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The Mn(II)Mn(III) multiline EPR signal from the water oxidising complex of photosystem II is attributed to a S_{-2} state.

Ch. Goussias[1], G. Schansker[2], V. Petrouleas[2] , & A.W. Rutherford[1].

[1] *Service de Bioenergetique, CEA Saclay, 91191 Gif-sur-Yvette, France.*
Fax +33169088717, email: haris314@yahoo.com

[2] *Institute of Materials Science, NCSR ¨Demokritos¨, 15310 Athens, Greece.*

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Introduction

The multiline EPR signal arising from the very slow interaction of nitric oxide with Photosystem II at –30°C (Goussias et al., 1997) is a reduced state of the manganese cluster, attributed to a magnetically isolated Mn(II)Mn(III) dimer (Sarrou et al. 1998). The reduction procedure in PSII was studied in (Ioannidis et al. 1998), indicating a state below S_{-1}, tentatively S_{-2}, but without a specific S-state assignment. In the present study we attempt to assign this reduced form of the Mn-cluster to a specific S-state.

Materials and methods

PSII-enriched membranes were isolated from spinach using standard techniques (Berthold et al., 1981) Nitric oxide was added to dark-adapted PSII anaerobically at 0°C. The maximum Mn(II)Mn(III) multiline signal was obtained after an 18h incubation at –30°C. Removal of NO, after maximum signal generation, was performed by pumping at 0°C.

The flash oxygen measurements were carried out using a Joliot-type electrode at a polarization voltage of 650 mV. Fluorescence measurements were performed as described in (Ioannidis el al., 2000). Electron paramagnetic resonance spectroscopy was performed using a Bruker ER-200-SRC spectrometer. Flash illumination of the EPR samples was performed with a Spectra Physics GCR-230-10 Nd:YAG laser (532 nm, 550mJ, 8ns pulse) at room temperature.

One rather peculiar property of this state is that its EPR signal is very sensitive to temperatures above 0 °C: it very quickly disappears, only to be restored by a brief incubation at –30°C (Goussias et al. 1997). Under our experimental protocol, the flash oxygen experiment, the chlorophyll α fluorescence and the EPR measurements, have as starting material PSII membranes that is characterised by the EPR-silent form of the Mn(II)Mn(III) state.

Results

Flash oxymetry

The appearance of a considerable amount of oxygen on the 6th and 7th flash (with an estimated miss factor of 15-20%), indicates that the number of centres, which can still turn over is in a S_{-2} state. Saturating pre-flashes cause a downward shift of the number of the maximum O_2

evolution peak, by one for each preflash, up to the third. The fourth preflash, yielding the S_2 state, results in no further reduction of the number of the maximum O_2 peak; this had mostly decayed to the S_1 before the measurement started.

EPR measurements.

Figure 1 shows the EPR spectra recorded after flashing the Mn(II)Mn(III) samples, incubated with 0.5mM p-benzoquinone and 1% methanol. The starting level of the Mn(II)Mn(III) is shown in Figure 1A. A fraction of centres displays an S_2 multiline upon the 4th flash (Figure 1E) (43% of the control in fig. 1H), with lesser amounts appearing on the 1st, 2nd, 3rd and 5th flash (Fig 1A-C, F). In order to assess the maximum S_2 of the sample illuminated with 4 flashes, this was further dark-adapted for 5 hours at 10-15°C, and then illuminated for 4min at 200K. This results in the S_2 multiline in figure 1G. This spectrum is approximately 75% of the untreated control sample of figure 1H. The smaller S_2 on the 4th flash can be explained on the basis of a mixture of lower S-states but higher than the Mn(II)Mn(III), as indicated by the small amounts of S_2 multiline, observed for flash numbers other than the fourth. Assuming a small loss in the form of free Mn^{2+} and the unavoidable misses when flash illuminating, this percentage seems to correlate rather well with the control.

