Conversion of the Spin State of the Manganese Complex in Photosystem II Induced by Near-Infrared Light

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ABSTRACT: The manganese complex (Mn_4) which is responsible for water oxidation in photosystem II is EPR detectable in the S_2 state, one of the five redox states of the enzyme cycle. The S_2 state is observable at 10 K either as a multiline signal (spin $^1/_2$) or as a signal at $g = 4.1$ (spin $^3/_2$ or spin $^5/_2$). It is shown here that at around 150 K the state responsible for the multiline signal is converted to that responsible for the $g = 4.1$ signal upon the absorption of infrared light. This conversion is fully reversible at 200 K. The action spectrum of this conversion has its maximum at 820 nm (12 200 cm^{-1}) and is similar to the intervalence charge transfer band in di-μ-oxo-($Mn^{III}Mn^{IV}$) model systems. It is suggested that the conversion of the multiline signal to the $g = 4.1$ signal results from absorption of infrared light by the Mn cluster itself, resulting in electron transfer from Mn^{III} to Mn^{IV}. The $g = 4.1$ signal is thus proposed to arise from a state which differs from that which gives rise to the multiline signal only in terms of this change in its valence distribution. The near-infrared light effect was observed in the S_2 state of Sr^{2+}-reconstituted photosystem II and in Ca^{2+}-depleted, EGTA (or citrate-)-treated photosystem II but not in ammonia-treated photosystem II. Earlier results in the literature which showed that the $g = 4.1$ state was preferentially formed by illumination at 130 K are reinterpreted as being the result of two photochemical events: the first being photosynthetic charge separation resulting in an S_2 state which gives rise to the multiline signal and the second being the conversion of this state to the $g = 4.1$ state due to the simultaneous and inadvertent presence of 820 nm light in the broad-band illumination given. There is therefore no reason to consider the state responsible for the $g = 4.1$ signal as a precursor of that which gives rise to the multiline signal.

Photosystem II (PSII)[1] catalyzes light-driven water oxidation, resulting in oxygen evolution. The reaction center of PSII is made up of two membrane-spanning polypeptides (D1 and D2) analogous to the L and M subunits of the purple photosynthetic bacterial reaction center [Michel and Deisenhofer (1988) for a review]. Absorption of a photon leads to a charge separation between a chlorophyll molecule, designated P_{680}, and a pheophytin molecule. The pheophytin anion transfers the electron to a quinone Q_A, and P_{680}^+ is reduced by a tyrosine residue, TyrZ. A cluster of four Mn located in the reaction center of PSII probably acts both as the active site and as a charge-accumulating device of the water-splitting enzyme. During the enzyme cycle, the oxidizing side of PSII goes through five different redox states that are denoted S_n, with n varying from 0 to 4. The oxygen is released during the S_3 to S_0 transition in which S_4 is a transient state [Debus (1992) and Rutherford (1989) for reviews].

In oxygen-evolving PSII, of the five S states, only the S_2 state gives rise to EPR signals which are detectable using conventional EPR. The first signal detected from PSII was a multiline signal at close to $g = 2$. This signal is spread over roughly 1800 G and is made up of at least 19 lines, each separated by approximately 80 G. These hyperfine lines appear to be superimposed on a broad gaussian-shaped signal. The similarities of the S_2 EPR signal with the signals of spin $^1/_2$ mixed valence di-μ-oxo-($Mn^{III}Mn^{IV}$) dimers (Cooper et al., 1978; Hagen et al., 1988) were taken as evidence of a similar structure for the biological complex (Dismukes & Siderer, 1981; Hansson & Andréasson, 1982). Currently, the most commonly favored origin for the multiline S_2 signal is that it arises from a tetramer of Mn which includes a di-μ-oxo-($Mn^{III}Mn^{IV}$) dimer motif [reviewed in Britt (1996), Brudvig (1989), and Dismukes (1993)].

In addition to the usual S_2 multiline signal, a series of different multiline signals with altered spectroscopic characteristics can be observed in PSII after certain inhibitory treatments. In ammonia-treated PSII, a 200 K illumination induces the formation of a normal multiline signal, but after warming of the sample, ammonia can bind to the Mn cluster, resulting in a multiline signal exhibiting 21−22 lines with reduced hyperfine spacing (68 G) (Beck et al., 1986). A similar, but not identical, multiline signal can be induced in a sample in which Sr^{2+} is substituted for Ca^{2+} (Boussac & Rutherford, 1988). An even more drastically modified multiline signal was generated by a Ca^{2+} depletion procedure done in the presence of a high concentration of chelators (Boussac et al., 1989, 1990b). This signal showed at least 26 lines spaced by 55 G and was stable in darkness for days.

