Effect of Near-Infrared Light on the S$_2$-State of the Manganese Complex of Photosystem II from *Synechococcus elongatus*[†]

Alain Boussac,*,‡ Helena Kuhl,§ Sun Un,‡ Matthias Rögner,§ and A. William Rutherford‡

Section de Bioénergétique, URA CNRS 2096, DBCM, CEA Saclay, 91191 Gif-sur-Yvette Cedex, France, and Lehrstuhl fuer Biochemie der Pflanzen, Fakultaet fuer Biologie, Ruhr-Universitaet Bochum, Universitaetsstrasse 150, 44780 Bochum, Germany

ABSTRACT: The Mn cluster of Photosystem II (PSII) from *Synechococcus elongatus* was studied using EPR. A signal with features between $g = 5$ and $g = 9$ is reported from the S$_2$-state. The signal is attributed to the manganese cluster in a state with a spin 5/2 state. Spectral simulations of the signal indicate zero field splitting parameters where the |E/D| was 0.13. The new signal is formed by irradiating PSII samples which contain the spin = 1/2 S$_2$-state using 813 nm light below 200 K. This effect is attributed to a spin-state change in the manganese cluster due to absorption of the IR light by the Mn-cluster itself. The signal is similar to that reported recently in PSII of plants [Boussac, A., Un, S., Horner, O., and Rutherford, A. W. (1998) *Biochemistry 37*, 4001−4007]. In plant PSII the comparable signal is formed at a lower temperature (optimally below 77 K), and gradual warming of the sample in the dark leads to the formation of the state responsible for the well-known $g = 4.1$ signal prior to formation of the spin 1/2 multiline signal. In the present work using cyanobacterial PSII, warming of the sample in the dark leads to the formation of the spin 1/2 multiline signal without formation of the $g = 4$ type signal as an intermediate. These observations provide a partial explanation for the long-standing "mystery of the missing $g = 4$ state" in cyanobacterial PSII. The observations are rationalized in terms of three possible states which can exist for S$_2$: (i) the spin 1/2 multiline signal, (ii) the state responsible for the $g = 4.1$ signal, and (iii) the new spin 5/2 state. The relative stability of these states differs between plants and cyanobacteria.

Photosystem-II[1] (PSII) 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 (for a review, see ref *1*). Absorption of a photon results in a charge separation between a chlorophyll (P$_{680}$) and a pheophytin. This charge separation is stabilized by electron transfer from the reduced pheophytin to a quinone Q$_A$ and by the reduction of P$_{680}^+$ by Tyr$_Z$, the tyrosine 161 of the D1 polypeptide (*2, 3*). Further stabilization of charges occurs by reduction of Tyr$_Z$• by the Mn complex and by the oxidation of Q$_A^-$ by the secondary quinone, Q$_B$.

The Mn complex constituted of four manganese ions (Mn$_4$) and 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$_n$, *n* varying from 0 to 4. Oxygen is released during the S$_3$ to S$_0$ transition in which S$_4$ is a transient state (*4–7*). In addition to Tyr$_Z$, there is a second redox active tyrosine in PSII, Tyr$_D$, the tyrosine 160 of the D2 polypeptide (*8–10*). In the dark, Tyr$_D$• slowly oxidizes the S$_0$-state, but in the other S-states Tyr$_D$• is stable (*11*). Tyr$_D$ is able to reduce the S$_2$- and S$_3$-states (*12–14*).

In oxygen evolving PSII, the S$_2$-state gives rise to EPR signals which are detectable using conventional EPR. The first signal detected from S$_2$ was a multiline signal near $g = 2$ (*15*). This signal is spread over roughly 1800 G and is made up of at least 18 lines, each separated by approximately 80 G. Currently, the most commonly favored origin for the S$_2$-multiline signal is that it arises from a Mn-tetramer which includes a di-μ-oxo(Mn^{III}Mn^{IV}) motif (reviewed in refs *4, 16–18*).

The S$_2$-multiline signal has been detected in PSII isolated from plants (*4, 16–18*); from the cyanobacteria, *Synechococcus* (*19, 20*) and *Synechocystis* (*21–23*); and from the green alga, *Scenedesmus obliquus* (*24, 25*). The S$_2$-multiline signals observed in these PSII samples of different origins were found to be similar.

