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The relationship between excitation energy transfer, trapping and antenna size in Photosystem II

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Introduction

Energy transfer and trapping in Photosystem II (PSII) may be investigated by breaking down the multi-pigment-protein complex into small components. Indeed, studies carried out on the smallest charge separating unit that can be isolated from PSII, the D1-D2-cyt *b*559 reaction centre, have allowed the primary reactions to be investigated without the added spectral congestion caused by the presence of antenna or secondary electron transfer pathways. These studies have enabled microscopic models to be produced, which are able to reproduce the details of the multiexponential energy transfer and trapping dynamics observed in both wild type and genetically modified PSII reaction centres (Durrant *et al*., 1995; Leegwater *et al*., 1997; Klug *et al*., 2001).

Questions have nevertheless been raised regarding how the photochemical properties of the isolated reaction centre relate to those observed from the system *in vivo*. A study of the time dependent absolute singlet state populations obtained from a range of different sized core particles isolated from PSII and studied with Q_A in a fully closed state, has however, suggested that the slow dynamics, associated with excitation energy trapping via the formation of a shallow primary radical pair state are a genuine feature of the bioenergetics of PSII (Barter *et al*., 2001a). It is not however obvious that one can rationalise these slow trapping dynamics with those reported from BBY core particles studied with Q_A in a fully open state. We have therefore extended this study in an attempt to probe the dynamics of Q_A reduction in PSII, using a comparison of calculation and experiment.

Materials and methods

Samples were isolated as described by (Barter *et al.*, 2001a) and references within, and were diluted using buffers to give a final concentration of ~3μg mL^{-1}. The time resolved single photon counting apparatus has been described in detail by (Booth *et al*., 1990) and (Barter *et al.*, 2001a). Briefly a mode-locked coherent YAG laser was used to synchronously pump a DCM dye laser (with a cavity dumped output of 5MHz and 8ps pulse duration). The samples were excited with an excitation wavelength of 620nm, and all of the emission was collected to the red of a 640nm high-pass filter. Rather than focusing on multiexponential lifetime analysis, absolute singlet state populations were recorded as detailed previously (Barter *et al.*, 2001a; Barter *et al*., 2001b).

Experiments carried out on samples with Q_A in a closed state used the incident light intensity to ensure that Q_A was in a fully reduced state. The experiments that investigated the dynamics of Q_A reduction employed a combination of low light intensity and the addition of 2mM $K_3Fe(CN)_6$ to keep Q_A in a fully open state. Power dependent studies were performed on all samples to ensure that Q_A was kept in either a fully open or closed state throughout the experiment.

Results

The absolute singlet state populations have been monitored in a range of samples studied with Q_A in a fully closed state using the technique of time resolved single photon counting. This has enabled the antenna size dependence of energy transfer and trapping to be investigated. The absolute singlet state populations from each sample at 250ps are shown in figure 1.

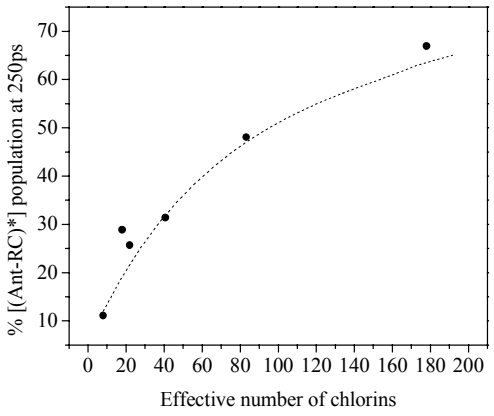

Figure 1. Percentage singlet state population at 250ps plotted as a function of the effective chlorophyll content for each sample studied with Q_A in a fully closed state. Populations obtained from fits to the experimental data (●) are compared with the modeled results (---).

A three state phenomenological trapping engine model, which allows inhomogeneous broadening of the primary radical pair free energies, was able to reproduce the trapping dynamics observed in wild type and genetically modified isolated PSII reaction centres. This trapping engine model was coupled to the antenna to enable it to predict the antenna size dependence of energy transfer and trapping as detailed in (Barter *et al.*, 2001a). Briefly, it was assumed that energy transfer between the antenna excited states and the reaction centre was fast, such that the antenna excited states formed a quasi-static equilibrium with the reaction centre excited states. The model was easily adapted to account for the effective antenna size by altering the equilibrium constant between the antenna and the reaction centre excited states. Figure 1 shows the predicted antenna size dependence of energy transfer and trapping obtained from these calculations, and enables a comparison with the experimental results obtained from a range of different sized core particles studied with Q_A in a fully closed state.

The absolute singlet state populations have also been obtained from BBY type PSII membranes studied with Q_A in a fully open state. The multiexponential fit to the singlet state decay is shown in both figures 2A and B.

