Ammonia Binds to the Manganese Site of the O_2-Evolving Complex of Photosystem II in the S_2 State

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Abstract: Binding of NH_3 to the S_2 state of the O_2-evolving complex of photosystem II (PSII) causes a structural change in the Mn site that is detectable with low-temperature electron paramagnetic resonance (EPR) spectroscopy. Untreated spinach PSII membranes at pH 7.5 produce a S_2 state multiline EPR spectrum when illuminated at either 210 K or at 0 °C in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) having an average hyperfine line spacing of 87.5 G. The temperature dependence of the S_2 state multiline EPR signal observed from untreated samples deviates from the Curie law above 5 K, with a maximum signal intensity at 6.9 K as has been previously observed. In contrast, 100 mM NH_4Cl-treated PSII membranes at pH 7.5 exhibit a new S_2 state EPR spectrum when illuminated at 0 °C in the presence of DCMU. The novel S_2 state EPR spectrum from NH_4Cl-treated PSII membranes has an average hyperfine line spacing of 67.5 G and a temperature dependence obeying the Curie law except for small deviations at low temperature. We assign the new S_2 state EPR signal from NH_4Cl-treated PSII membranes to a form of the S_2 state having one or more NH_3 molecules directly coordinated to the Mn site. NH_3 does not bind to Mn in the dark-stable S_1 state present before illumination, since generation of the S_2 state in NH_4Cl-treated PSII membranes by illumination at 210 K does not yield the new S_2 state EPR spectrum. Since inhibition of O_2 evolution activity in the presence of NH_4Cl probably occurs through binding of NH_3 to the O_2-evolving complex in competition with substrate H_2O molecules, these results indicate that the EPR-detectable Mn site functions as the substrate-binding site of the O_2-evolving complex.

The mechanism of photosynthetic O_2 evolution involves the accumulation of four oxidizing equivalents by the O_2-evolving complex, a membrane-bound enzyme complex that contains four Mn ions.[1] Sequential absorption of photons by the photosystem II (PSII) reaction center advances the O_2-evolving complex through its five oxidation states S_i, $i = 0$ through 4, with release of an O_2 molecule occurring with the conversion of the S_4 state to the S_0 state. The intermediate oxidation states S_2 and S_3 back-react during dark adaptation to form the dark-stable S_1 state.[2] The S_0 state is slowly oxidized in the dark to form the S_1 state through an undetermined mechanism.[3] The function of the bound Mn ions required for photosynthetic O_2 evolution activity[1] remains undetermined. Several possible functions for Mn ions have been suggested, among which are storage of oxidizing equivalents, binding and oxidation of substrate H_2O molecules, and electron transport on the donor side of PSII.[4]

Electron paramagnetic resonance (EPR) studies at low temperature have shown that the S_2 state exhibits a complex, multiline EPR signal with hyperfine line spacings characteristic of Mn ions in a binuclear or tetranuclear exchange-coupled configuration.[5-13] The S_2 state EPR signal produced by a series of light flashes followed by rapid freezing shows a period four intensity dependence on the number of flashes with the maximum yield observed after the first flash.[5,9] This EPR behavior is consistent with one function of the Mn site being the accumulation of oxidation equivalents. This interpretation is further supported by X-ray absorption edge studies which show oxidation of the Mn site in the S_1 to S_2 state transition.[14] These experiments do not, however, answer the question of the location of the substrate-binding site.

Most of the suggestions that the Mn site is the H_2O-binding site have been based on the observed inhibition of O_2 evolution activity by amines, which might be expected to coordinate to metal ions in the O_2-evolving complex. Sandusky and Yocum[15] demonstrated that a variety of primary amines inhibit O_2 evolution by competing with Cl^- for a common site on the O_2-evolving complex; depletion of Cl^- from PSII reversibly inhibits O_2 evolution activity.[16] Sandusky and Yocum[15] also found that only NH_3 binds additionally to a different site. They proposed that the amine/Cl^--binding site and the NH_3-specific site were both on the Mn site. Radmer and Ollinger[17] concluded from results of mass spectrometric measurements that hydroxylamine and substituted hydroxylamines bind to Mn and proposed a model for the shape of the H_2O-binding site based on steric arguments. Unfortunately, no direct spectroscopic evidence exists showing that inhibition of O_2 evolution by H_2O analogues occurs through binding directly to the Mn site. The results of Velthuys' luminescence measurements,[18] however, indicate that NH_3 binds to the O_2-evolving complex specifically in the S_2 and S_3 states, which associates specific changes in the oxidation state of the Mn site with binding of inhibitors.