The second signal attributed to the S$_2$-state is centered at $g = 4.1$ (*26–29*) or $g = 4.25$, depending on experimental or preparation conditions. The $g = 4.1$ signal has been

* Author to whom correspondence should be addressed. Phone: 33 1 69 08 72 06. Fax: 33 1 69 08 87 17. E-mail: boussac@dsvidf.cea.fr.
‡ URA CNRS 2096.
§ Ruhr-Universitaet Bochum.

[1] Abbreviations: P$_{680}$, chlorophyll (Chl) center of photosystem II (PSII); Tyr$_Z$, the tyrosine acting as the electron donor to P$_{680}$; Q$_A$, primary quinone electron acceptor of PSII; EPR, electron paramagnetic resonance; DCBQ, (2,6)dichloro benzoquinone; PPBQ, phenyl-*p*-benzoquinone; MES, 2-(*N*-morpholino) ethanesulfonic acid; β-DM, β-dodecyl maltoside; HPLC, high-performance liquid chromatography; IR, infrared.

proposed to arise from either a spin $= 3/2$ state (e.g., *16, 30, 31*) or a spin $= 5/2$ state (*32, 33*). Under some experimental conditions the S_2-state gives rise to the $g = 4$ type signal rather than the multiline signal. Several biochemical treatments favor formation of the $g = 4$ type signal in both the inhibited and the functional enzyme (reviewed in ref *34*, see also ref *35*). The $g = 4.1$ state can also be formed preferentially by illumination at 130 K (*26*). This effect was shown to be the result of two photochemical events: the first being photosynthetic charge separation resulting in a S_2-state which gives rise to the multiline signal, 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 (*36*). The formation of the $g = 4.1$ signal upon the absorption of IR light by the Mn cluster was optimal at 150 K. Warming in the dark of the IR-irradiated sample led the conversion of the $g = 4.1$ state to that responsible for the spin $= 1/2$ multiline signal (*36*, see also ref *26*).

In cyanobacterial PSII, the $g = 4.1$ signal has not been reported. The procedures (biochemical and photochemical) which lead to formation of the $g = 4.1$ signal in plant PSII have failed to yield a detectable $g = 4.1$ signal (*16, 20*). However, such treatments have, in some cases (*20*), yielded decreases in the spin 1/2 multiline signal which, in plant PSII, accompany formation of the $g = 4$ state, and thus could indicate the presence of an undetected state behaving like the $g = 4.1$ state. An explanation for the absence of the $g = 4.1$ signal in cyanobacteria has yet to be found.

Recently, a third signal in the S_2-state of plant PSII was discovered and attributed to a spin $= 5/2$ state (*37*). This state, with resonances in the region of $g = 10$ and $g = 6$, is optimally formed by IR illumination of the spin $= 1/2$ state below 77 K, a temperature where IR illumination leads to the loss of the spin $= 1/2$ signal but to no formation of the $g = 4.1$ state. The new state could be trapped in $\approx$40% of the PSII centers, and warming of the sample to temperatures between 77 and 150 K, in the dark, led to the loss of the $g = 10$ and $g = 6$ resonances with the corresponding appearance of the $g = 4.1$ signal. It was assumed that the earlier report of IR-induced conversion of the spin $= 1/2$ state into the $g = 4.1$ state at 150 K (*36*) involves the transient formation of the new $g = 10/g = 6$ state (*37*).

The existence of a third form of S_2 may help to explain the absence of a $g = 4.1$ type signal in cyanobacteria. In this report, the effect of near-IR illumination on the Mn_4 cluster has been investigated in PSII isolated from the cyanobacterium *Synechococcus elongatus*. It is shown that IR light converts the Mn_4 cluster into a state which exhibits characteristic EPR signals from a spin $= 5/2$ state. This new state is spectroscopically comparable to the recently discovered spin $= 5/2$ signal in plant PSII, but its stability with temperature is quite different. As a result, it seems that the $g = 4.1$ state in cyanobacteria, should it exist, is unstable relative to the new state and cannot be trapped.

MATERIALS AND METHODS

Untreated oxygen evolving Photosystem II-enriched membranes from spinach were prepared as in (*37*). PSII core complexes from *S. elongatus* were isolated and purified as previously described (*38, 39*) with some modifications.