The second EPR signal attributed to the S_2 state is centered at $g = 4.1$ (Casey & Sauer, 1984; de Paula et al., 1985; Zimmermann & Rutherford, 1984, 1986) and can be formed either by room temperature or 200 K illumination of

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[1] Abbreviations: P_{680}, chlorophyll (Chl) center of photosystem II (PSII); TyrZ, the tyrosine acting as the electron donor to P_{680}; Q_A, primary quinone electron acceptor of PSII; EPR, electron paramagnetic resonance; EXAFS, extended X-ray absorption fine structure; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; PPBQ, phenyl-*p*-benzoquinone; Mes, 2-(*N*-morpholino)ethanesulfonic acid.

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membranes in the presence of sucrose (Zimmermann & Rutherford, 1984, 1986) or by illumination at 130 K (Casey & Sauer, 1984; de Paula et al., 1985). The $g = 4.1$ signal was proposed to arise from either a spin $^3/_2$ state [reviewed by Brudvig (1989), de Paula et al. (1986a), and Smith et al. (1993) and references therein] or a spin $^5/_2$ state (Astashkin et al., 1994; Haddy et al., 1992).

When a sample showing the $g = 4.1$ signal generated at 130 K was warmed to 200 K, the $g = 4.1$ signal was lost while the amplitude of the multiline signal increased (Casey & Sauer, 1984). This behavior was interpreted as indicating that the $g = 4.1$ signal arose from a state which was a precursor of that giving the multiline signal. Different views of the nature of the precursor have been put forward. It was proposed that the two signals observed in the S_2 state could result from two separate but sequential electron transfer components [Hansson et al., 1987; see also Casey and Sauer (1984) and Zimmermann and Rutherford (1984)]. In one such model, it was suggested that a single Mn^{IV} gave rise to the $g = 4.1$ signal while a $Mn^{III}Mn^{IV}$ dimer was responsible for the multiline signal (Hansson et al., 1987). This model was modified to a monomer/trimer model (Pecoraro, 1988; Hansson & Wydrzynski, 1990; Penner-Hahn et al., 1990) in order to account for indications that the multiline signal arose from a cluster which was bigger than a dimer. However, the more recent observation of hyperfine lines in the $g = 4.1$ signal in oriented ammonia-treated PSII argued strongly against the $g = 4.1$ signal arising from a monomeric Mn^{IV} (Kim et al., 1992).

An alternative and now more commonly held view of the nature of the $g = 4.1$ signal is that both the $g = 4.1$ and the multiline signal arise from the same multinuclear Mn complex in different structural environments (de Paula et al., 1986a; Zimmermann & Rutherford, 1986). It was suggested that the $g = 4.1$ conformation is transiently formed in all centers upon formation of S_2 and, in most of the centers, this converts into the multiline conformation in a temperature-dependent reaction. To explain the fraction of centers showing the stable $g = 4.1$ signal, it was suggested that this represents those centers existing in a different conformation in the dark (i.e. in S_1) which is interconvertible with the dominant conformation (Zimmermann & Rutherford, 1986).

The fraction of centers giving rise to the $g = 4.1$ signal that is stable at $T \geq 200$ K is dependent on the pretreatment of the enzyme, being markedly increased by (i) having sucrose present in the medium, (ii) the removal of chloride (vanVliet & Rutherford, 1996) or its replacement by F^- (Casey & Sauer, 1984), amines (Beck et al., 1986), or NO^{3-} (Ono et al., 1987), and (iii) replacing Ca^{2+} with Sr^{2+} (Boussac & Rutherford, 1988). The $g = 4.1$ signal stable at $T \geq 200$ K is suppressed by the presence of glycerol, ethylene glycol, and ethanol (Zimmermann & Rutherford, 1986).

In the present study, it is demonstrated that the $g = 4.1$ signal which is formed by illumination at 130 K is generated by near-infrared excitation of an absorption band which is presumed to originate from the Mn cluster itself.

MATERIALS AND METHODS

Oxygen-evolving photosystem II-enriched membranes from spinach were prepared as described in Boussac and Rutherford (1988). The membranes were then submitted to two additional washings in 25 mM Mes (pH 6.5), 20 mM NaCl, and 0.1 mM EDTA. These membranes are designated untreated PSII. Sr^{2+} reconstitution, ammonia treatment, and NaCl−EGTA treatment of PSII were done as reported previously (Boussac & Rutherford, 1988; Boussac et al., 1989, 1990a,b). Citrate treatment at pH 3 was done as described by Kodera et al. (1995) except that, after the pH was increased to 6.5, washings of the membranes were done in room light to allow the binding of citrate to occur (Boussac et al., 1990b).

Then, the PSII preparations were put in quartz EPR tubes (at 6−8 mg of Chl/mL). After dark adaptation for 1 h, at 0 °C, PPBQ, dissolved in dimethyl sulfoxide (DMSO), was added as an artificial electron acceptor. For the Sr^{2+}-reconstituted membranes, the PPBQ added was dissolved in ethanol instead of DMSO. The presence of ethanol favored the formation of the multiline signal by a 200 K illumination at the expense of the $g \approx 4$ signal [see Zimmermann and Rutherford (1986)]. After the addition of PPBQ, the samples were immediately frozen in the dark at 200 K in a CO_2−ethanol bath and then transferred to liquid nitrogen (77 K). CW-EPR spectra were recorded at liquid helium temperatures with a Bruker ER 200D or ESP300 X-band spectrometer equipped with Oxford Instruments cryostats.