If the Mn site is the substrate-binding site of the O_2-evolving complex, then the structure of the Mn site should be sensitive to

(1) (a) For a review, see: Amesz, J. *Biochim. Biophys. Acta* **1983**, *726*, 1-12. (b) Yocum, C. F.; Yerkes, C. T.; Blankenship, R. E.; Sharp, R. R.; Babcock, G. T. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 7507-7511. (c) Murata, N.; Miyao, M.; Omata, T.; Matsunami, H.; Kuwabara, T. *Biochim. Biophys. Acta* **1984**, *765*, 363-369.

(2) Joliot, P.; Kok, B. In *Bioenergetics in Photosynthesis*; Govindjee, Ed.; Academic Press, New York, 1975; pp 387-412.

(3) (a) Vermaas, W. F. J.; Renger, G.; Dohnt, G. *Biochim. Biophys. Acta* **1984**, *764*, 194-202. (b) Hanssum, B.; Dohnt, G.; Renger, G. *Biochim. Biophys. Acta* **1985**, *806*, 210-220.

(4) (a) Velthuys, B. R. *Annu. Rev. Plant. Physiol.* **1980**, *31*, 545-567. (b) Wydrzynski, T. J. In *Photosynthesis: Energy Conversion by Plants and Bacteria*; Govindjee, Ed.: Academic Press, New York, 1982; Vol 1, pp 469-506.

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

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

(7) Brudvig, G. W.; Casey, J. L.; Sauer, K. *Biochim. Biophys. Acta* **1983**, *723*, 366-371.

(8) Brudvig, G. W.; Casey, J. L.; Sauer, K. In *The Oxygen Evolving System of Photosynthesis*; Inoue, Y., et al., Eds.: Academic Press: Tokyo, 1983; pp 159-164.

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

(10) Casey, J. L.; Sauer, K. *Biochim. Biophys. Acta* **1984**, *767*, 21-28.

(11) Beck, W. F.; de Paula, J. C.; Brudvig, G. W. *Biochemistry*, **1985**, *24*, 3035-3043.

(12) de Paula, J. C.; Brudvig, G. W. *J. Am. Chem. Soc.* **1985**, *107*, 2643-2648.

(13) de Paula, J. C.; Innes, J. B.; Brudvig, G. W. *Biochemistry* **1985**, *24*, 8114-8120.

(14) Goodin, D. B.; Yachandra, V. K.; Britt, R. D.; Sauer, K.; Klein, M. P. *Biochim. Biophys. Acta* **1984**, *767*, 209-216.

(15) (a) Sandusky, P. O.; Yocum, C. F. *FEBS Lett.* **1983**, *162*, 339-343. (b) Sandusky, P. O.; Yocum, C. F. *Biochim. Biophys. Acta* **1984**, *766*, 603-611.

(16) Kelley, P.; Izawa, S. *Biochim. Biophys. Acta* **1978**, *502*, 198-210.

(17) (a) Radmer, R. *Biochim. Biophys. Acta* **1979**, *546*, 418-425. (b) Radmer, R.; Ollinger, O. *FEBS Lett.* **1982**, *144*, 162-166. (c) Radmer, R.; Ollinger, O. *FEBS Lett.* **1983**, *152*, 39-43.

(18) Velthuys, B. R. *Biochim. Biophys. Acta* **1975**, *396*, 392-401.

