**S22-027**

# ESR studies on Photosystem II samples and Core Complexes from Higher Plants. Implications from studies on the g4.1 signal.

P.J. Smith[1], S. Peterson[2], E. Krausz[1], T. Wydrzynski[1] and R.J. Pace[1].

[1] *Australian National University, Canberra 0200, Australia, Paul.Smith@Anu.edu.au, Ron.Pace@Anu.edu.au*

[2] *Biochemistry, University of Lund, Lund, Sweden.*

*Keywords:* photosystem II, Core complexes, S States, Electron Spin Resonance.

## Introduction

The water oxidase centre (WOC) of Photosystem (PS)II contains at least four Mn ions, one calcium and chloride. The structural organisation of these is yet unresolved. Three intermediate S states of the WOC in functional PSII display Electron Spin Resonance (ESR) signals from Mn containing paramagnetic centres. The most thoroughly characterised S state is $S_2$.

Two independent Mn derived paramagnetic states may be generated in $S_2$. These are the multiline (ML) $S_2$ state, characterised by a broad resonance centred near g=2, featuring 18 to 22(+) hyperfine lines and the g4 $S_2$ state, exhibiting a relatively featureless signal centred near g = 4.1. Both have been shown to be ground spin states under appropriate generation conditions (Pace et al 1991, Smith et al 93). The ML $S_2$ is a physiological intermediate state within the catalytic cycle of the WOC. The ground g4 $S_2$ state appears by either low temperature illumination (130K, visible light) of dark $S_1$ state PSII (Casey and Sauer 1984), or by low temperature illumination (145K IR light) of PSII set in to the ML $S_2$ state prior to the IR illumination (Boussac et al 1996). Both methods of generating the g4 $S_2$ allow interconversion of the g4 $S_2$ state to ML $S_2$ state by short dark annealing at 200K.

In PSII samples illuminated at 130K, the g4 $S_2$ state has been shown to develop a radical signal with apparent excited state temperature behaviour (Smith et al 96). The illumination regime and presence of the excited state radical were shown to be dependent on the presence of ethylene glycol (EG) in the sample buffer. Recent experiments by the others (Boussac and Rutherford 2000) using direct illumination or IR interconversion to form the g4 $S_2$ state in PSII samples not containing polyalcohol failed to observe the excited state radical species. Experiments on lower temperature IR illumination of higher plant PSII samples (65K) led to the observation of signals at higher g values (6 to 10) (Boussac et al 1998) which displayed complex temperature dependence.

We have recently developed a new protocol for preparation of PSII Core Complexes from higher plants. This material retains oxygen evolution rates ($VO_2$) equivalent to the parent PSII membrane samples on a per reaction centre basis. ESR studies show that the complexes retain the ability to interconvert the ML $S_2$ state to the g4 $S_2$ state by low temperature (140 to 145K) far red / near IR (FR/NIR) illumination ($\lambda > 710$nm). Here we discuss the ESR properties of the resulting g4 $S_2$ states formed in a range of sample buffer conditions. The signals observed and their spin state temperature dependences are discussed in terms of a single model and are related to the observations for the PSII membrane samples reported by other workers.

## Methods

All PSII preparation protocols were undertaken at or below 4C under faint green light. Procedures for the preparations of PSII membrane and Core samples were as per Smith et al (2001 submitted). $O_2$ evolution rates ($V_{O2}$), chlorophyll (Chl) content and a/b ratios, and detailed pigment content examined using HPLC are detailed in Smith et al (2001 submitted).

Core complex samples ($VO_2$ > 3.6mmoles $O_2$ per hour per mg Chl and an a/b ratio > 30:1) were stored in a buffer containing 0.4M sucrose, 20mM BisTris, 10mM $MgCl_2$, 5mM $CaCl_2$ and 90mM $MgSO_4$ (BTS). Sample illuminations used a temperature controlled nitrogen flow cryostat : ML $S_2$ (255K, 20 s through blue filter, 370nm to 590nm window, 10cm water), and interconversion to g4 $S_2$ (145K , 15 min FR/NIR, 710nm cutoff, no water path) with 250W lamp source. Temperature dependence studies measured the g4 $S_2$ as the initial state, followed by the ML $S_2$ subsequently developed by a 100 second 200K annealing in a $CO_2$/ethanol bath. Signal quantitation was by extrapolation to zero power of double integral / root power plots (as per Smith et al 93).