Formation of the S_2 state in untreated PSII, ammonia-treated PSII, and Sr^{2+}-reconstituted PSII was done by illumination of the samples with a 800 W tungsten lamp through water (which absorbs between 900 and 1050 nm) and infrared filters (cut off above 750 nm) in a nonsilvered dewar filled with ethanol and cooled to 200 K with solid CO_2. Further illuminations of the samples were then given in some experiments as described below: (1) at 80 K, directly in the EPR cavity, or (2) from 85 to 200 K in a nitrogen gas flow system (Bruker, B-VT-1000). At 80 K, illumination was done for 15 min with a laser diode emitting at 820 nm and with a power of approximately 50 mW measured at the grill of the EPR cavity. For 85−200 K, illumination of the samples was done for 5−10 min either with a 800 W tungsten lamp through a 2 cm water filter but in the absence of infrared filters or with an argon laser (Coherent, Innova 70) working in the all-lines mode. The laser beam was defocused, and the power was adjusted so that it was approximately 1 W at the level of the sample, i.e. a power equivalent to that obtained with the tungsten lamp. Finally, for the experiment depicted in Figure 3, the sample was placed at 150 K in the N_2 gas flow system. Illumination was done at different wavelengths by using a Ti-sapphire laser (Spectra Physics model 3900S). The laser beam was defocused, and the power was adjusted so that the multiline to $g = 4.1$ conversion was observed in about 50% of the centers after illumination with 820 nm light for 5 min.

RESULTS

Spectrum a, in Figure 1, was recorded at 10 K on dark-adapted, untreated PSII, and spectrum b was recorded after a 200 K illumination of the same sample. The illumination resulted in the formation of the S_2 multiline signal and a signal at $g = 1.9$ (≈3600 G) from $Q_A^-Fe^{2+}$. Virtually no signal at $g = 4.1$ was formed under these conditions. A second illumination of the sample with the 800 W tungsten lamp (through 2 cm of water but without infrared filters, see below) but at 150 K resulted in the appearance of a signal at close to $g = 4$ with a parallel loss of the multiline signal (spectrum c in Figure 1).

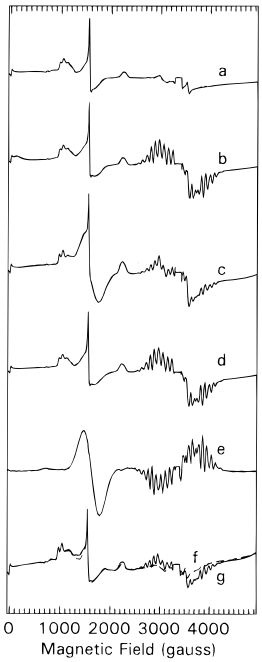

FIGURE 1: EPR spectra of untreated PSII recorded at 10 K on dark-adapted membranes [spectra a and f (dashed line)], after a 200 K illumination for 1 min (spectrum b), and after a second illumination, with no infrared filters, for 5 min at 150 K (spectrum c). Spectrum d was recorded after a warming of the sample at 200 K, in the dark. Spectrum e is the difference: spectrum c minus spectrum d (×2). Spectrum *g* was recorded after an illumination with an argon laser for 5 min at 130 K of dark-adapted membranes. The instrument settings were as follows: modulation amplitude, 25 G; microwave power, 20 mW; microwave frequency, 9.4 GHz; and modulation frequency, 100 KHz. The central part of the spectra corresponding to the TyrD° region was deleted.

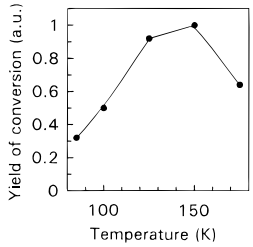

FIGURE 2: Yield of the mutiline signal converted into the $g = 4.1$ signal as a function of the temperature at which the sample was illuminated. For this, the multiline form was first produced as described in spectrum b of Figure 1. Then, the second illumination was done at different temperatures by a short period of illumination with the tungsten lamp (in the absence of infrared filters). The EPR spectrum was recorded, then the sample was warmed to 200 K in the dark, and the second EPR spectrum was recorded. The yield of the multiline to $g = 4$ conversion was determined by estimating the proportion of the multiline converted into the $g = 4.1$ signal which was restored by the warming at 200 K. It was observed that several cycles of illumination followed by warming to 200 K did not induce any decrease in the signal amplitudes. The yield of the conversion was normalized to 1 at 150 K, the temperature at which it is maximal.