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binding of NH_3 in the S_2 state. In this paper, we have used low-temperature EPR spectroscopy to monitor the structure and magnetic properties of the Mn site in the presence of NH_3. Because Ghanotakis et al.[19] found that the dependence of the inhibition constant for O_2 evolution measured in the presence of various amines was proportional to the basicity of the amine, it is likely that NH_3 coordinates to Mn in the free base form, requiring studies to be performed in alkaline media. We first address the effects on the S_2 state EPR signal caused by changing the pH from 6.0 to 7.5, since our previous studies[11-13] were performed at pH 6.0. Next, we demonstrate that binding of NH_3 to the O_2-evolving complex causes large changes in the S_2 state multiline EPR signal trapped after illumination at 0 °C, indicating that NH_3 probably binds directly to the Mn site in the S_2 state. However, NH_3 does not perturb the S_2 state EPR signal if the S_2 state is produced by illumination at 210 K, a temperature at which ligand exchange is inefficient. These results demonstrate that coordination of NH_3 to the Mn site occurs after formation of the S_2 state. Our findings provide direct spectroscopic evidence that the Mn site of the O_2-evolving complex exchanges ligands, which suggests that the Mn site binds substrate H_2O molecules during the turnover of the O_2-evolving complex. More importantly, the finding that the structure of the Mn site in the O_2-evolving complex can be affected in an EPR-detectable manner by the binding of NH_3 raises new possibilities for study of the coordination chemistry of the Mn site. Such studies may be expected to provide considerable insight into the structure and function of the Mn site on the electron donor side of PSII.

Experimental Section

Materials. $^{15}NH_4Cl$, with a stated isotopic purity of 99 atom % ^{15}N, was used as received from MSD Isotopes. 2,5-Dichloro-*p*-benzoquinone (DCBQ), from Eastman Kodak, was recrystallized twice from 95% ethanol, and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), obtained from Sigma, was recrystallized four times from 95% ethanol before use. DCBQ and DCMU solutions were prepared in 95% ethanol immediately prior to use. 2-(*N*-Morpholino)ethanesulfonic acid (MES) and *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid (HEPES), from Research Organics, and Triton X-100, from Sigma, were used as received.

Methods. PSII membranes were isolated from market spinach and assayed as described previously[11] and were stored at 77 K suspended at 5 mg of chlorophyll (Chl)/mL in a buffer solution containing 20 mM MES–NaOH, 15 mM NaCl, and 30% (v/v) ethylene glycol, pH 6.0. All steps in the isolation procedure were performed in darkness. The PSII membrane preparations used in these experiments evolved O_2 at rates between 450 and 800 μmol of O_2 (mg of Chl h)$^{-1}$.

We previously showed that the hyperfine line pattern and magnetic properties of the S_2 state multiline EPR spectrum produced by continuous low-temperature (150–240 K) illumination strongly depended on the length of time that PSII membranes were dark adapted at 0 °C before freezing.[11,12] The PSII membranes prepared for use in this study had experienced a total of 6 h of darkness at 0 °C during the isolation procedure. With use of our previously introduced nomenclature, a homogeneous population of the resting state configuration of the O_2-evolving complex is found in such extensively dark-adapted PSII membrane samples.[11,12] The oxidation state of the O_2-evolving complex in these preparations is exclusively the S_1 state.[3] All experiments discussed in this paper were performed with PSII membranes that were not preilluminated before treatment with reagents.

PSII membranes which had been stored at 77 K at pH 6.0 were thawed on ice for 30 min and then equilibrated in a solution containing 20 mM HEPES–NaOH, 15 mM NaCl, and 30% (v/v) ethylene glycol, pH 7.5 (suspension buffer), through two resuspension and centrifugation cycles. The resulting pellet of PSII membranes was resuspended in the suspension buffer to the desired concentration for EPR experiments. All samples contained 250 μM DCBQ and 50 μM DCMU added from 20 mM solutions in ethanol, unless otherwise noted. NH_4Cl-treated samples were made by adding a 1.25 M solution of NH_4Cl in the suspension buffer to the PSII membranes to obtain a final concentration of 100 mM. The EPR samples were frozen in liquid N_2 following incubation on ice for 10 min after treatment. All of the steps used in preparing the EPR samples were conducted in darkness.

(19) Ghanotakis, D. F.; O'Malley, P. J.; Babcock, G. T.; Yocum, C. F. In *The Oxygen Evolving System of Photosynthesis*; Inoue, Y., et al., Eds.; Academic Press: Tokyo, 1983; pp 91–101.