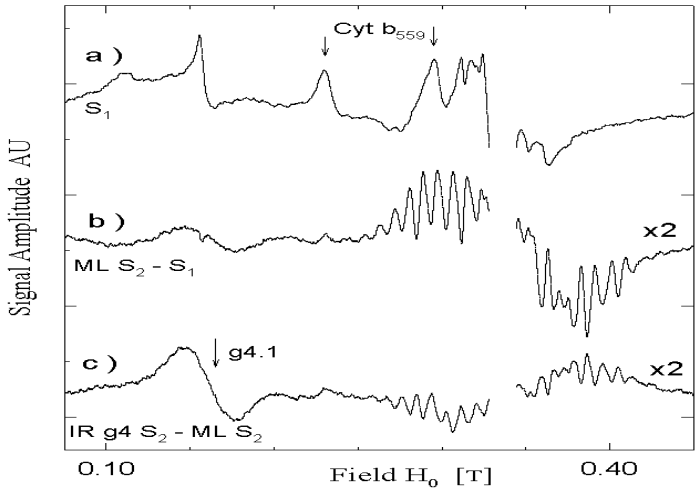



**Figure 1**. ESR spectra recorded from PSII Core samples containing EG/Glyc in the sample buffer. (a) Dark $S_1$ state spectra. Cyt $b_{559}$ is fully oxidised in these samples, with both the g~3.0 and g~2.26 turning points present in all spectra (indicated). (b) Illuminated – dark $S_2$ state signals generated by 255K illumination. (c) FR/NIR illumination (g4 $S_2$) minus ML $S_2$ (b) difference spectra showing signals arising from FR/NIR illumination of ML $S_2$ state samples. ESR conditions: Temperature 8K, microwave power 5mW, frequency 9.42GHz, modulation 100KHz at 20 gauss.

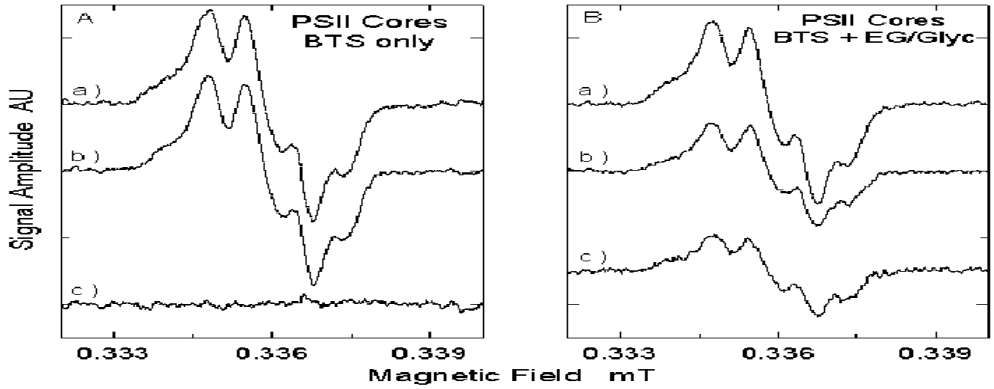



**Figure 2** ESR spectra of g=2.00 region for PSII Core samples with (A) BTS only and (B) BTS + EG/Glyc. Spectra for each buffer condition are (a) post FR/NIR illumination, g4 signal present , (b) post 200K anneal, in the ML $S_2$ state (no g4) and the difference of these (c). ESR Conditions T = 22K, microwave power 5μW, modulation 100KHz at 2G

## Results and Discussion

Four sample buffer were examined; PSII cores with i) buffer only (BTS), ii). with pPBQ and 1% DMSO added to the sample (DMSO, pPBQ), iii). with 3% ethanol added to the sample (EthOH), and iv). with ethylene glycol and glycerol (20%v:v each) added to the sample (EG/Glyc).

For each of the four buffer conditions examined, illumination at 255K with blue filtered light generated a ML signal with amplitude close to one spin per reaction centre. Figure 1 shows the ESR spectra for the $S_1$ state (a) and ML signal generated by 255K illumination (illum – dark) (b). Re-illumination of the ML $S_2$ state samples with NIR light at 145K reduced the ML amplitude in all samples with a g4.1 signal observed to develop, figure 1(c) (FR/NIR illum g4 $S_2$ minus ML $S_2$) (conversion 45 to 75%). The overall hyperfine and superhyperfine features of the ML signals and the width ($\Delta H_{p-p}$) and apparent g value of the g4 signals developed were observed to differ somewhat between the differing buffer conditions (not shown). Illuminations < 145K (130 to 100K) resulted in lower interconversion efficiency, but no higher g value signals were seen.

