

S10-012

Studies on the interaction between tyrosine Y_Z and the Mn complex.

JHA Nugent, IP Muhiuddin, MCW Evans

Photosynthesis Research Group, Department of Biology, Darwin Building, University College London, Gower Street, London WC1E 6BT, United Kingdom.

Fax: +44 (0) 207 679 7096

Email: j.nugent@ucl.ac.uk

Keywords: oxygen evolution, photosystem II, water oxidation, tyrosine, manganese

Introduction

$P680^+$ extracts an electron from the WOC via the electron donor Y_Z , (tyrosine-161) on the D1 polypeptide to form Y_Z^\bullet , the neutral tyrosine radical (see Nugent, 1996). The reduction kinetics of $P680^+$ by Y_Z and subsequently of Y_Z^\bullet by the WOC is dependent on the S-state. A large fraction of $P680^+$ is reduced by Y_Z in nanoseconds but slower phases occur. To explain the slower phases we suggested a role for proton coupled electron transfer processes, in which net deprotonation from the region completes reduction of $P680^+$, resulting in a progressive change in the equilibrium between Y_Z/Y_Z^\bullet and $P680/P680^+$ towards Y_Z^\bullet formation (Schilstra et al., 1998; Nugent et al., 2001).

During one cycle to oxidise two molecules of water to oxygen and protons, the WOC passes through redox states termed S-states, electrons being removed on each step from S_0 to S_4 and O_2 being evolved at the transient S_4 state forming S_0 . Oxidation of the WOC must be an almost electroneutral process with proton release to the lumen during the S-state cycle providing the necessary charge balance to electron transfer. Y_Z is close to the Mn cluster and when both are paramagnetic i.e. Y_Z^\bullet plus a paramagnetic Mn cluster; they can interact to give complex split electron paramagnetic resonance (EPR) signals at cryogenic temperatures, which have been assigned to a $S_2Y_Z^\bullet$ state. We have already reported experiments showing that interaction between Y_Z^\bullet and the Mn cluster ($S_2Y_Z^\bullet$) could be observed in uninhibited PSII and that this is related to formation of S_3 (Nugent et al., 1997). This work has been extended by Petrouleas and co-workers (Ioannidis and Petrouleas, 2000), showing that infra-red (IR) illumination of the S_3 state also forms $S_2Y_Z^\bullet$.

In this study we find evidence that the S_1 to S_2 step can operate at liquid helium temperatures, a result that has important implications for the nature of Y_Z and the models of water oxidation.

Materials and Methods

PSII membranes were prepared from market spinach or freshly cropped 10-14 day old pea seedlings using Triton X-100, with the modifications of Ford and Evans (1983). The membranes were resuspended in 20 mM MES, 15 mM NaCl, 4 mM $MgCl_2$, 1 mM $CaCl_2$, pH 6.5 plus cryoprotectant (0.4 M sucrose or 20-25% v/v glycerol). For EPR, 0.3 - 0.4 mL samples (approx 6-10 mg Chl/mL; 25-40 μM PSII) were placed in calibrated 3 mm quartz EPR tubes. Treated or untreated samples were dark-adapted for a 4h on ice before being frozen to 77 K in the dark (total dark adaptation 4h). This

produced samples initially in the S_1 Qa state. During experiments, samples were illuminated at a variety of temperatures. IR light was excluded from illuminated samples where necessary by using an Ealing Electro-optics long wavelength cut-off filter (50% transmission at 661.6 nm, ~ zero above 700 nm). Samples were examined by EPR at cryogenic temperatures using a Jeol RE1X spectrometer fitted with an Oxford Instruments cryostat.

Results

We have detected a broad radical EPR signal (S1 split signal) during illumination of S_1 state oxygen-evolving PSII at < 10 K (Nugent and Evans, 1998). The characteristics of this signal indicate that it arises from an interaction between an organic radical and the Mn cluster of PSII; we assign it to $S_1 Y_Z^\bullet$. The signal decays in the dark following illumination ($t = 1/2 \sim 230$ s at 11 K) either by back-reaction with $Qa^{\bullet-}$ or by forward electron transfer to the Mn cluster. The forward electron transfer results in the formation at 77 K of a multiline signal similar but distinct from other S_0 and S_2 state multiline forms. An approximate quantitation of the yield of these new signals, using the S_2 state multiline generated by 200 K illumination, indicates that up to 40-50% of PSII centres can form the “S1 split signal”. Ethanol addition removes the ability to observe the “S1 split signal” but the multiline signal is still formed at 77 K. The multiline forms with < 700 nm light, and is not affected by near infra-red (IR) light. The results indicate that we are detecting electron transfer in centres not affected by IR illumination and that these centres are capable of forming the S_2 state at very low temperatures.