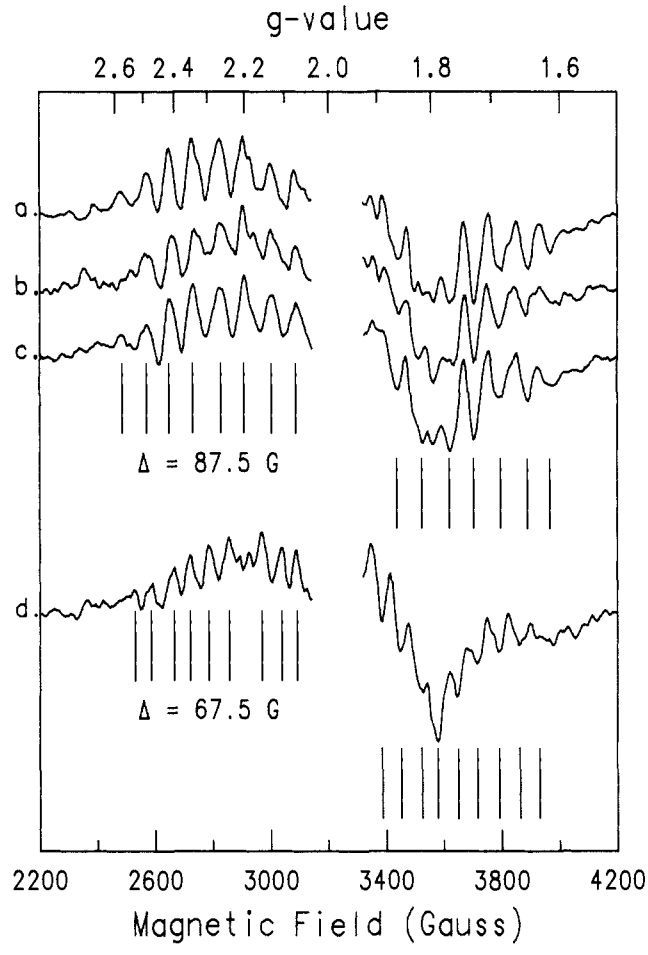

Figure 1. Comparison of S_2 state EPR spectra produced in NH_4Cl-treated and untreated PSII membranes at pH 7.5. The spectra shown are the difference between the spectrum after illumination and the spectrum of the same dark-adapted sample. The $g = 2.0$ region, which is interfered with by EPR Signal II_s, is not shown. EPR spectrometer conditions: microwave frequency 8.92 GHz; microwave power 200 μW; field modulation frequency 100 kHz; field modulation amplitude 20 G; temperature 8.0 K. PSII membranes (5 mg Chl/mL) were treated with 50 μM DCMU and 250 μM DCBQ: (a) untreated PSII membranes, illuminated for 120 s at 210 K; (b) 100 mM NH_4Cl-treated PSII membranes, illuminated for 120 s at 210 K; (c) untreated PSII membranes, illuminated for 30 s at 0 °C; (d) 100 mM NH_4Cl-treated PSII membranes, illuminated for 30 s at 0 °C. The vertical lines below spectra c and d show the positions of the hyperfine lines; Δ is the average of the hyperfine line spacings for the indicated spectrum. Each spectrum is the average of two scans.

The S_2 state EPR spectrum was generated by using the continuous illumination method previously employed with PSII membrane samples at pH 6.0.[11,12] The presence of DCMU in the samples ensured that the S_2 state was formed in high yield from the S_1 state initially present.[11] Samples that were illuminated at 0 °C were equilibrated at 0 °C in the dark for 3 min before illumination, illuminated for 30 s, and then rapidly cooled in the dark in a solid CO_2/ethanol bath followed by cooling to 77 K in liquid N_2. Samples that were illuminated at 210 K were equilibrated at 210 K in the dark for 3 min before illumination, illuminated for 120 s, and then cooled in the dark in liquid N_2. EPR spectra were obtained at 4–25 K with the instrumentation previously described.[11,12] Difference spectra (light minus dark) were obtained through computer subtraction of the dark background spectrum from the post-illumination spectrum obtained under the same measurement conditions.