It was reported previously that, after the $g = 4.1$ signal had been generated at 130 K, subsequent warming of the sample to 200 K resulted in the conversion of the $g = 4.1$ signal to the multiline signal (Casey & Sauer, 1984). Spectrum d in Figure 1 was recorded on the sample which was briefly (5−10 s) incubated at 200 K in the dark after the illumination at 150 K. This warming to 200 K clearly restores the multiline signal at the expense of the $g = 4.1$ signal. The conversion of the multiline signal to the $g = 4.1$ signal by low-temperature illumination is illustrated by spectrum e which is the difference spectrum c minus d. The $g = 4.1$ signal formed in these conditions has a peak to trough width of 320 G. At 150 K, the $g = 4.1$ signal converted back to the multiline signal with a half-time of about 30 min (not shown).

The temperature at which the light-induced multiline to $g = 4.1$ conversion occurs with the highest yield is illustrated in Figure 2 and was maximal around 140−150 K.

During the first 200 K illumination, the formation of the S_2 multiline state is accompanied by the reduction of the Q_A electron acceptor (Figure 1b). Therefore, the second illumination at 150 K is not expected to produce a second charge stabilization. Indeed, no increase in the $Q_A^-Fe^{2+}$ signal around $g = 1.9$ occurred upon this second illumination (see Figure 1c and below). The multiline to $g = 4.1$ conversion thus appeared to be unrelated to stable charge separation in the PSII reaction center. Therefore, we investigated the possibility that the multiline to $g = 4.1$ conversion was the result of photochemistry unrelated to photosynthetic charge separation in the PSII reaction center. Since the tungsten lamp used for the illumination experiments described above emits significant near-infrared radiation in addition to visible light, we tested the effect of using both visible and near-infrared light.

Spectrum f in Figure 1 (dashed line) was recorded on untreated, dark-adapted PSII membranes, and spectrum *g* was recorded after illumination at 130 K with an argon laser emitting only blue/green light. Under these conditions, the multiline signal was formed and no $g = 4.1$ signal was generated. The amplitude of the multiline signal formed at 130 K was lower than that formed at 200 K (see spectrum b). This is expected since at this temperature the oxidation of the Mn cluster is in competition with other electron donors such as Cyt b_{559} or the monomeric chlorophyll (de Paula et al., 1986b). It can be concluded that the blue/green light used is not capable of inducing the multiline to $g = 4.1$ conversion.

The involvement of infrared light in the conversion of the multiline signal to the $g = 4.1$ signal was first tested by using samples in which the multiline signal was present and illuminating then at 150 K using the white light from the tungsten lamp with and without infrared filters. It was found that the $g = 4.1$ signal was formed much more slowly in the presence than in the absence of infrared filters (not shown but see below). This was taken as a strong indication that infrared light is responsible for the conversion of the multiline signal to the $g = 4.1$ signal. This was verified below using infrared laser excitation.

The action spectrum of the light-induced multiline to $g = 4.1$ conversion was recorded in the near-infrared region. The result is shown in Figure 3. The action spectrum determined under these conditions shows a maximum around 820 nm and a half-width at half-maximum of about 60 nm.

The multiline to $g = 4.1$ conversion triggered by near-infrared light was investigated in Ca^{2+}-depleted and chelator-treated PSII membranes which exhibit a modified multiline signal. This material also has the advantage of being in the S_2 state after a dark adaptation period. Panels A and B in Figure 4 show spectra obtained with EGTA-treated PSII, and panels C and D show spectra obtained with citrate-treated PSII. Spectra in panels A and C and in panels B and D

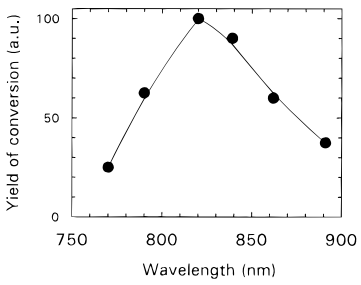

FIGURE 3: Action spectrum of the multiline to $g = 4.1$ conversion. The multiline form was first produced as described in Figure 1, spectrum b. Then, the samples were further illuminated at 150 K, at different wavelengths with a Ti-sapphire laser. The proportion of the multiline signal which was converted into the $g = 4.1$ signal was estimated with a protocol similar to that used in Figure 2. The yield of the conversion was normalized to 100 at 820 nm, the wavelength at which it is maximal.