Essentially, after extraction of the thylakoid membranes the sucrose density gradient was omitted and PSII was purified by two HPLC column steps, yielding pure and homogeneous dimeric PSII core complexes. For the core complexes, the oxygen evolution activity using 2 mM DCBQ as electron acceptor was 2500−3000 μmol O_2/(h * mg Chl) at 25 °C. The preparation was frozen in MES buffer (20 mM, pH 6.5) containing 10 mM $CaCl_2$, 10 mM $MgCl_2$, 0.5 M mannitol, 0.03% β-DM and stored at −70 °C prior to use. The details of this preparation will be provided elsewhere (Kuhl et al., manuscript in preparation).

The PSII preparations were put in quartz EPR tubes (at 6−8 mg of Chl/mL for PSII from spinach and 3−4 mg Chl/mL for PSII from *S. elongatus*) and dark-adapted for 1 h at 0 °C. After the dark adaptation, PPBQ, dissolved in ethanol 95% (Carlo Erba), was added as an artificial electron acceptor. After the addition of PPBQ, the samples were immediately frozen in the dark at 200 K in a CO_2−ethanol bath. Then, the samples were degassed at 200 K and placed under a He atmosphere, then transferred to liquid nitrogen (77 K).

CW-EPR spectra were recorded at liquid helium temperatures with a Bruker ER 200D X-band spectrometer equipped with an Oxford Instruments cryostat.

Formation of the S_2-state was achieved by illumination of the samples with an 800 W tungsten lamp, light from which was filtered through water (which absorbs above 900 nm) and IR filters (cut off above 750 nm), in a nonsilvered dewar at 200 K (ethanol, solid CO_2). Above 80 K, near-IR illumination of the samples was done in a nitrogen gas flow system (Bruker, B-VT-1000). At and below 65 K, IR illumination of the sample was done in the EPR cavity. IR illumination was provided by a laser diode emitting at 813 nm (Coherent, diode S-81-1000C) with a power of 600 to 700 mW at the level of the sample. In all the conditions tested, the duration of the near-IR illumination (30−60 s) was adjusted to produce the spin state conversion in the maximum amount of reaction centers.

RESULTS

The spectra labeled a, in Figure 1, were recorded on dark-adapted PSII isolated from *S. elongatus*. The spectra labeled b were recorded after a 200 K illumination of the same sample. Spectra in Panel A were recorded at 10 K, while spectra in Panels B, C, and D were recorded at 15 K.

Spectrum a in Panel A exhibits features at $\approx$2230, $\approx$3030, and $\approx$4650 G which are the g_z, g_y, and g_x resonances, respectively, arising from low spin heme Fe^{3+} of oxidized cytochrome. The g_z value measured in the dark-adapted PSII ($g_z = 3.01$, Figure 1A) is higher than that found for the relaxed form of the low-potential $Cytb_{559}$ (*40*), and the amplitudes of the cytochrome signals in Figure 1A seem greater than expected. Although this must be verified by quantitative studies, it is possible that the signals may contain at least a contribution from $Cytc_{550}$ which is known to be associated with PSII in cyanobacteria (*41−45*) and is present in this preparation (Kuhl et al., manuscript in preparation). In spectrum a of Figure 1A, the signals below 1600 G arise from high-spin Fe^{3+}. At $\approx$1090 and $\approx$1575 G, the signals arise from high-spin Fe^{3+} which is present as a contaminant in most of the biological samples. The two other features