Results

In Figure 1, the S_2 state EPR spectra produced by continuous illumination at 0 °C and at 210 K in both untreated and 100 mM NH_4Cl-treated PSII membranes are compared. The spectra are characterized by a series of hyperfine lines spread over nearly a 2000 G magnetic field range centered at $g = 2.0$. Superimposed on the hyperfine line pattern from the S_2 state multiline EPR spectrum is an EPR signal with a turning point at approximately

$g = 1.9$ from the reduced primary quinone electron acceptor of PSII, Q_A^-, which is magnetically coupled to a Fe^{2+} ion.[20] Also present, but not shown in Figure 1, is a radical EPR signal at $g = 2.0$ known as EPR Signal II_s, which is attributed to a radical plastoquinone cation species having an unknown function.[19] The intensity of the S_2 state multiline EPR spectra produced by illumination at 210 K of PSII membranes at pH 7.5 was comparable to the intensity produced in the same PSII membrane preparation at pH 6.0 (data not shown), in accord with the previous findings of Damoder and Dismukes.[21] Moreover, the number, position, and relative intensity of the hyperfine lines of the S_2 state multiline EPR spectrum from untreated PSII membranes at pH 7.5 are the same as those of the spectra produced in PSII membranes at pH 6.0 with the same procedure. This result shows that there is no significant structural change in the Mn site between pH 6.0 and 7.5. In support of this conclusion are the data in Figure 3a which show that the temperature dependence of the S_2 state EPR signal produced in PSII membranes at pH 7.5 is exactly the same as that reported previously for PSII membranes at pH 6.0.[12] Hence, the methods for the photochemical generation of the S_2 state which were used previously at pH 6.0[11-13] are equally applicable to studies at pH 7.5.

We also show in Figure 1 that the S_2 state multiline EPR signal produced by illumination of untreated samples at 0 °C (Figure 1c) is identical to the S_2 state multiline EPR signal produced by illumination of untreated samples at 210 K (Figure 1a). This finding shows that the S_2 state can be produced in high yield with continuous illumination at temperatures as high as 0 °C as long as DCMU is present to block further S-state advancement. Therefore, it is possible to monitor the binding of inhibitors specifically to the S_2 state by comparing the S_2 state EPR signal produced at temperatures at which ligand exchange cannot occur (210 K, for example) with the S_2 state EPR signal produced at 0 °C, a temperature at which ligand exchange can occur. We have employed this method to determine the effects of NH_4Cl treatment on the S_2 state EPR signal.

The S_2 state EPR spectra formed in 100 mM NH_4Cl-treated PSII membrane samples at pH 7.5 depend strongly on the illumination temperature. Figure 1b shows the spectrum produced in 100 mM NH_4Cl-treated samples illuminated at 210 K. Although slightly reduced in intensity compared to the control spectrum in Figure 1a, the hyperfine line pattern exactly matches that observed in untreated samples. In contrast, the spectrum shown in Figure 1d from 100 mM NH_4Cl-treated PSII membranes illuminated at 0 °C differs markedly from the S_2 state EPR spectrum observed in untreated PSII membrane samples; the observed average spacing of the hyperfine lines is reduced to 67.5 G compared to the average spacing of 87.5 G observed in untreated samples. The average line width of the hyperfine lines is similarly reduced in the NH_4Cl-treated sample's S_2 state EPR spectrum compared to the spectrum from the control sample (34.0 and 44.6 G, respectively). Further, more hyperfine lines are resolved in the S_2 state EPR signal from NH_4Cl-treated samples. We have indicated with vertical lines 18 hyperfine lines in the spectrum shown in Figure 1d, which is to be compared to the 15 hyperfine lines of comparable relative intensity in the control spectrum shown in Figure 1c. It is reasonable to expect that there are additional hyperfine lines in the $g = 2.0$ region not shown in the spectra in Figure 1 due to interference from EPR Signal II_s. Thus, the addition of 100 mM NH_4Cl to PSII membrane samples causes a change in the number and spacing of the hyperfine lines of the S_2 state EPR spectrum, but this change is observed only if the S_2 state is generated at 0 °C.

