680}{}^{+}Q_A{}^{-}$, in manganese depleted BBY-type PSII preparations, assumed that the reorganisation energy remained unchanged (330 meV), whereas, the free energy was increased to 475 meV [3]. The result of this calculation shows that the rate of Q_A reduction would decrease by a factor of 1.6 in manganese depleted samples compared to the reduction rate in intact preparations, as a result of the 145 meV shift in the free energy.

The trapping dynamics observed in intact BBY-type PSII membranes studied with Q_A in a fully open state should be reproducible using a kinetic model that is able to represent the dynamics in closed cores and isolated reaction centres. Such a kinetic model has already been shown to reproduce the trapping dynamics in the isolated reaction centre as well as a range of different sized core particles studied with Q_A in a fully closed state [14]. Using these parameters, the data obtained from open core particles may be reproduced if the model is adapted to include a fast rate of Q_A reduction (to be published shortly).

A surprisingly rapid intrinsic rate constant of Q_A reduction may be estimated as 24 ps^{-1} from the edge-to-edge distance (R) between pheophytin and Q_A (~7.3 Å) obtained from the recently published crystal structure of the PSII reaction centre [32]. To reproduce the singlet state decay in BBY-type PSII membranes, using this 24 ps^{-1} value of the intrinsic rate constant for Q_A reduction, a shift of the $P_{680}{}^{+}Phe^{-}$ radical pair free energy by ~160 meV is required (this will be discussed in more detail in a paper to be published shortly).

If however, it is assumed that the primary radical pair has the same free energy as that observed in the isolated reaction centre and in core particles studied with Q_A in a fully closed state, then a rate constant of 1.5 ps^{-1} is required to reproduce the experimental data from the BBY-type particles studied with Q_A in a fully open state. A 1.5 ps^{-1} rate constant could be obtained if the edge-to-edge distance between the pheophytin and Q_A, that has been estimated from the crystal structure, was out by 1.9 Å. Indeed, this is a perfectly plausible possibility, given the size of Q_A and the resolution of the crystal structure [32].

Both of these cases have, therefore, been investigated to estimate the effect on the singlet state decay that would result from a 145 meV shift of the Q_A redox potential, and the results are shown in Figs. 2 and 3. Indeed, Fig. 2 shows that there is a small, but significant difference in the results obtained from the model, which has a shallow radical pair free energy (as observed in the isolated reaction centre), and allows the rates of Q_A reduction to be 1.5 ps^{-1} in intact and 2.4 ps^{-1} in manganese depleted samples (i.e. a 1.6 change in rate that results from the 145 meV shift in the redox potential of Q_A). Fig. 3 shows, however, that if the free energy of the primary radical pair is large, a significant effect on the singlet state decay would not be expected following a 145 meV shift in the redox potential of Q_A.

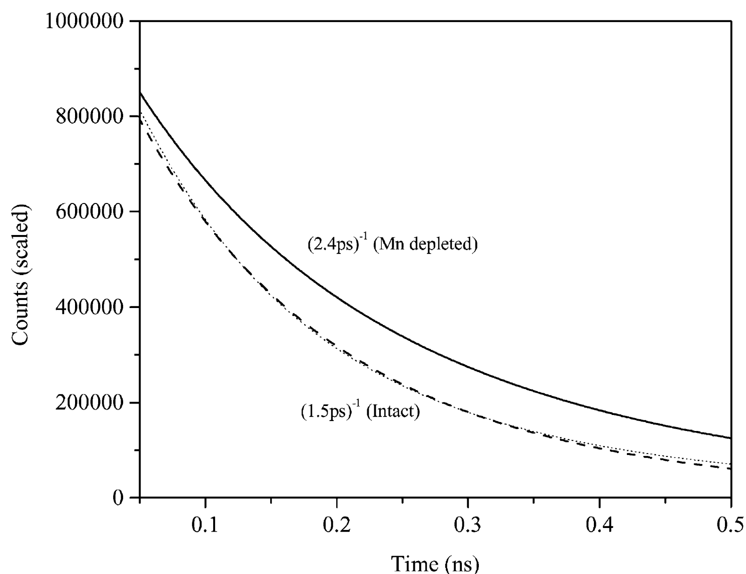

Fig. 2. A model (---) that assumes a shallow equilibrium (using the parameters described in Barter and coworkers [14,27]) may reproduce the fit to the data obtained from intact BBY-type core particles studied in a fully open state (···), if an intrinsic rate constant of Q_A reduction of 1.5 ps^{-1} is employed. The result obtained from the model, which alters the rate of Q_A reduction by a factor of 1.6 (see text) is also shown (—) to demonstrate the effect that a 145 meV change in the free energy of Q_A would have on the trapping dynamics.