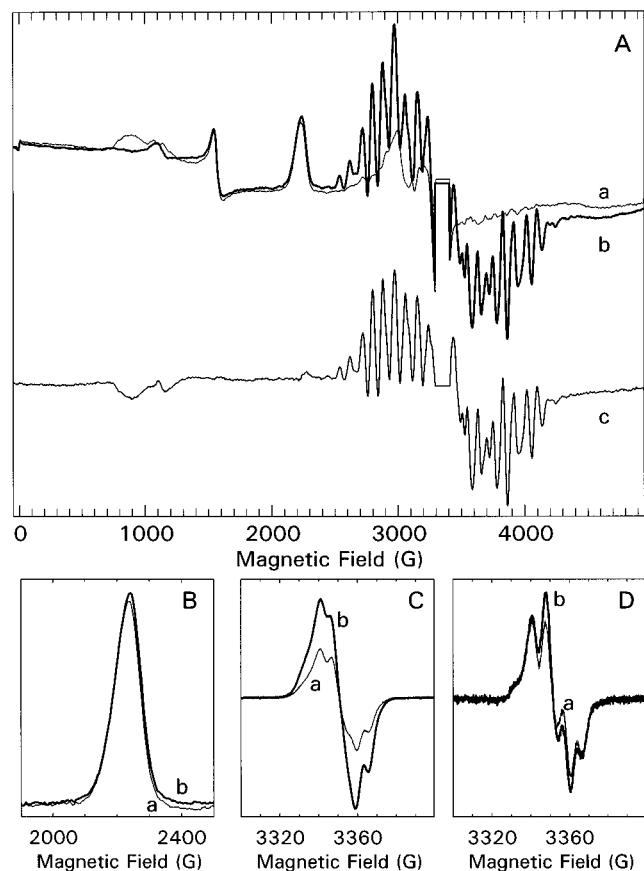FIGURE 1: Panel A; EPR spectra of PSII isolated from S. elongatus and recorded at 10 K. Spectrum a, dark-adapted sample. Spectrum b, sample illuminated at 200 K. Spectrum c is the difference spectrum: spectrum b *minus* spectrum a. Instrument settings: modulation amplitude, 25 G; microwave power, 20 mW; microwave frequency, 9.4 GHz; modulation frequency, 100 kHz. The central part of the spectra corresponding to the $Tyr_D^•$ region was deleted. Panel B: EPR spectra recorded at 15 K before (a) and after (b) the 200 K illumination. Instrument settings: modulation amplitude, 32 G; microwave power, 10 mW; microwave frequency, 9.4 GHz; modulation frequency, 100 kHz. Panels C and D: $Tyr_D^•$ spectra recorded at 15 K before (a) and after (b) the 200 K illumination. Instrument settings: modulation amplitude, 2.8 G; microwave power, 4 mW for Panel C and 0.63 μW for Panel D; microwave frequency, 9.4 GHz; modulation frequency, 100 kHz.

at $\approx$900 G ($g \approx 7.5$) and $\approx$1150 G ($g \approx 5.8$) originate from the oxidized form of the non-heme iron Q_AFe^{3+} (*46*). Indeed, even after a long dark-adaptation period, the electron acceptor side of PSII appeared still largely reduced (not shown). In these conditions, it is known that addition of PPBQ in the dark results in the oxidation of the reduced quinones and in the oxidation of the non-heme iron (*47*).

Spectrum b in Figure 1A was recorded after an illumination at 200 K. The changes induced by such an illumination are show in spectrum c of Figure 1A (spectrum c is the difference: spectrum b *minus* spectrum a). The 200 K illumination resulted in the formation of a normal spin = 1/2 S_2 multiline signal and in the disappearance of the signals which originate from the quinone-associated Fe^{3+} at $\approx$900 G ($g \approx 7.5$) and $\approx$1150 G ($g \approx 5.8$). Panel B in Figure 1 shows that the cytochrome g_z signal recorded under nonsaturating conditions is not significantly modified by the 200 K illumination. Panels C and D in Figure 1 show the $Tyr_D^•$ signal recorded under saturating and nonsaturating conditions, respectively, before (spectra a) and after (spectra b) the 200
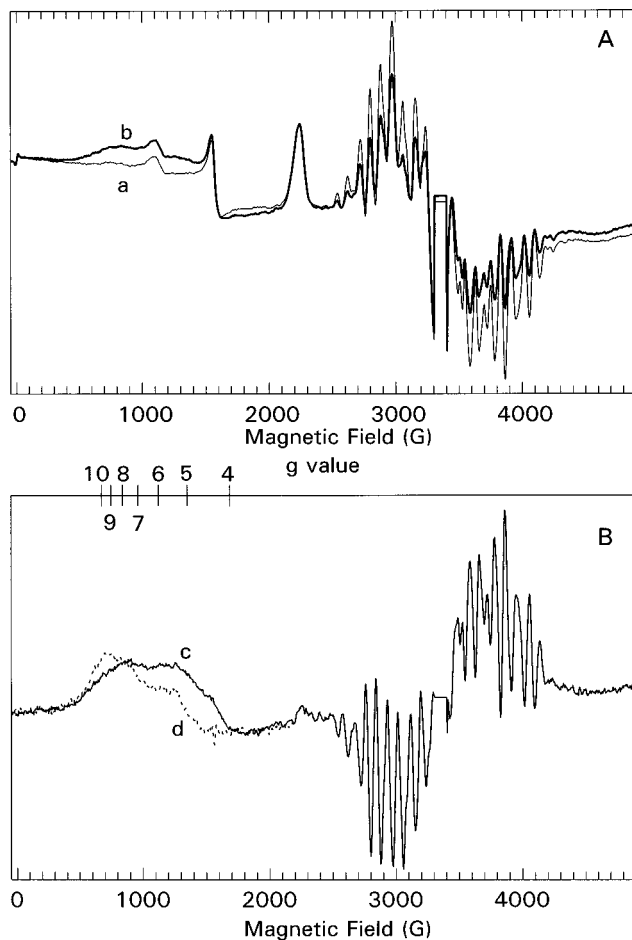