The changes in the S_2 state EPR spectrum produced by illumination of PSII membranes at 0 °C in the presence of NH_4Cl suggest that one or more NH_3 molecules bind directly to the Mn site under these conditions. The observation that illumination of PSII membranes in the presence of NH_4Cl at 210 K produces

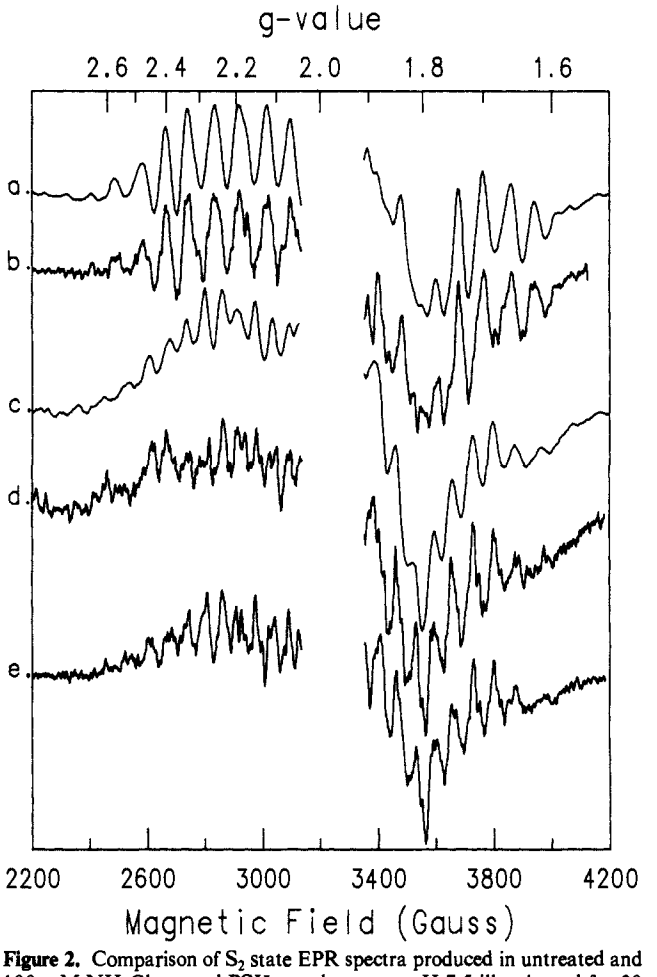

Figure 2. Comparison of S_2 state EPR spectra produced in untreated and 100 mM NH_4Cl-treated PSII membranes at pH 7.5 illuminated for 30 s at 0 °C. The spectra shown are the difference between the spectrum after illumination and the spectrum of the same dark-adapted sample. EPR spectrometer conditions are as in Figure 1 except as noted below. PSII membranes (20 mg Chl/mL) were treated with 200 μM DCMU and 250 μM DCBQ and were suspended in the presence of 0.05% (w/v) Triton X-100: (a) untreated PSII membranes, 20 G field modulation amplitude; (b) as in spectrum a, 8 G field modulation amplitude; (c) $^{15}NH_4Cl$-treated PSII membranes, 20 G field modulation amplitude; (d) $^{14}NH_4Cl$-treated PSII membranes, 8 G field modulation amplitude; (e) as in spectrum c, 8 G field modulation amplitude. Spectra recorded at 20 G field modulation amplitude were the average of two scans; spectra recorded at 8 G field modulation amplitude are the average of four scans, with the spectrum being recorded in two 1000-G sections.

a normal S_2 state EPR spectrum (Figure 1b) can be explained if ligand exchange proceeds slowly at this temperature. Further, it is clear that NH_3 does not bind to Mn in the S_1 state since incubation of PSII membranes at 0 °C in the presence of NH_4Cl for as long as 30 min in the dark failed to induce an altered S_2 state multiline EPR spectrum when samples were illuminated at 210 K (data not shown). Increasing the NH_4Cl concentration from 100 to 200 mM had little further effect on the S_2 state EPR spectrum produced by illumination at 0 °C (data not shown), suggesting that the O_2-evolving complex is nearly saturated with NH_3 when 100 mM NH_4Cl is present. Inhibition of O_2 evolution activity by NH_4Cl at pH 7.5 also occurs in the 100 mM concentration range.[15]

Binding of NH_3 to the Mn site could cause additional hyperfine structure in the S_2 state EPR spectrum due to the presence of a ^{14}N nuclear hyperfine interaction. S_2 state EPR spectra from untreated PSII membrane preparations show additional hyperfine structure when recorded at lower field modulation amplitude, as is shown in Figure 2, a and b. Similar results were obtained previously with untreated spinach thylakoid membranes,[5,8] but lower signal-to-noise ratios were obtained. The structure resolved at higher resolution in S_2 state EPR spectra from untreated

(20) Rutherford, A. W.; Zimmermann, J. L. *Biochim. Biophys. Acta* **1984**, *767*, 168–175.
(21) Damoder, R.; Dismukes, G. C. *FEBS Lett.* **1984**, *174*, 157–161.