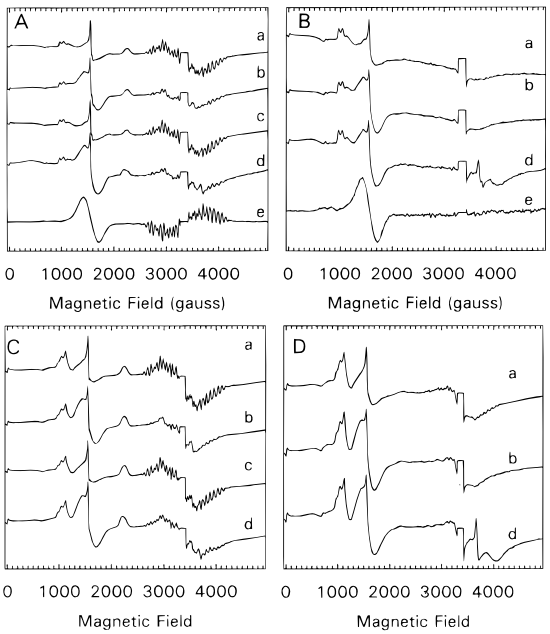

FIGURE 4: EPR spectra of NaCl-washed, EGTA-treated, polypeptide-reconstituted PSII (A and B) or citrate-treated PSII (C and D) recorded at 10 K (A and C) or 4 K (B and D). Spectra a were from dark-adapted membranes. Spectra b were recorded after illumination at 820 nm for 20 min at 80 K in the EPR cavity. Spectra c were recorded after a warming of the samples at 200 K in the dark, following the 820 nm illumination. Spectra d were recorded after a second illumination at 85 K with the tungsten lamp with no infrared filters. Spectra e are the difference: spectra b minus spectra a (×2). The instrument settings for A and C were as follows: modulation amplitude, 22 G; microwave power, 20 mW; microwave frequency, 9.4 GHz; and modulation frequency, 100 KHz. The instrument settings for B and D were as follows: modulation amplitude, 32 G; microwave power, 32 mW; microwave frequency, 9.4 GHz; and modulation frequency, 100 KHz. The central part of the spectra corresponding to the TyrD° region was deleted.

were recorded at 10 and 4 K, respectively. Spectra a were recorded in dark-adapted samples. The characteristic modified multiline signal can be observed at 10 K (panels A and C). At 4 K, the multiline signal was no longer observable (panels B and D), and the absence of any signal between 3500 and 4500 G shows that the primary electron acceptor Q_A is totally oxidized. Spectra b were recorded after illumination of the samples at 80 K, directly in the EPR cavity, with a laser diode emitting at 820 nm. Spectra b in panels A and C show that the multiline signal largely disappeared and that a $g \approx 4$ signal was generated. This is clearly illustrated in spectrum e of panel A which shows the near-infrared light minus dark spectrum generated by the 820 nm illumination. In contrast to the conditions for untreated PSII, it was found that 80 K was the best temperature to induce the multiline to $g = 4.1$ conversion in Ca^{2+}-depleted and chelator-treated PSII. Spectra b in panels B and D show that this conversion is not associated with a spectral change between 3500 and 4500 G demonstrating that no $Q_A^-Fe^{2+}$ was formed during the illumination. Since chlorophyll does not absorb at 820 nm, this result is expected and is a further confirmation that the conversion of the multiline signal into the $g \approx 4$ signal is not associated with a charge separation process in the PSII reaction center. Spectra b in panels B and D show that the $g \approx 4$ signal formed in these conditions is easily observable at 4 K. The $g \approx 4$ signal has not previously been reported in such preparations. The signal formed in these membranes by this treatment has a g value equal to 4.25 and a peak to trough width of 280 G. These characteristics are similar to those exhibited by the signal generated by 200 K illumination of ammonia-treated PSII (Andréasson & Hansson, 1987).

Spectra c in Figure 4 were recorded on the samples which were briefly (5−10 s) incubated at 200 K in the dark after the illumination at 820 nm. This warming to 200 K clearly restored the multiline signal at the expense of the $g \approx 4$ signal. More than 10 conversion cycles on the same sample did not change the amplitude of the signals, indicating that this process induced no damage to PSII (not shown).

The EGTA- and citrate-treated PSII were then illuminated at 85 K with a 800 W tungsten lamp with no infrared filters (spectra d in Figure 4). The multiline to $g = 4.1$ conversion occurred with a high yield. Concomitant with this conversion was the appearance of the characteristic $Q_A^-Fe^{2+}$ signal (spectra d, panels B and D) and a large Chl^+ signal (not shown). This was expected since this kind of illumination is able to induce a charge separation.

After an ammonia treatment which results in the formation of a specific modified multiline signal, we were unable to observe the multiline to $g = 4.1$ conversion whatever the temperature (below 200 K) at which the illumination was given (not shown). In contrast, in Sr^{2+}-reconstituted samples which exhibit a similar (but not identical) modified multiline signal, the multiline to $g = 4.1$ conversion induced by low-temperature illumination (150 K) was easily observed (not shown).

DISCUSSION

The results show that the state responsible for the multiline EPR signal in PSII is converted to that responsible for the $g = 4.1$ signal by excitation with near-infrared radiation given at approximately 150 K. Warming of the sample to 200 K in the dark reversed this effect. These results have obvious implications on the long-held view that the $g \approx 4$ state represents a precursor of that responsible for the multiline signal.