FIGURE 2: Spectrum a of this figure is identical to spectrum b in Figure 1A. Spectrum b was recorded after a further 813 nm illumination given at 150 K. Panel B shows the spectra obtained by subtracting the spectrum recorded before the IR illumination from that recorded after the IR illumination (spectrum c). Spectrum d (dotted line) corresponds to the difference spectrum obtained by IR illumination at 65 K of PSII isolated from spinach (*37*). Spectra c and d were scaled to the same multiline signal amplitude as estimated by a double integration. Instrument settings as in Figure 1A.

K illumination. In nonsaturating conditions (panel D), it can be seen that the 200 K illumination induced a free radical signal, usually attributed to a Chl^+ (*2*), in about 10% of the reaction centers. In saturating conditions, formation of the S_2-state results in the well-known saturation enhancement effect on $Tyr_D^•$ (*48*–*50*).

In summary, the PSII complex isolated from *S. elongatus* exhibits no significant difference with that characterized in PSII-enriched membranes from spinach with the exception of the redox state of the quinone-complex and the apparently greater amplitude of the cytochrome signal.

The effects of near-infrared illumination in the S_2-state of PSII isolated from *S. elongatus* are shown in Figure 2. Spectrum a (Panel A) was recorded in the S_2-state after the 200 K illumination (this spectrum is identical to spectrum b in Figure 1A). Spectrum b (Figure 2A) was recorded after a further illumination at 813 nm done at 150 K. Spectrum c in Figure 2B is the difference spectrum: spectrum b *minus* spectrum a. The signals which disappear upon IR illumination appear negative, and the IR-induced signals appear positive. Figure 2 shows that IR illumination at 150 K resulted in the disappearance of the spin = 1/2 multiline

signal in about 50% of the reaction centers and that signals with g values ≈ 8.5 and 5.5 are simultaneously induced. After a short incubation (a few seconds) at 200 K in the dark, the signals at $g \approx 8.5$ and 5.5 disappeared and the multiline signal was fully restored (not shown).

For comparison, spectrum d, in Figure 2B, shows the signals which are induced in PSII isolated from spinach by IR illumination below 77 K. Spectra c and d were normalized to the same amount of PSII susceptible to IR light. This was done using measurements of the double integral of the multiline signal which disappeared upon the IR illumination in both kinds of sample. From Figure 2B it appears that IR light induces similar but not identical signals in PSII from spinach and from *S. elongatus*. The resonances with g values ≈ 8.5 and 5.5, in spectrum c, are characteristics of a system arising from a spin $= 5/2$ state.

In PSII isolated from spinach, the $g = 4.1$ signal is the main signal which is induced by IR illumination at 170 K (*36, 37*). Below this temperature, the yield of the $g = 4.1$ signal formation decreased while the signals at $g = 10$ and 6 increased (*37*). The yield of the spin conversion (as estimated by the amplitude of the multiline signal which disappeared) is maximum at 150 K (*36, 37*) and decreases above and below this temperature. Similarly, the yield of the spin conversion (as estimated by the amplitude of the multiline signal which disappeared) is maximum at 150 K in PSII isolated from *S. elongatus* and decreases above and below this temperature (not shown).[2]

Upon raising the temperature above 77 K, the Mn_4-state responsible for the features at $g = 10$ and $g = 6$ in PSII isolated from spinach was converted to a state responsible for a $g = 4$ type signal (*37*). At higher temperatures, the state responsible for the $g = 4.1$ signal decayed to form the spin 1/2 state that is responsible for the multiline signal. In PSII isolated from *S. elongatus*, we were unable to detect a $g = 4.1$ signal formation irrespective of the temperature at which the IR illumination was performed (between 175 and 10 K)(not shown).