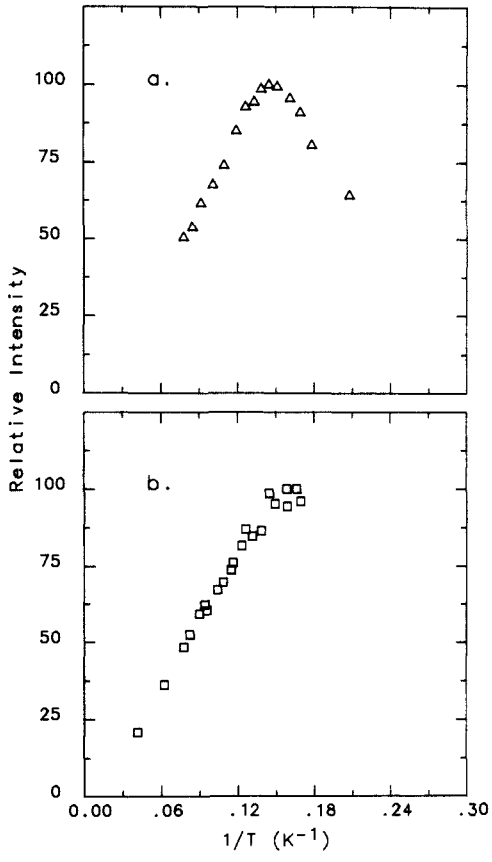

Figure 3. Comparison of the temperature dependences of the S_2 state EPR spectra produced in (a) untreated and (b) 100 mM $^{15}NH_4Cl$-treated PSII membranes by illumination for 30 s at 0 °C. EPR spectrometer conditions were as described in Figure 1, except the the microwave power was at 25 μW. The S_2 state EPR spectra for the PSII membrane samples used for these measurements are shown in Figure 2a,c. The intensity of the S_2 state EPR spectrum was determined by the sum of the peak-to-peak heights of the hyperfine lines marked in Figure 1c for the untreated sample and marked in Figure 1d for the $^{15}NH_4Cl$-treated sample.

preparations has been previously attributed to nuclear hyperfine interactions from endogenous ligands of the Mn site.[8] We compared the spectra from $^{14}NH_4Cl$-treated and $^{15}NH_4Cl$-treated samples illuminated at 0 °C in order to determine if a ^{14}N or a ^{15}N nuclear hyperfine interaction from bound NH_3 molecules could be resolved. These results are shown in Figure 2, d (100 mM $^{14}NH_4Cl$) and e (100 mM $^{15}NH_4Cl$), which were recorded with an 8 G field modulation amplitude. A comparison of the two spectra reveals few, if any, differences in the overall hyperfine pattern. Recording the spectra at still lower field modulation amplitudes (as low as 4 G, data not shown) did not resolve additional structure, showing that the ^{14}N and ^{15}N nuclear hyperfine couplings in the spectra of NH_4Cl-treated PSII membranes are too small to be resolved. It also appears that the line widths of individual hyperfine lines are unchanged in Figure 2d,e. The additional hyperfine structure resolved in Figure 2d, may then be attributed to either ^{55}Mn nuclear hyperfine interactions or hyperfine interactions with endogenous nuclei.