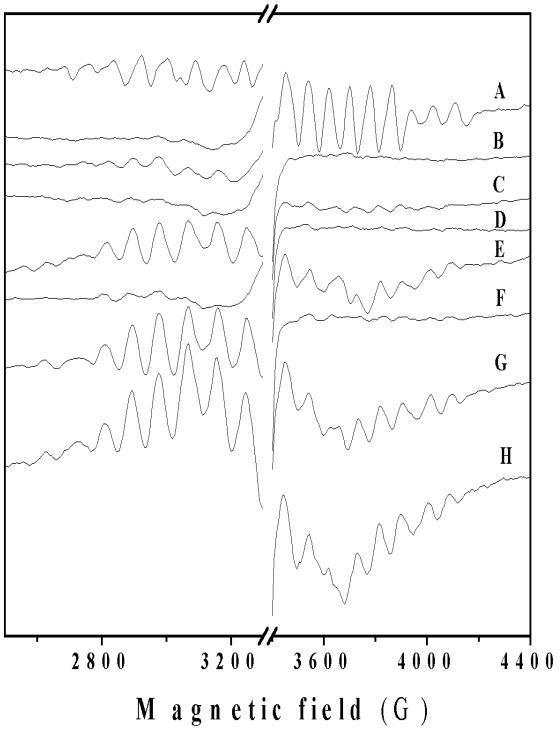

Figure 1. EPR spectra of different PSII samples treated with NO, in the presence of 0.5mM pBQ and 1% methanol. A, the level of the Mn(II)Mn(III) multiline for all the samples, before illumination; B, one saturating flash; C, two flashes; D, three flashes; E, four flashes; F, five flashes; G, 200K illumination of the sample at 4E, following its dark adaptation for 5 hours at 10°C; H, S_2 multiline of a control sample, containing 1% methanol and 0.5mM pBQ.

Tyrosine $D^{\cdot}$ was also monitored for each sample and for each flash. Given its redox equilibrium with the Mn-cluster, a change of its amplitude as a function of flash number would introduce a degree of uncertainty to the assignment of an S-state to the Mn(II)Mn(III). Although the amount of tyrosine $D^{\cdot}$ present varied from sample to sample, no significant change to the size of the tyrosine $D^{\cdot}$ EPR signal were observed in any of the samples, before and after the train of flashes.

These results also indicate that the NO-reduced state corresponds to S_{-2}. The appearance of small S_2 levels on the second and third flash, shows that at the point of complete Mn(II)Mn(III) formation, a fraction of centres is still in a more oxidised state. Despite the fact that methanol was used as the exogenous quinone solvent, no S_0 spectrum was observed on the second flash. Given the small size of the S_0 signal, this could easily be hidden under the residual S_2 multiline (figure 1C). Alternatively, we cannot exclude the possibility of a different valence distribution of the four Mn ions when hydrazine is used as a reductant, as opposed to NO.

Flash-fluorescence measurements

The S-state of the Mn(II)Mn(III)-multiline can also be determined using a chlorophyll *a* fluorescence technique. In figure 2, the results are summarized in 3 panels. In panel a., a control sample is compared with a Mn(II)Mn(III) sample. Since they are both period-4 oscillations, this indicates that the fluorescence pattern of the NO-treated sample was shifted by three flashes, relative to the control sample. In panel b., it is demonstrated that by giving 1 to 3 saturating pre-flashes (pre-flash frequency, 1Hz) the fluorescence pattern will shift by 1on each pre-flash. Finally, in panel c., it is demonstrated that if the time between the pre-flashes and the flash series is increased to 5 minutes (long enough for the S_2 state to decay back to S_1) there is very little difference between giving 3 and 4 pre-flashes. These data are consistent with an S_{-2} state of the manganese cluster.

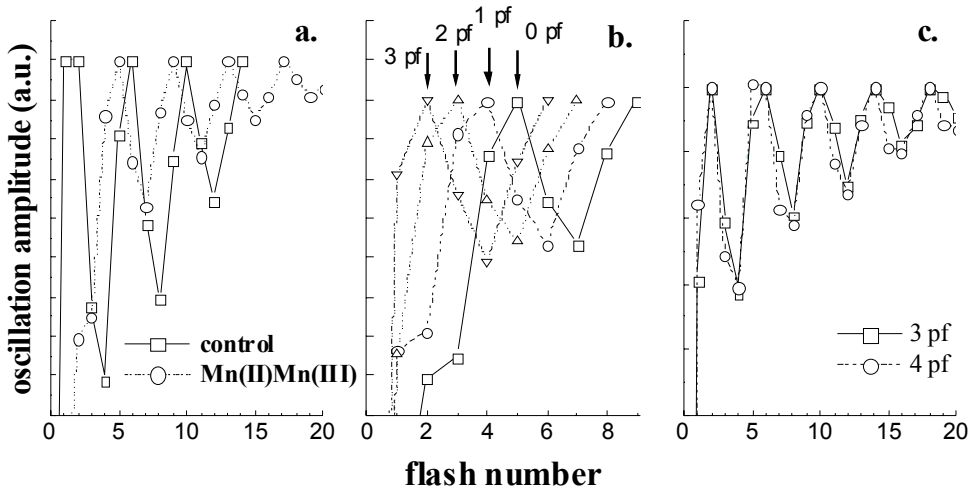

Figure 2. Period-4 oscillations of PSII samples that exhibit the NO-induced multiline EPR spectrum. Panel a., the period-4 oscillations of a NO-treated sample are compared with those of a control sample; panel b., the shift of the period-4 oscillations by 0-3 saturating pre-flashes at 1Hz; panel c., comparison of the period-4 oscillations of NO-treated samples after 3 or 4 pre-flashes and a 5min dark adaptation.

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