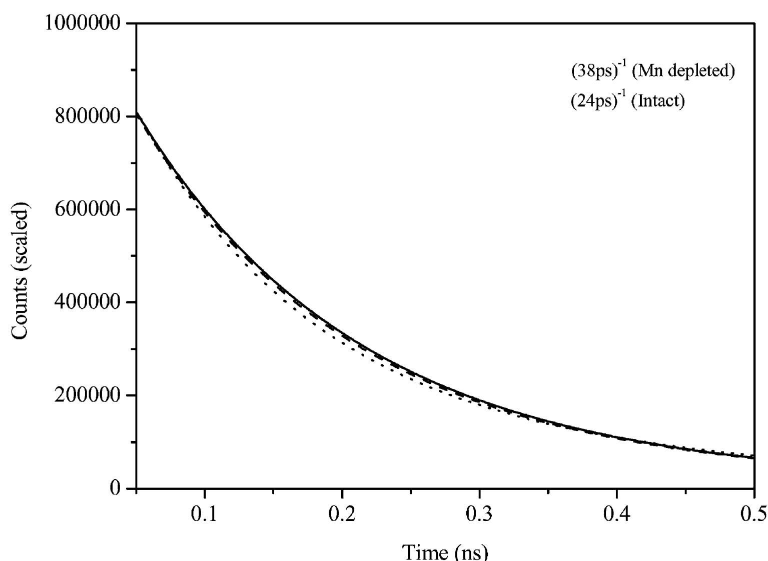

Fig. 3. The intrinsic rate constant of Q_A reduction may be estimated to be 24 ps^{-1} from the recently published structure of the PSII reaction centre. A 160 meV shift of the primary radical pair free energy with respect to that observed in the isolated reaction centre (as well as in a range of different sized core particles studied with Q_A in a fully closed state) is required to reproduce the data from BBY-type core particles with Q_A in a fully open state, if this Q_A reduction rate is employed. The result obtained from this model is shown (---) and compared with the fit to the experimental data (···) obtained from intact BBY-type PSII membranes. The result obtained from the model, which alters the rate of Q_A reduction by a factor of 1.6 (see text) is shown (—) to demonstrate the effect that a 145 meV change in the free energy of Q_A would have on the trapping dynamics.

4. Discussion

Following the loss of the water splitting activity, a 145 meV shift in the redox potential of Q_A is observed [1,2]. It has been suggested that the different midpoint potentials of Q_A/Q_A^- could determine the charge recombination pathway for $P_{680}^+Q_A^-$ [3]. When the low potential form of Q_A is present, charge recombination is likely to occur via $P_{680}^+Phe^-$. However, this route may be thermodynamically unfavourable when the high potential form is present. This method of regulation of the electron transfer and charge recombination pathways has been suggested to

provide a photoprotective mechanism during photoactivation [3,33].

The work presented in this paper has employed the shift in the Q_A redox potential, as a tool to probe the effect that the different free energies of Q_A have on the trapping dynamics in PSII. Time resolved fluorescence measurements have been used to monitor the dynamics and the absolute time-dependent singlet state populations in all of the samples studied. The results obtained from two types of manganese depleted samples, which were prepared using different methods, have been compared with those obtained from intact BBY-type PSII membranes. Somewhat surprisingly, the singlet state fluorescence decays, observed in both of the intact and the manganese depleted samples, show no significant differences, despite the 145 meV shift in the Q_A redox potential that occurs with the loss of water splitting activity (see Fig. 1).

It is expected that, the rate of Q_A reduction would decrease by a factor of 1.6 as a result of a 145 meV shift in the Q_A redox potential. The effect of this decrease in the electron transfer rate to Q_A has been investigated within the framework of two plausible cases. The first case assumed a shallow primary radical pair free energy, as observed in the isolated reaction centre and also in a study of a range of core particles investigated with Q_A in a fully closed state [14]. This model required a fast 1.5 ps^{-1} rate of Q_A reduction to enable the experimental data to be reproduced. The second case invoked a ~160 meV shift in the free energy of the primary radical pair relative to the parameters that are required to reproduce data from the isolated reaction centre (as well as a range of different sized core particles with Q_A in a fully closed state) [14]. This shift is, however, necessary to reproduce the data obtained from open BBY-type PSII complexes if a 24 ps^{-1} rate of Q_A reduction, estimated from the published PSII reaction centre crystal structure, is employed in the model.

The results obtained from the mathematical modelling provide a number of plausible explanations for the lack of sensitivity to the redox potential of Q_A shown by the experimentally observed singlet state dynamics. Indeed, the similarity of the trapping dynamics shown by the model that shifts the free energy of the primary radical pair ($P_{680}{}^{+}Phe^{-}$) by 160 meV with respect to the energy required to reproduce the trapping dynamics in the isolated reaction centre (see Fig. 3), could indicate that in open core particles $P_{680}{}^{+}Phe^{-}$ acts as a deep trap. It is nevertheless important to note that the trapping dynamics observed in a range different sized core particles, studied either without Q_A present, or with Q_A in a fully closed state, may be reproduced using the shallow primary radical equilibrium as observed in the isolated reaction centre [14].

There is a possibility that the 145 meV shift in the Q_A redox potential, that has been observed on the loss of the manganese complex [1–3] is time-dependent, and, therefore, on the time scale of these experiments it is too small to be observed.

Inhomogeneity must also be considered as its importance in PSII due to the shallow equilibrium has been demonstrated previously [27,34,35]. Indeed, it is possible that the presence of inhomogeneous broadening of the $P_{680}{}^{+}Phe^{-}/P_{680}{}^{+}Q_A{}^{-}$ free energy gap could reduce the observed effect of the 145 meV shift of the Q_A redox potential on the singlet state trapping dynamics. This, however, has not yet been resolved and will be the subject of a future paper.

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