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Evidence that the Mn complex of the photosystem II reaction centre is exposed to water in the S_1 and S_2 states.

MCW Evans, AM Rich, IP Muhiuddin, JHA Nugent

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: mike.evans@ucl.ac.uk*

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Introduction

Water oxidation by photosystem II is thought to involve a Mn complex, probably containing 4Mn atoms. Turnover of the photosystem II reaction centre is a 1 electron process while water oxidation is a 4 electron process. Oxidation of water therefore requires the operation of an accumulator. This process was demonstrated in classical experiments showing that when water oxidation is driven by single turnover flashes oxygen evolution occurs on every fourth flash. The phenomenon is described by the S state hypothesis which proposes the water oxidation system has five redox states S_0 to S_4. S_0 is the most reduced while oxygen is released during the dark conversion of S_4 to S_0. The dark stable state is S_1. There is strong evidence that the Mn complex undergoes redox changes in step with the S states with complex multiline EPR signals associated with the S_0 and S_2 states while integer spin signals associated with S_1 and S_3 have been identified by parallel mode EPR. A wide range of models of the physical structure of the Mn complex and of redox changes during the S states have been proposed. It is generally accepted that the basic structural unit is a μ-oxo bridged Mn dimer. It is also accepted that there are two Mn-Mn vectors at different angles to the thylakoid membrane. Many of the models which have been proposed to encompass these factors apparently are not compatible with the preliminary X-ray crystal structure and a definitive model awaits a more detailed structure.

The mechanism of water oxidation is unknown. However if the Mn complex is the redox accumulator for water oxidation the majority of models suggest, it, or a complex of the Mn complex and redox active amino acid residues, is also the site of water oxidation. Until recently there has been little direct evidence for interaction of the Mn with water. The characteristic EPR spectra of the S_0 and S_2 states offer the possibility of detecting interaction of water with these states using water labelled with ^2H of ^{17}O to investigate magnetic interaction between these nuclei in water and the Mn complex. Andreasson (Andreasson 1989) identified small shifts in the CW EPR spectrum of S_2 in the presence of $H_2{}^{17}O$. Nugent (Nugent 1987) reported very small changes in samples in ^2H_2O, but these can be interpreted as reflecting overall exchange of deuterium into the protein. Electron spin echo envelope modulation (ESEEM) offers a more specific and sensitive technique to detect specific binding of magnetic nuclei to redox centres or interaction with nuclei in the immediate environment of the centre. Initial experiments with ^2H_2O and $H_2{}^{17}O$ did not detect specific interactions with the S_2 state induced either by 200K or room temperature illumination (Turconi et al 1997). However subsequent work showed that interaction with deuterated methanol could be detected (Evans et al. 1999: Force et al. 1998). We found that the component giving rise to the EPR signal showing interaction with methanol was metastable,

decaying even at 77K over a period of weeks. We have now shown that non-specific interaction with ^2H_2O (Evans et al. 2000) can be detected and preliminary results indicate more specific binding of $H_2{}^{17}O$.

Materials and Methods

Oxygen evolving photosystem II subchloroplast membranes were prepared from laboratory grown pea seedlings by a method based on that of Ford and Evans (Ford & Evans 1983. Preparations were transferred into ^2H_2O by pelleting the preparation from the protonated medium and resuspending into medium containing 5mM $MgCl_2$, 20mM 2-(N-morpholino)ethanesulfonic acid, 15mM NaCl, 0.33M sucrose prepared in 99.9% ^2H_2O adjusted to pD 6.7 with NaOH. Samples in $H_2{}^{17}O$ were pelleted and suspended in $H_2{}^{17}O$ The samples were converted to the S_2 state by continuous illumination at 200K in an ethanol/solid CO_2 bath for 10 min. CW EPR spectra were recorded using a JEOL RE1X spectrometer with an Oxford Instruments liquid helium cryostat. ESEEM spectra were recorded on a Bruker ESP380E X-band pulsed spectrometer equipped with a Bruker 1052 DLQ-H8907 variable Q dielectric resonator and an Oxford Instruments CF395 cryostat. Data analysis was performed using Bruker WINEPR software. Measurements were routinely made using $\tau = 128$ns. Preliminary experiments using two other values of τ did not show any effect of τ on detection of the ^2H modulation. The pulse train repetition rate was 10Hz.