Figure 2 shows the Signal II (Sig. II) region (across g = 2.00) for core samples containing BTS buffer only (A) and EG/Glyc (B). Spectra were recorded at 22K for the NIR illuminated samples (+g4 signal) (a) and samples annealed at 200K to the ML $S_2$ state (b). The data for figure 2A are consistent with the observations of Boussac and Rutherford (2000). The g4 $S_2$ state shows no additional intensity within the Sig. II region, the g4 signal is a thermally isolated spin state. This occurs also for samples containing DMSO and pPBQ (fig. 3). For samples with alcohol present (3% EthOH or EG/Glyc (20% each v:v)) additional radical signal intensity overlies Sig. II in the presence of the g4 signal developed by NIR illumination. At ~20K this additional signal intensity is dependent on the interconversion from ML to g4 state. The line shape is consistent with the additional signal intensity arising from a tyrosyl radical, similar in nature to $Y_D^+$, fig 2Bc.

The relative amplitudes of the Sig. II region are compared for the four buffer conditions examined in figure 3. Data (illuminated and annealed) are presented as Spin populations (signal intensity * temperature) vs 1/T. The data are scaled to the Sig. II intensity (spin population) recorded at 6 K for each sample. A spin population of 1 for all temperatures examined defines Curie behaviour. This is observed for the radical signals of the ML $S_2$ state in <u>*all*</u> buffer regimes, indicating *no* additional radical signal development over the temperature range ~5K to 30K (ie Sig. II in isolation). In samples containing BTS + EthOH and BTS + EG/Glyc, the relative spin population of the Sig. II region is observed to <u>increase</u> with increasing temperatures *when* the g4 signal is *present*. This suggests a thermally accessible excited state associated with the g4 signal centre in the PSII samples. This excited state appears as a radical signal, and is tyrosine related.

To give rise to an S ≥3/2 g4 signal and an excited S = 1/2 radical state, interaction between at least three spin centres is required. A model we have proposed (Smith et al 96) is an oxidisable centre (tyrosine) bridging an even spin Mn dimer (fig 4A). With antiferromagnetic coupling between the Mn ions, and ferromagnetic coupling between the Mn ions and the tyrosine, the energy levels for the spin states ($S_T$, $S_D$) is given by $E(S_T,S_D) = ½(J_D-J')[S_D(S_D+1)-12] + ½J'[S_T(S_T+1)-51/4]$, where $S_D = 0, 1, 2,..$, $S_T = S_{radical} + S_D$. Different buffer /cryoprotectant conditions may allow variation in each of J' and $J_D$, allowing differing spin states to be observed at a given temperature.

This model could account for all the observations considered here. For PSII (membranes and Cores) without alcohol, the thermally isolated g4 $S_2$ state would have the $J'/J_D$ ratio within region 2 on fig 4B ($|J'/J_D| \sim 3$). For PSII membranes with EG and Cores with alcohol, the $J'/J_D$ ratio lies within region 1 on fig 4B ($2<|J'/J_D| \leq 2.6$). The g4 signal is observable as a

ground state signal ($S_D = 1$) and the spin ½ radical signal arises as the excited state with the dimer spin ($S_D$) populating the $S_D = 0$ level. For the lower temperature IR illumination (~65K) of PSII membranes forming higher g value signals (Boussac et al 1998), the $|J'/J_D|$ ratio will lie towards region 3 on fig 4B ($|J'/J_D|$ ~3.5 to 4). In this region the energy difference between the $S_D = 2$ ($S_T = 5/2$) and the $S_D = 1$ ($S_T = 3/2$) states is low, with either the g4 being observed as ground state and higher g signals observed as excited state signals or the higher g signals observable as the ground state (Boussac et al 1998). The lower temperature illumination may disallow protein conformational changes required during the spin and electron redistribution (induced by IR illumination) and lock the Mn system into the higher spin conformation. Annealing at 140K then allows the conformational changes interconverting this form back to the g4 ground state system.