This view stems from the frequently reproduced observation that the $g \approx 4$ signal is preferentially formed by illumination at 130 K and that it converts to the multiline signal upon being warmed to 200 K in the dark (Casey & Sauer, 1984). Above we have shown that, when illumination at 130 K is done using light which lacks any infrared component, only the multiline signal is formed (Figure 1g).

The results show that infrared illumination is required before any $g \approx 4$ signal is formed at these temperatures. In light of the results presented here, it seems reasonable to conclude that all previous studies on the formation of the $g \approx 4$ signal at around 130 K were in fact monitoring two distinct photochemical events: (a) the formation of the S_2 state (detectable as the multiline signal) through PSII reaction center photochemistry which is triggered by visible light and (b) the subsequent conversion of the S_2 state from the multiline form to the $g \approx 4$ form due to simultaneous and inadvertent excitation with infrared light as part of the broad-band illumination given. We further conclude that there is no evidence to suggest that the $g \approx 4$ signal represents a state that is a precursor of that giving rise to the multiline signal.

The action spectrum triggering the multiline to $g = 4.1$ conversion in the near-infrared region presents a maximum around 820 nm. The spectrum shown in Figure 3 can be compared to that observed in the same spectral region in di-μ-oxo-($Mn^{III}Mn^{IV}$) model systems. This absorption was attributed to an intervalence charge transfer band [Cooper & Calvin, 1977; see also Hush (1967) and Blondin and Girerd (1990)]. It seems reasonable to suggest a similar origin for the absorption band reported here.

The spectrum in Figure 3 may be related to that reported by Dismukes and Mathis (1984) upon the S_1 to S_2 transition and attributed to the formation of a mixed-valence state in S_2. Although this report was questioned (Velthuys, 1998), the present work leads us to suggest that further studies like those initiated by Dismukes and Mathis (1984) may be worthwhile.

If the 820 nm band reported here corresponds to an intervalence charge transfer band as suggested above, then this suggests that the multiline and $g \approx 4$ signals represent states which differ from each other in terms of a redistribution of valence within the cluster. In terms of the dimer of dimer model for the Mn cluster [e.*g*. de Rose et al. (1994)], the simplest example of such a valence redistribution involves the swapping of the valence in $Mn^{III}Mn^{IV}$ dimer that is responsible for the main characteristics of the multiline signal, for example

$$(Mn_a^{III}Mn_b^{IV})-(Mn_c^{IV}Mn_d^{IV}) \quad \underset{\text{multiline}}{\text{to}} \quad (Mn_a^{IV}Mn_b^{III})-(Mn_c^{IV}Mn_d^{IV}) \quad g \approx 4$$

The most favored explanation of the multiline/$g \approx 4$ signals is that they arise from the same cluster but in slightly different conformations. These are proposed to result in modifications of the exchange interactions within the cluster, resulting in either the spin $^1/_2$ (multiline) or the spin, $^3/_2$ (or $^5/_2$) ($g = 4.1$) ground states (Brudvig, 1989). We suggest that it is a redistribution of valence rather than a conformation change that is responsible for the modified exchange interactions. This valence change is predicted to result in specific changes in the bond lengths and the geometry of the orbitals around the Mn ions, but these should be seen as secondary effects and not the cause of the multiline to $g \approx 4$ conversion.

Under some circumstances, the $g \approx 4$ signal can be generated at room temperature with a single flash and has a lifetime comparable to that of the multiline signal (Zimmermann & Rutherford, 1984; van Vliet & Rutherford, 1996). Clearly, there can be no role for infrared light in the formation of the signal under these conditions. In the context of the current discussion, we suggest that this stable $g \approx 4$ state reflects a stable form of the species which is generated with infrared light at low temperatures, i.e. a state which differs from the multiline signal in terms of a valence swap, as described above. In this case, however, the free energy level of the $g \approx 4$ state is not higher than that of the multiline state; instead, it must be stabilized in some way to make it the energetically preferred state at room temperature. What could be responsible for the stabilization of the $g \approx 4$ state is open to speculation, but it is easy to imagine minor changes that would favor the two different valence distributions of the type suggested. Since it seems that changes in the binding of ions (halides, amines, hydroxyl, and protons) influence the distribution of the multiline and stable $g \approx 4$ signals, it is reasonable to suggest that these may induce differential modifications in the electrostatic environment of the Mn ions involved in the putative valence swap. Such changes could easily be responsible for stabilizing one form or the other.

In principle, the study of intervalence charge transfer bands can provide structural information. In model systems, the intensity, the half-width at half-maximum, and the peak position of this band taken together with the $Mn^{III}-Mn^{IV}$ interdistance allowed the estimation of the delocalization coefficient of the electron between Mn^{III} and Mn^{IV} [Cooper & Calvin, 1977; see Hush (1967)]. In the same way, using the results reported here, the Mn−Mn distance from the EXAFS studies (de Rose et al., 1994), and the very approximate extinction coefficient from the work of Dismukes and Mathis (1984), we can estimate the delocalization to be on the order of 10^{-3}. Before such a calculation can be taken seriously, we require a greater degree of certainty not only concerning the extinction coefficient of the band but also in terms of its origin.