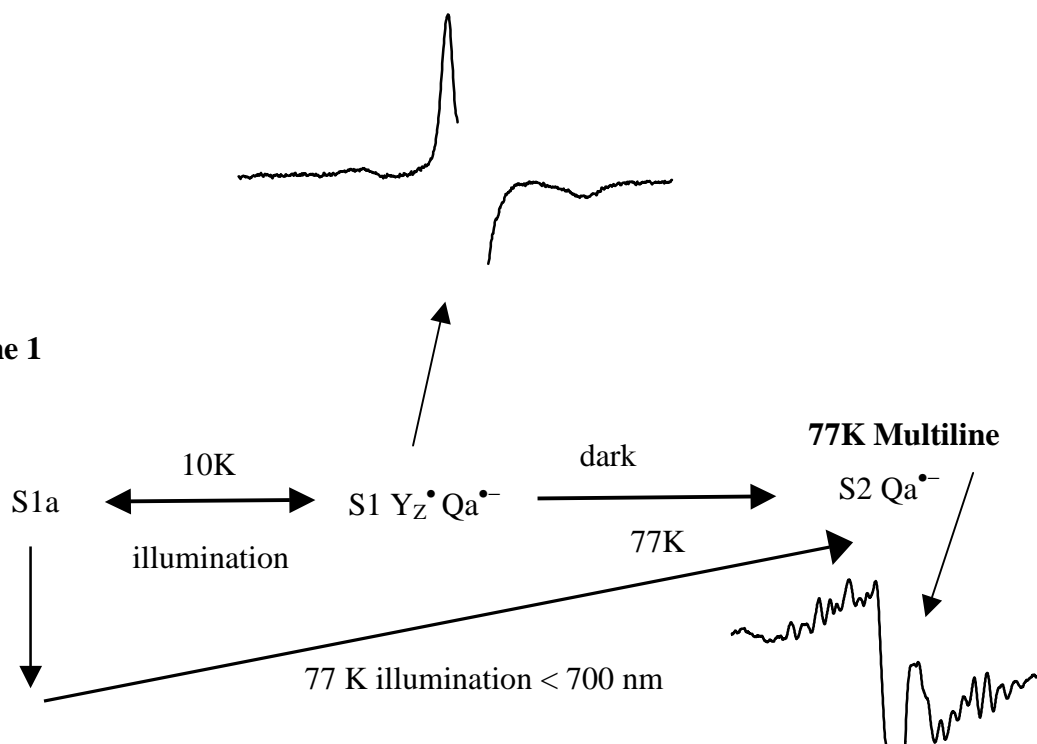
Discussion

In recent years a number of new EPR signals have been characterised that give us probes of the turnover the WOC. These have included signals from interaction of the Yz component with the Mn cluster. We now show the characterisation of a signal we attribute to the interaction between paramagnetic S_1 state and Y_Z^\bullet , an intermediate between the S_1 and S_2 states. The results suggest that a substantial number of centres are able to operate the S_1 to S_2 step through Yz at very low temperatures, confirming the unusual temperature profile of this step and supporting the tyrosinate like nature of Yz at least in S_1 .

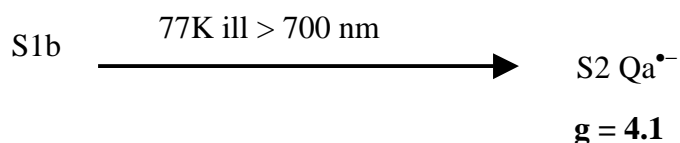
If Y_Z is effectively deprotonated, or if it were involved in a permanent hydrogen bond that is modified between Y_Z redox states, Y_Z would be able to be oxidised at temperatures where proton movements are prevented, as observed. Y_Z would be effectively negative in the reduced form and neutral when oxidised, accounting for EPR and electrochromic shift data. Y_Z is rapidly oxidised by $P680^+$, the transition is

Scheme 1

A.