Figure 3 illustrates the relative stability of the $g > 5$ type signals in PSII isolated from *S. elongatus* at 150 K. In this experiment, the IR illumination was done at 150 K. Then, after different periods of incubation at 150 K in the dark, the samples were quickly transferred at 77 K. The closed squares correspond to the kinetic of the $g > 5$ signals to multiline signal transition in PSII isolated from *S. elongatus*, and the closed circles correspond to the kinetic of the $g = 4.1$ signal to multiline signal transition in PSII isolated from spinach. The state responsible for the $g = 4.1$ signal in PSII isolated from spinach is considerably less stable than the state responsible for the $g > 5$ type signals in PSII isolated from *S. elongatus*.

In metallic systems with a spin $\geq 5/2$ (and a small D value), the relative amplitudes of the resonances at $g > 4$
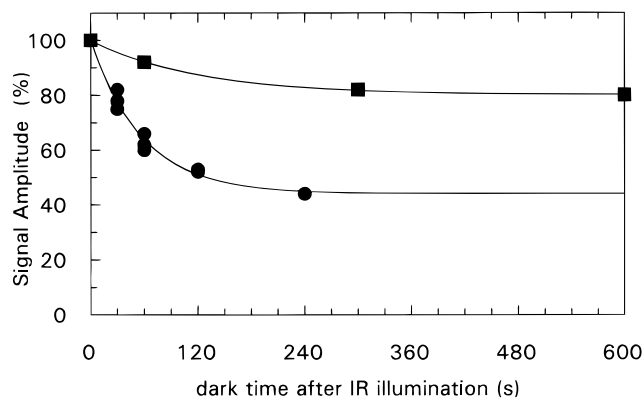

FIGURE 3: Amplitude of the $g > 5$ type signals in PSII isolated from *S. elongatus* (closed squares) and of the $g = 4.1$ signal in PSII isolated from spinach (closed circles) versus the time of incubation in the dark, at 150 K, following the IR illumination given at 150 K. The amplitude of the signals was normalized to 100% for $t \approx 2$ s (the estimate of the minimum time required to transfer the sample to 77 K after the dark incubation at 150 K).

are expected to be strongly temperature dependent. Figure 4 shows the temperature dependence of the signals induced at 50 K by IR light and recorded with a nonsaturating microwave power (i.e., 20 mW).[3] As expected, the amplitude of the $g \approx 8.5$ and $g \approx 5.5$ resonances varies significantly with the temperature at which they are recorded (from 4.2 to 30 K).

The new signal in *Synechococcus* is spectroscopically comparable to that detected in plant PSII after IR illumination below 77 K (*37*). In plant PSII, the signal was attributed to a S $= 5/2$ state and spectral simulations indicated a E/D ratio of -0.05 with $|D| \leq 1$ cm^{-1}. The resonances at $g = 10$ and $g = 6$ corresponded to the g_z of the $\pm 5/2$ and $\pm 3/2$ transition, respectively. In the same way, the IR-induced spectrum in *Synechococcus* has been tentatively simulated assuming that it arises from a spin 5/2 state (*37, 51–53*). From the field position of the observed resonances, a good simulation can be obtained with a $|E/D|$ ratio of 0.13 which gives the g values for the three transitions shown in the inset of Figure 4.

The g_z of the $-5/2$ to $+ 5/2$ transition which is the most intense signal in PSII from spinach (*37*) (see also the dotted lines in Figure 2 and Figure 4) is not detected in PSII from *S. elongatus*. A similar situation has been reported earlier in polycrystalline samples of Fe^{3+}-containing ferrichrome A (*52*), where the $\pm 5/2$ transition (i.e., the ground-state transition) was not seen at 4.2 K, but was observed at 1.4 K. Presumably, this is due to relaxation effects. The lack of a $g = 10$ signal precludes the estimate of the D value. Nevertheless, the spectrum recorded at 4.2 K indicates that the $\pm 3/2$ and $\pm 1/2$ sublevels are significantly populated and that the D value is small (<3 cm^{-1}).