The phenomenological model has also been employed to investigate whether the fast dynamics of Q_A reduction, observed in BBY particles with Q_A in an open state, can be reconciled with the slow trapping dynamics observed in isolated reaction centres as well as in a range of different sized core particles studied with Q_A in a closed state. The three state model was therefore extended further, to include a

unidirectional Q_A reduction pathway from either pheophytin excited or pheophytin anion states. The intrinsic rate of Q_A reduction is not however known to any high degree of accuracy, although it may be estimated using the edge-to-edge distance between the Pheophytin and Q_A obtained from the 3.8Å resolution crystal structure of the PSII reaction centre (Zouni *et al*., 2001). A simplified form of non-adiabatic electron transfer theory, given by (Page *et al*., 1999), predicts a surprisingly fast $(24ps)^{-1}$ rate of Q_A reduction if electron transfer is assumed to be activationless. A shift of the primary radical pair free energies of the order of 160meV is nevertheless required to reproduce the singlet state decay observed in open BBY type membranes using this $(24ps)^{-1}$ intrinsic rate of Q_A reduction as shown in figure 2A. If however, it is assumed that the primary radical pair has the same free energy as that observed in isolated reaction centres and core particles with Q_A studied in a fully closed state, then a rate constant of $(1.5ps)^{-1}$ is required to reproduce the experimental data from open BBY type membranes (figure 2B).

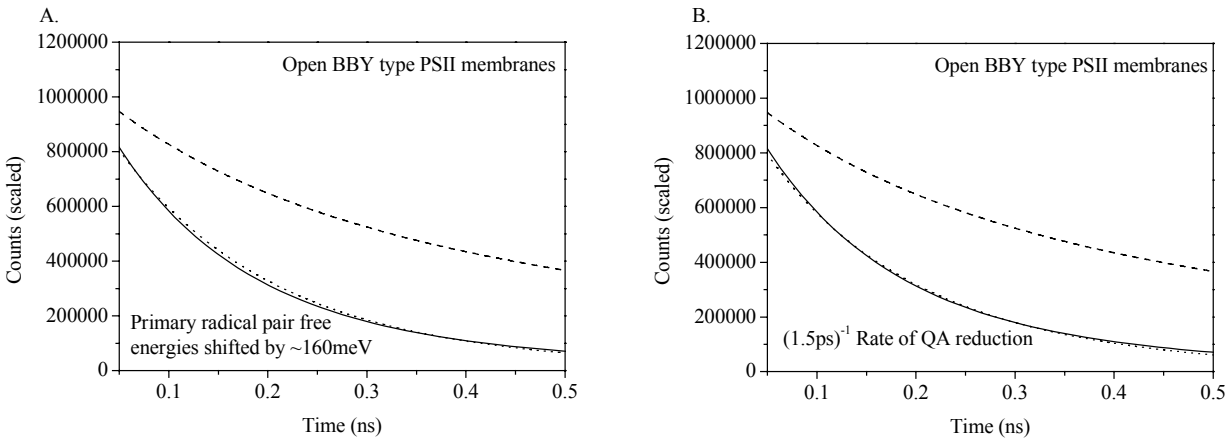

Figure 2. The intrinsic rate of Q_A reduction may be estimated to be $(24ps)^{-1}$ using the edge-to-edge distance between pheophytin and Q_A taken from the published crystal structure of the PSII reaction centre (Zouni *et al*., 2001). The multiexponential fit to the experimental data obtained from BBY type PSII membranes studies with Q_A in a fully open state (—) cannot however be reproduced by a model that keeps the primary radical pair free energies as those observed in the isolated reaction centre if a $(24ps)^{-1}$ rate of Q_A reduction is assumed (---). **A.** The model demonstrates that a shift of the primary radical pair free energies by ~160meV is required to reproduce the experimental data if the rate of Q_A reduction is assumed to be $(24ps)^{-1}$ (····). **B.** The calculations have also shown that a Q_A reduction rate of $(1.5ps)^{-1}$ may be employed to reproduce the experimental data if the primary radical pair free energies are kept as those observed in isolated reaction centres as well as in a range of core particles studied with Q_A in a fully closed state (····).

Discussion

A comparison between the experimental and modeled results obtained from an investigation of the antenna size dependence of energy transfer and trapping observed in core particles studied with Q_A in a fully closed state has suggested that the slow trapping of excitation energy via the formation of the shallow primary radical pair state $P_{680}^{+}Pheo^{-}$ seems to be a genuine feature of the bioenergetics of PSII.

It has been suggested that energy transfer from the antenna to the reaction center is rate limiting (vanGrondelle *et al*., 1994; Visser *et al*., 1996; Dekker & Grondelle, 2000). Our work however strongly suggests that energy transfer from the antenna to the reaction centre is not the rate limiting step.

The extension of the phenomenological model has indicated that even if the surprisingly fast $(24ps)^{-1}$ rate of Q_A reduction (predicted using the edge-to edge distance between the pheophytin and Q_A (Zouni *et al*., 2001)) is included in the model a shift primary radical pair free energies of the order of ~160meV is required to reproduce the open BBY data. The need to invoke such a large shift of the free energy gap between the excited singlet states and primary radical pair states is not implausible, but is nevertheless somewhat surprising in light of the results obtained from isolated reaction centres and closed cores. Our work has also shown that the singlet state decay obtained from open BBY membranes could be reproduced using a $(1.5ps)^{-1}$ intrinsic rate of Q_A reduction. This fast rate would be predicted if the edge-to-edge distance, estimated from the crystal structure of the PSII reaction centre (Zouni *et al.*, 2001), was out by only 1.9Å, which seems perfectly plausible given the small size of Q_A and the resolution of the published structure. Interestingly a similarly fast value of the intrinsic rate of activationless electron transfer between A_0 and A_1 in Photosystem I, has been estimated to be only $(0.8ps)^{-1}$ (Klukas *et al*., 1999).

Acknowledgments

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