B.



almost insensitive to H/D substitution and has a low activation barrier (Haumann et al., 1999). These are characteristic of electron transfer reactions and in our view the rate limitation of proton transfer is absent in native PSII but can be caused by structural change in cofactor depleted PSII.

Our conclusions can be summarised by Scheme 1. We propose that there are two slightly different S_1 states that can be resolved by their EPR characteristics. The S_1 state is known to have different electronic arrangements, which result in the different EPR signals and are most easily explained, as for S_2 state, as different arrangements of the Mn^{3+} and Mn^{4+} ions of the cluster (Peloquin and Britt, 2001). The configuration is affected by relatively small changes that could be caused for example by the presence / absence of a proton or H_2O/OH^- binding as ligands to the Mn cluster. The absence of an effect of IR illumination on the 77 K multiline indicates that the Mn^{3+} atom presumed to be affected by IR might be the one that has been oxidised in these centres. The yield of the S_1 split signal and 77 K multiline vary from preparation to preparation in both spinach and pea PSII, as well as by choice of cryoprotectant or electron acceptor. This undefined heterogeneity was also seen in studies of the IR induced $g = 4.1$ S_2 signal (20-70% of PSII responding to IR illumination (Boussac, 1997). Comparison of the conditions tested in this study and (Boussac, 1997) suggests that we are observing those centers not sensitive to IR illumination.

We favour an electrostatically controlled electron transfer mechanism, where at physiological temperatures the changes in pKa induced by the initial oxidation of P680 cause a wave of deprotonation and progressive reduction in redox potential, firstly of Y_Z and then the WOC (Nugent et al., 2001). Y_Z is rapidly oxidised by $P680^+$, and at the same time deprotonation from the WOC occurs to complete the oxidation of Y_Z and this deprotonation in turn lowers the redox potential in the Mn cluster, promoting its oxidation by Y_Z^\bullet . Domino deprotonation through a hydrogen-bonding network leads to proton release into the lumen. This would be a delicate mechanism, the electrostatic balance being easily shifted by slight perturbations to the PSII centre, that would account for the variation in proportion of centres having the S1 split signal. The balance would be easily destroyed by more substantial changes, for example involving removal of manganese or calcium cofactors.

In summary, we have detected a “split” electron paramagnetic resonance (EPR) signal during illumination of dark-adapted (S_1 state) oxygen-evolving photosystem II (PSII) at <20 K. The characteristics of this broad radical indicate that it arises from $S_1Y_Z^\bullet$. The signal decays in the dark following illumination either by back-reaction with $Qa^{\bullet-}$ or by forward electron transfer to the Mn cluster. The forward electron transfer results in the formation at 77 K of a multiline signal slightly different from other well characterised multiline forms. This data provides important new information about the mechanism of the water oxidising complex (WOC), showing that electron transfer from the Mn cluster to P680 can occur without proton movement.

Acknowledgements

We thank the UK BBSRC and the Leverhulme Trust for financial support.

References

- Boussac A (1997) *Journal of Biological Inorganic Chemistry* **2**, 580-585.
- Ford RC, Evans MCW (1983) *FEBS Letters* **160**, 159-163.
- Haumann M, Mulkidjanian A, Junge W (1999) *Biochemistry* **38**, 1258-1267.
- Ioannidis N, Petrouleas V (2000) *Biochemistry* **39**, 5246-5254.
- Nugent JHA (1996) *European Journal of Biochemistry* **237**, 519-531.
- Nugent JHA, Turconi S, Evans MCW (1997) *Biochemistry* **36**, 7086-96.
- Nugent JHA, Evans, MCW (1998) in *Photosynthesis: Mechanisms and Effects* (Garab, G, Ed.) Vol. II pp 1379-1382 Kluwer Academic Publishers The Netherlands.
- Nugent JHA, Rich AM, Evans MCW (2001) *Biochimica et Biophysica Acta* **1503**, 38-146.
- Peloquin JM, Britt RD (2001) *Biochimica et Biophysica Acta* **1503**, 96-111.
- Schilstra MJ, Rappaport F, Nugent JHA, Barnett CJ, Klug DR (1998) *Biochemistry* **37**, 3974-3981.