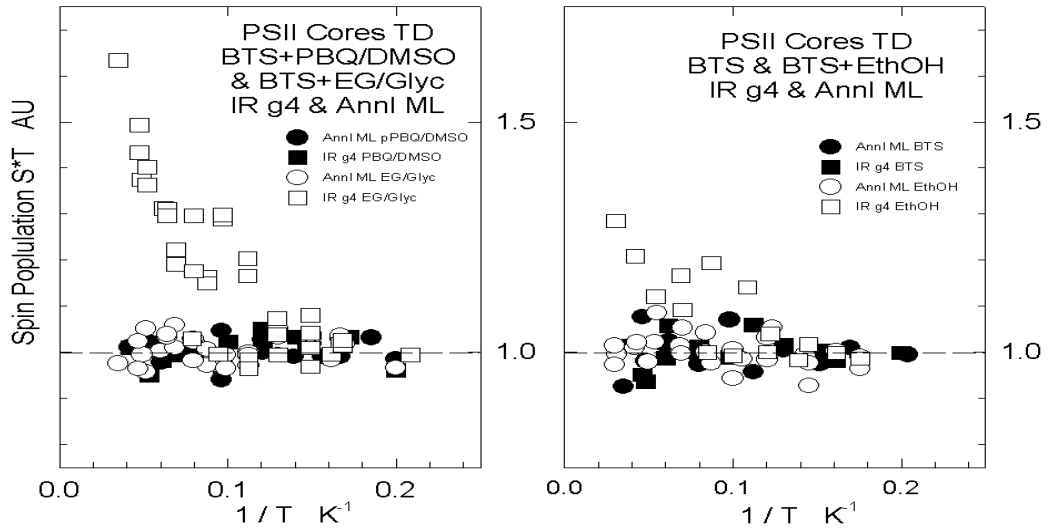



**Figure 3.** Temperature dependences of Signal II region signals of PSII Core samples presented as Relative Spin Populations (see text). Signal amplitudes from extrapolation of double integral plots to zero microwave power (see text). Annl ML is post 200K annealed ML $S_2$ state (no g4), IR g4 is FR/NIR illuminated g4 $S_2$ state (with g4)

In this model the g4 must arise from an S=3/2 system, otherwise observation of the S=1/2 radical excited state would be improbable. The interaction of the (tyrosine) bridged dimer with the other Mn might be weak if the dimers are spatially isolated or if one is strongly coupled and ESR silent. Our major conclusion for the Mn cluster structure is that the ground state g4, excited state radical and higher g signals require a Mn pair equally coupled to an oxidisable (tyrosine) moiety.

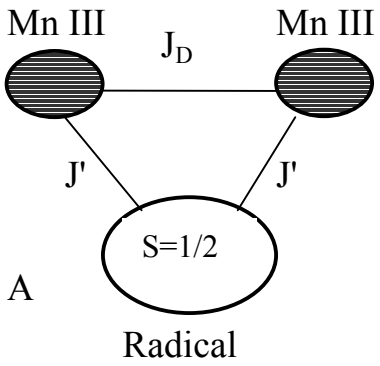
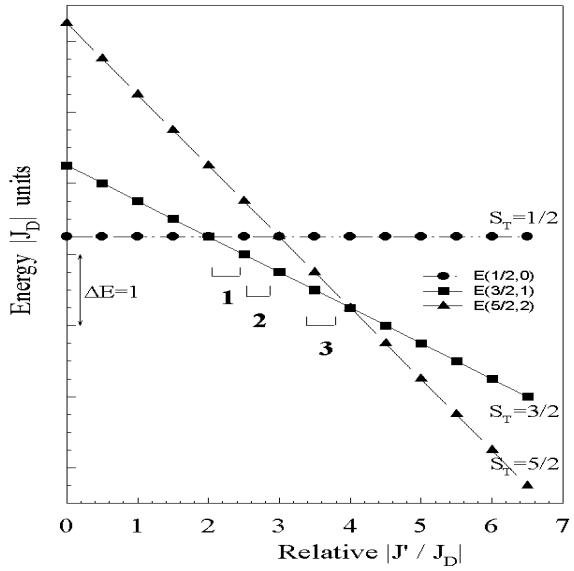



**Figure 4.** Model for the Mn centre associated with the g4 $S_2$ state signal. The model requires the bridging of an even spin Mn dimer by an oxidisable radical (A). Buffer and protein environment influence magnitudes of J' and $J_D$. Relative energy levels for the states (1/2,0), (3/2, 1) and (5/2,2) (B) are dependent only on the ratio $|J'/J_D|$. The couplings J' must be equivalent.




## References:

Boussac, A., Girerd, J.-J. and Rutherford, A.W. (1996) *Biochemistry* **35**, 6984 - 6989.
Boussac, A., Un, S., Horner, O. and Rutherford, A.W. (1998) *Biochemistry* **37**, 4001 - 4007.
Boussac, A. and Rutherford, A.W. (2000) *Biochimica et Biophysica Acta* **1457**, 145 - 156.
Casey, J.L. and Sauer, K (1984) *Biochimica et Biophysica Acta* **767**, 21 - 28.
Pace, RJ, Smith, P, Bramley, R and Stehlik, D (1991) *Biochimica et Biophysica Acta* **1058,** 161-170
Smith, P.J. and Pace, R.J. (1996) *Applied Magnetic Resonance* **11**, 443 - 460.
Smith, P.J., Peterson, S., Masters, V.M., Wydrzynski, T., Styring, S., Krausz, E. and Pace, R.J. (2001) *Biochemistry* Submitted Manuscript.