The temperature dependence of the multiline to $g \approx 4$ conversion shows high, and low-temperature limits. Above the high-temperature limit, the reconversion of the $g \approx 4$ state back to the multiline state presumably occurs. This temperature dependence presumably represents an activation energy for the back reaction. The low-temperature limit could have several explanations. One possibility is that thermal energy is required to overcome an activation barrier between unstable intermediates (should they exist) and the stable $g \approx 4$ state. In this case, the intermediate could be the state formed after electron transfer but prior to the stabilization of the new valence distribution by changes in the coordination sphere. Alternatively, the state excited by 820 nm light could be an excited state (with $S = {}^3/_2$ or $^5/_2$) which would become depopulated at low temperatures [see Decurtins et al. (1984)]. Such an excited state has been estimated to lie 30−36 cm^{-1} above the multiline signal (Hansson et al., 1987; Lorigan & Britt, 1994). Studies of the kinetics and temperature dependence of the multiline to $g = 4.1$ transition should provide a more complete picture of its thermodynamics and its mechanism.

In Ca^{2+}-depleted chelator-modified PSII, a much lower temperature maximum was found, while in ammonia-treated PSII, we were unable to find conditions for the conversion to occur. This is consistent with the intervalence transition model presented above, since any structural modification of the Mn cluster could change the energy levels of the states

involved in the intracluster electron transfer event. In ammonia-treated PSII, ammonia is thought to replace one of the oxygens of the di-μ-oxo bridge (Britt et al., 1989), resulting in an elongation of what is presumably the Mn^{III}—Mn^{IV} distance by 0.15 Å (Dau et al., 1995). It is not surprising then that the ammonia-modified cluster behaves differently than the other cases tested.

In Sr^{2+}-reconstituted PSII, in which the Mn−Mn distance seems to be unmodified compared to that of untreated PSII (Latimer et al., 1995), the light-induced multiline to $g \approx 4$ conversion was observed. This confirms that the states responsible for the modified multiline signals in ammonia-treated PSII and in Sr^{2+}-reconstituted PSII are different.

From the present work, we have gained new insight on the nature of the EPR signals arising from the Mn cluster in PSII. In contrast to the current generally held view, there is now no reason to believe that the $g \approx 4$ state represents a precursor of the multiline state. The conversion of the multiline signal to the $g \approx 4$ signal occurs through the absorption of 820 nm radiation, and we tentatively assign this to an intervalence charge transfer event. We suggest then that the $g \approx 4$ signal represents a state which differs from the state giving rise to the multiline only in terms of its valence distribution. The room temperature stable $g \approx 4$ signal is suggested to arise from the same state except that it is energetically favored by a minor change in the (electrostatic?) environment of the Mn cluster which is induced by a range of biochemical treatments. The presence of the intervalence charge transfer band as suggested here should serve as a spectroscopic tool for future research. In addition, the method described here can be used for the quantitative generation of the $g \approx 4$ state for detailed spectroscopic studies, something which has not been possible to date. Furthermore, the novel low-temperature Mn photochemistry provides another characteristic to be looked for in biomimetic Mn clusters.

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REFERENCES

Andréasson, L.-E., & Hansson, Ö. (1987) in *Progress in Photosynthesis Research* (Biggins, J., Ed.) Vol. I, pp 503−510, Martinus Nijhoff Publishers, Dordrecht.

Astashkin, A. V., Kodera, Y., & Kawamori, A. (1994) *J. Magn. Reson. 105*, 113−119.

Beck, W. F., de Paula, J. C., & Brudvig, G. W. (1986) *J. Am. Chem. Soc. 108*, 4018−4022.

Blondin, G., & Girerd, J.-J. (1990) *Chem. Rev. 90*, 1359−1376.

Boussac, A., & Rutherford, A. W. (1988) *Biochemistry 27*, 3476−3483.

Boussac, A., Zimmermann, J.-L., & Rutherford, A. W. (1989) *Biochemistry 28*, 8984−8989.

Boussac, A., Rutherford, A. W., & Styring, S. (1990a) *Biochemistry 29*, 24−32.

Boussac, A., Zimmermann, J.-L., & Rutherford, A. W. (1990b) *FEBS Lett. 277*, 69−74.

Britt, R. D. (1996) in *Oxygenic photosynthesis: The light reactions* (Ort, D. R., & Yocum, C. F., Eds.) Kluwer Academeic Publishers, Dordrecht.

Britt, R. D., Zimmermann, J.-L., Sauer, K., & Klein, M. P. (1989) *J. Am. Chem. Soc. 111*, 3522−3532.