DISCUSSION

The results presented in this work show that in PSII isolated from *S. elongatus*, the spin $= 1/2$ state responsible for the S_2-multiline EPR signal is converted to a state that

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[2] The possibility of a warming of the sample by strong IR light may be considered despite the weak absorption at 813 nm of PSII and H_2O. Nevertheless, the temperature dependence of the spin state conversions were tested and found similar (i) in experiments performed in D_2O (which absorbs at longer wavelengths than H_2O does) and (ii) by reducing the IR intensities and increasing the time of illumination correspondingly (not shown). In experiments in which the temperature of the sample is maintained by a low flow of gas, an increase of the temperature by 10–15 K during the IR illumination may be observed.

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[3] In PSII from spinach, at 4.2 K, the $g = 4.1$ signal induced by IR illumination has a half-saturation at a microwave power ≈ 1.1 mW, and the signal at g ≈ 9 has a half-saturation at a microwave power ≥ 40 mW (not shown).

g value

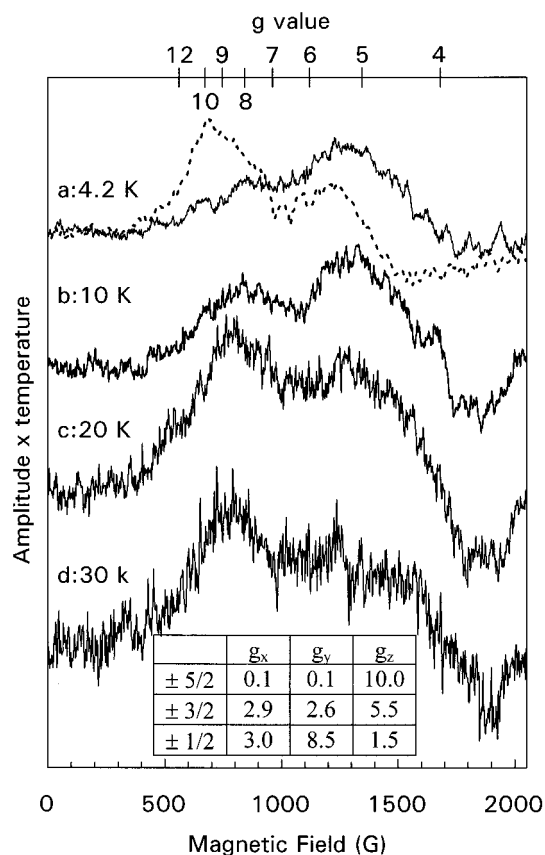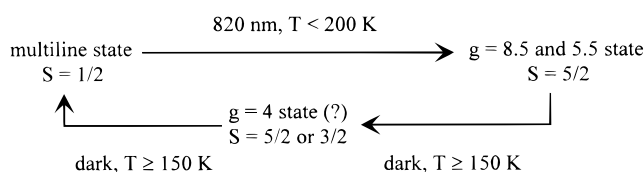|	g_x	g_y	g_z
± 5/2	0.1	0.1	10.0
± 3/2	2.9	2.6	5.5
± 1/2	3.0	8.5	1.5

FIGURE 4: Spectra obtained by subtracting the spectrum recorded before the IR illumination from that recorded after the IR illumination given at 50 K directly in the EPR cavity. The EPR spectra were recorded at 4.2 K (spectrum a), at 10 K (spectrum b), at 20 K (spectrum c), at 30 K (spectrum d). The amplitude of the spectra has been multiplied by the temperature value. Same instrument settings as in Figure 1A. The inset shows some of the parameters used in the simulation. Spectrum with a dotted line corresponds to the difference spectrum measured at 4.2 K and obtained by IR illumination at 65 K of PSII isolated from spinach (*37*) (spectrum a and spectrum with a dotted line are normalized so that the amplitude of the multiline signals measured at 10 K and which disappeared upon IR illumination are equivalent as estimated by double integration).