Results

ESEEM spectra of freshly illuminated samples in ^2H_2O show strong modulation at the Larmor frequency of ^2H. This modulation is observed around g=2.00 and can be detected to approximately 50mT on either side of g=2.00, Fig. 1. No modulation is seen in the dark adapted samples and the appearance of modulation is correlated with the induction of the multiline S_2 signal. On storage at 77K the intensity of modulation decreases and is essentially completely lost in 2-4 weeks. The loss of modulation correlates with a loss of intensity of the multiline signal. However only part of the multiline signal decays, about half being stable after eight weeks, Fig 2.

Similar experiments with ^{17}O labelled water have detected modulation of the EPR signal on the high field side of g=2.00 induced by 200K illumination. The modulation is lost on storage at 77K as is the modulation from deuterated water. Preliminary experiments show ^{17}O specific modulation is observed at 1.6-1.8MHz with very weak signals also in the 7-8Mhz region. Increased modulation intensity is observed in both ^{17}O and ^{16}O samples in the 4-6MHz probably corresponding to the modulation of the multiline signal by N in a histidine ligand reported by Britt and coworkers.

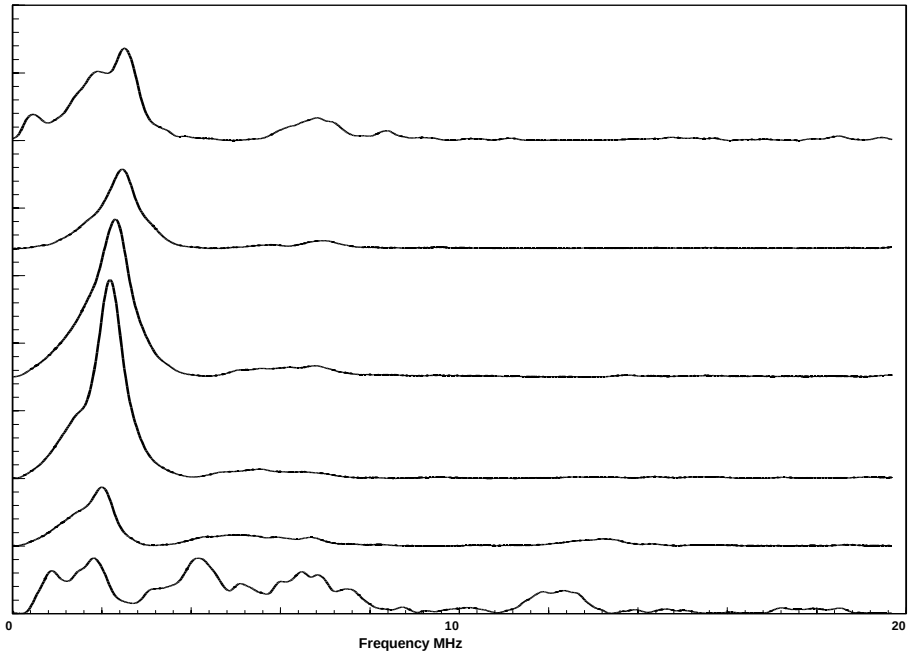

Fig 1 the EPR spectrum of photosystem II in ^2H_2O after 200K illumination. Three pulse ESEEM spectra were recorded at different field positions as described in section 2 immediately following the illumination at 200K of a dark adapted sample. (1top) 398.5 mT. (2) 378.5 mT. (3) 358.5 mT. (4) 337.5 mT (5) 317.5 mT. (6, bottom) 297.5 mT Spectra 1 & 6 are expanded *10. (frequency = 9.71 GHz g=2.00 is at 346.9 mT).