Brudvig, G. W. (1989) in *Advanced EPR: Applications in Biology and Chemistry* (Hoff, A. J., Ed.) Chapter 24, pp 839−864, Elsevier, Amsterdam.

Casey, J. L., & Sauer, K. (1984) *Biochim. Biophys. Acta 767*, 21−28.

Cooper, S. R., & Calvin, M. (1977) *J. Am. Chem. Soc. 99*, 6623−6630.

Cooper, S. R., Dismukes, G. C., Klein, M. P., & Calvin, M. (1978) *J. Am. Chem. Soc. 100*, 7248−7252.

Dau, H., Andrews, J. C., Roelofs, T. A., Latimer, M. J., Liang, W., Yachandra, V. K., Sauer, K., & Klein, M. P. (1995) *Biochemistry 34*, 5274−5287.

Debus, R. J. (1992) *Biochim. Biophys. Acta 1102*, 269−352.

Decurtins, S., Gütlich, P., Köhler, C. P., Spiering, H., & Hauser, A. (1984) *Chem. Phys. Lett. 105*, 1−4.

de Paula, J. C, Innes, J. B., & Brudvig, G. W. (1985) *Biochemistry 24*, 8114−8120.

de Paula, J. C., Beck, W. F., & Brudvig, G. W. (1986a) *J. Am. Chem. Soc. 108*, 4002−4009.

de Paula, J. C., Li, P. M., Miller, A.-F., Wu, B. W., & Brudvig, G. W. (1986b) *Biochemistry 25*, 6487−6494.

de Rose, V. J., Mukerji, I., Latimer, M. J., Yachandra, V., Sauer, K., & Klein, M. P. (1994) *J. Am. Chem. Soc. 116*, 5239−5249.

Dismukes, G. C. (1993) in *Polynuclear manganese enzymes, Bioinorganic catalysis* (Reedijk, J., Ed.) pp 317−346, Marcel Dekker, Inc., New York.

Dismukes, G. C., & Siderer, Y. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 274−278.

Dismukes, G. C., & Mathis, P. (1984) *FEBS Lett. 178*, 51−54.

Haddy, A., Dunham, W. R., Sands, R. H., & Aasa, R. (1992) *Biochim. Biophys. Acta 1099*, 25−34.

Hagen, K. S., Armstrong, W. H., & Hope, H. (1988) *Inorg. Chem. 27*, 969−971.

Hansson, Ö., & Andréasson, L.-E. (1982) *Biochim. Biophys. Acta 679*, 261−268.

Hansson, Ö., & Wydrzynski, T. (1991) *Photosynth. Res. 23*, 131−162.

Hansson, Ö., Aasa, R., & Vänngärd, T. (1987) *Biophys. J. 51*, 825−832.

Hush, N. S. (1967) *Prog. Inorg. Chem. 8*, 391−444.

Kim, D. H., Britt, R. D., Klein, M. P., & Sauer, K. (1992) *Biochemistry 31*, 541−547.

Kodera, Y., Hara, H., Astashkin, A. V., Kawamori, A., & Ono, T.-A. (1995) *Biochim. Biophys. Acta 1232*, 43−51.

Latimer, M. J., DeRose, V. J., Mukerji, I., Yachandra, V. K., Sauer, K., & Klein, M. P. (1995) *Biochemistry 34*, 10898−10909.

Lorigan, G. A., & Britt, R. D. (1994) *Biochemistry 33*, 10272−12076.

Michel, H., & Deisenhofer, J. (1988) *Biochemistry 27*, 1−7.

Ono, T.-A., Nakayama, H., Gleiter, H., Inoue, Y., & Kawamori, A. (1987) *Arch. Biochem. Biophys. 256*, 618−624.

Pecoraro, V. L. (1988) *Photochem. Photobiol. 48*, 249−264.

Penner-Hahn, J. E., Fronko, R. M., Pecoraro, V. L., Yocum, C. F., Betts, S. D., & Bowlby, N. R. (1990) *J. Am. Chem. Soc. 112*, 2549−2557.

Rutherford, A. W. (1989) *Trends Biochem. Sci. 14*, 227−232.

Rutherford, A. W., Zimmermann, J.-L., & Boussac, A. (1992) in *The photosystems: Structure, function and molecular biology* (Barber, J., Ed.) Chapter 5, pp 179−229, Elsevier Science Publishers, New York.

Smith, P. J., Åhrling, K. A., & Pace, R. J. (1993) *J. Chem. Soc., Faraday Trans. 89*, 2863−2868.

VanVliet, P., & Rutherford, A. W. (1996) *Biochemistry 35*, 1829−1839.

Velthuys, B. R. (1988) *Biochim. Biophys. Acta 933*, 249−257.

Zimmermann, J.-L., & Rutherford, A. W. (1984) *Biochim. Biophys. Acta 767*, 160−167.

Zimmermann, J.-L., & Rutherford, A. W. (1986) *Biochemistry 25*, 4609−4615.

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