1) PSII of cyanobacteria

820 nm, T < 200 K

multiline state $\longrightarrow$ g = 8.5 and 5.5 state
S = 1/2   S = 5/2

g = 4 state (?)
S = 5/2 or 3/2

dark, T ≥ 150 K   dark, T ≥ 150 K

2) PSII of plants

820 nm, T ≤ 77 K

multiline state $\longrightarrow$ g = 10 and 6 state
S = 1/2   S = 5/2

g = 4 state
S = 5/2 or 3/2

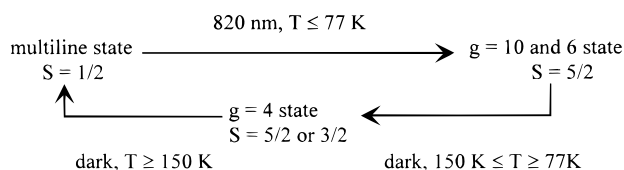dark, T ≥ 150 K   dark, 150 K ≤ T ≥ 77K

FIGURE 5: Relationship between the different states of S_2 in PSII from plants (Scheme 2) and cyanobacteria (Scheme 1).

PSII preparations, the structural differences in the Mn_4 cluster which are at the origin of the two different S_2-multiline signals (the spectral differences and the difference in stability) are not manifest in the spectra of the high spin states of the manganese complex. It is shown here that the structural differences in the Mn_4 cluster which are at the origin of the different signals at g > 4 (the spectral differences and the difference in stability) in PSII from spinach and PSII from *S. elongatus* are not manifest in the S = 1/2 multiline signal which seems very similar in both PSII preparations.

The stability of the spin = 5/2 state in cyanobacteria is quite different from that in plant PSII. In plant PSII, the spin 5/2 state is formed below 77 K, converts to the g = 4.1 state at temperatures above 77K, and the g = 4.1 signal in turn converts to the spin 1/2 multiline signal at temperatures of 150 K and above (*37*). By contrast, in *S. elongatus*, the spin = 5/2 state is formed by IR illumination at temperatures ≤ 150 K and decays directly to the state responsible for spin 1/2 multiline signal. Moreover, the spin 5/2 state in *S. elongatus* is more stable at 150 K than is the state responsible for the g = 4.1 signal in spinach. Should a g = 4.1 state exist in *S. elongatus* comparable to that in PSII of plants, its stability at 150 K relative to the spin 5/2 state would render it undetectable. Overall then these observations provide an explanation why the g = 4.1 signal has remained undetectable in cyanobacterial PSII.

Extending this explanation, we propose that the biochemical procedures which are known to favor formation of the state responsible for the g = 4.1 signal in plant PSII probably give rise to the g = 8.5/g = 5.5 state in cyanobacteria. This should be verifiable experimentally.

Figure 5 summarizes the relationship between the different states of S_2 as discussed above. Scheme 2 of Figure 5 for plant PSII is similar to that given earlier (*37*) while Scheme 1 of Figure 5, for cyanobacteria, is derived from that. The presence of an intermediate responsible for a g = 4.1 signal is proposed in cyanobacteria by analogy to Scheme 2, although no experimental evidence for this state exists. It is predicted that this state will not be detectable unless the rate constants are perturbed by, for example, some kind of biochemical modification of the cluster. For both plants and cyanobacterial PSII, it is assumed that at temperatures of 200 K and higher these reactions occur upon absorption of

gives rise to EPR signals with g values above g = 5 upon excitation with near-IR radiation given at temperatures lower than 200 K. The formation of a g = 4 type signal was not detected under any of the conditions used in this work.

The new signal is spectroscopically comparable to the spin 5/2 signal recently reported in plant PSII (*37*). The IR-induced signals reported here are characteristic of resonances arising from a spin = 5/2 state. Spectral simulations indicate a |*E/D*| ratio of 0.13. The resonances at g = 8.5 and g = 5.5 correspond to the g_y of the ± 1/2 transition and to the g_z of the ± 3/2 transition, respectively. The value of the *E/D* ratio for this state found in PSII isolated from *S. elongatus* is more rhombic than that found for the spin = 5/2 state in PSII from *spinach*, for which we found a *E/D* ratio equal to − 0.05 (*37*). This difference would imply, for example, differences in the ligand environment structure of the Mn_4 cluster.

We have shown previously (*37*) that IR illumination of Ca^{2+}-depleted/EGTA-treated/polypeptides-reconstituted PSII at 50 K and of Sr^{2+}-reconstituted PSII at 65 K resulted in the same EPR spectra. This suggested that, in these two

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IR light by the Mn cluster but that the multiline state is the stable state and that the other states are only transiently formed.

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