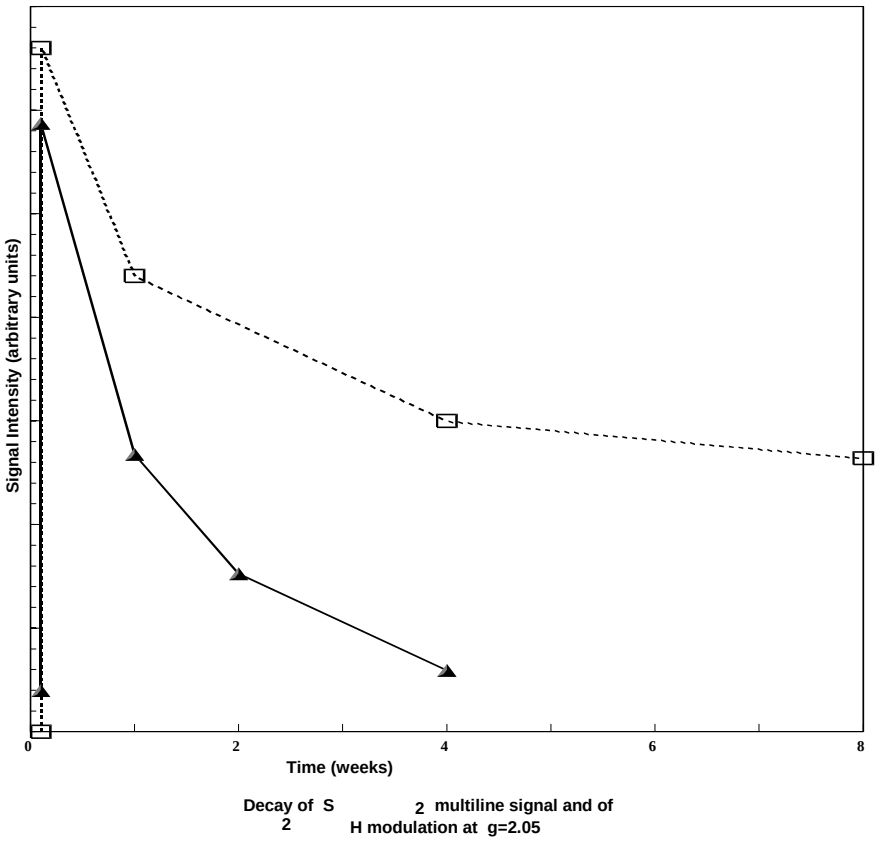

Fig 2 ▲ Changes in the intensity of the ^2H modulation of the spectra shown in Fig 1 at 337.5 mT with time. The intensity of the modulation was measured at 2.2MHz. The lower symbol on the y axis is the initial dark intensity, the upper symbol the intensity immediately after illumination at 200K. □ Intensity of the multiline signal in the same sample region.

Discussion

The results presented here, together with earlier experiments with deuterated methanol, indicate that photosystem II preparations contain a component giving rise to an EPR signal following induction of the S_2 state by 200K illumination which is accessible to the aqueous medium. The experiments with methanol and with ^2H_2O indicate that that component is part of the Mn complex giving rise to the multiline signal. However it is not the S_2 state as normally defined as the component is metastable decaying even at 77K, while the multiline signal has generally been observed to be stable over long periods. Modulation of the multiline signal by ^2H_2O has also been reported by Britt and coworkers (Britt et al. 2000). The existence of heterogeneity in the Mn complex giving rise to the multiline signal is also suggested by the effects of infra red radiation observed by Boussac (Boussac (1997) and work in our laboratory reported at this meeting (S10-012).

The modulation due to deuterium observed at the Larmor frequency indicates weak magnetic interaction between the water hydrogen and the Mn complex. The way in which the S_2 state is induced at 200K shows that water has access to the site in S_1. The modulation observed with ^{17}O at 1.7 MHz would also seem to suggest weak interaction, however the small modulation observed at 7-8 MHz may indicate strong interaction suggesting binding of water to the complex through oxygen. However these results are preliminary and ESEEM is not the best technique for detection of strong magnetic interaction. The similar stability of the modulation seen with both isotopes suggest they may reflect interaction with the same component. However ^{17}O modulation has not been observed across the full width of the multiline signal and the possibility of interaction with other photosystem II components cannot be excluded..

The relatively rapid decay of the multiline component interacting with water compared to the stability of the component which does not show modulation suggest that there are two Mn environments one exposed to water, the other not. A number of models to explain this can be suggested. The work of Wyrdzynski's group (Messinger et al. 1998) has shown that water can exchange readily with the water oxidising complex even in S_3. The exchange implies that there are two states of the complex with water bound and without water. In the present experiments centres frozen with water bound would show modulation and the S_2 state reflected by the multiline signal would be unstable, those without water would show a stable unmodulated S_2 state multiline. This model requires that a large fraction of centres do not have water bound. An alternative model would require that the Mn centre has two components in different environments. One would be in an anhydrous environment within the membrane giving rise to the stable multiline signal, the other would be exposed to water and have a less stable multiline signal. In the S_2 state the oxidising equivalent may reside on either component to give rise to a multiline signal. The distribution on freezing would represent the redox equilibrium between the two centres. The outer centre would be accessible to water or methanol, the inner one would not. This model would be compatible with EXAFS data showing two Mn-Mn dimer orientations in the membrane and that only part of the Mn complex is affected by Ca depletion (Yachandra et al 1992; MacLachlan et al. 1994).

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