In Figure 3, the temperature dependence of the S_2 state multiline EPR spectra produced by illumination at 0 °C of both untreated (Figure 3a) and 100 mM NH_4Cl-treated (Figure 3b) PSII membranes are compared. The S_2 state multiline EPR spectrum from the untreated sample exhibits a temperature dependence deviating from the Curie law, having a maximum intensity at 6.9 K when recorded under nonsaturating conditions (Figure 3a). As mentioned above, the temperature dependence of the S_2 state multiline EPR spectrum produced by illumination at 0 °C of PSII membrane samples at pH 7.5 is identical with that previously observed for PSII membrane preparations at pH 6.0. In order to account for the observed maximum in the temperature dependence of the S_2 state multiline EPR spectrum from untreated (pH 6.0 or 7.5) PSII membranes, the EPR signal must arise from an excited $S = {}^1/_2$ state populated from lower lying states as the temperature increases.[12] In contrast, the S_2 state multiline EPR spectrum from the NH_4Cl-treated sample in Figure 3b exhibits a temperature dependence without a maximum when recorded under nonsaturating conditions (Figure 3b), suggesting that the spectrum originates from a low-lying or ground $S = {}^1/_2$ state. The slight degree of curvature at the lower temperatures of the temperature dependence plot for the NH_4Cl-treated sample may be due to the presence of lower lying states which depopulate the EPR active level as the temperature is lowered. A similar temperature dependence was previously observed for the S_2 state EPR signal produced by illumination at 160 K of PSII membranes in the active state.[12] It was concluded in that case that the energy gap was smaller between the EPR-active $S = {}^1/_2$ excited state and the ground state such that the system was in the high temperature limit at temperatures above 5 K.[12]

Discussion

The presence of NH_3 alters the hyperfine line spacing and temperature dependence of the S_2 state multiline EPR signal, but only when the S_2 state is generated at temperatures high enough to allow facile ligand exchange. These results demonstrate that ligand exchange occurs during the S_1 state to S_2 state transition, altering the environment of the Mn site. Velthuys[18] has previously argued, based on luminescence experiments, that NH_3 does not bind to the S_1 state but does bind to both the S_2 and S_3 states. He also showed that the redox potential of the S_2 and S_3 states is altered in the presence of NH_3, as evidenced by the lengthening of the lifetimes of the S_2 and S_3 states. Thus, binding of NH_3 to the O_2 evolving complex occurs in a specific manner dependent on the oxidation state of the Mn site and affecting the redox potential and structure of the Mn site. Although the possibility exists that NH_3 binds to a distinct, EPR-silent site that strongly influences the environment of the Mn site, it is more likely that the observed effects on the properties of the Mn site in the S_2 state are caused by ligation of one or more NH_3 molecules directly to the Mn site.

The binding of NH_3 to the O_2-evolving complex inhibits electron transport from H_2O to the primary electron donor of PSII.[22] Considering the analogy between the structures of H_2O and NH_3, we propose that NH_3 binds to the water-oxidizing site in the S_2 state and that this substrate-binding site is located on the EPR-detectable Mn site, in agreement with the previous proposal of Sandusky and Yocum.[15] The determination by Hannson et al.[23] that the presence of $H_2{}^{17}O$ causes broadening of the hyperfine lines of the S_2 state multiline EPR signal is in line with this proposal. Our suggestion that NH_3 binds to the H_2O-binding site in the S_2 state is also compatible with the recent results of Radmer and Ollinger,[24] who found using mass spectrometric methods that the isotopic composition of O_2 evolved by spinach chloroplasts reflected the isotopic composition of H_2O present in the sample subsequent to the formation of the S_3 state. Their findings demonstrate that the S_1 and S_2 states do not bind a non-exchangeable intermediate oxidation product of H_2O.

The effect of the binding of NH_3 to the Mn site in the S_2 state is to reduce the observed hyperfine line spacing in the S_2 state multiline EPR spectrum by more than 20 %. Such a large reduction in the apparent hyperfine constant possibly could be related to an increase in the overall covalency of the metal–ligand complex. For example, Mn^{2+} complexes show reduced ^{55}Mn nuclear hyperfine coupling constants as the covalency of the host lattice or ligands increases.[25] The result of increasing covalency in a metal–ligand complex is to delocalize some of the electron spin density from the central metal ion onto the ligands. The presence of ligand superhyperfine coupling in the EPR spectrum is con-

(22) Yocum, C. F.; Babcock, G. T. *FEBS Lett.* **1981**, *130*, 99–102.
(23) Hansson, Ö; Andréasson, L.-E.; Vänngård, T. *FEBS Lett.* **1986**, *195*, 151–154.
(24) (a) Radmer, R.; Ollinger, O. *FEBS Lett.* **1980**, *110*, 57. (b) Radmer, R.; Ollinger, O. *FEBS Lett.* **1986**, *195*, 285–289.
(25) Goodman, B. A.; Raynor, J. B. *Adv. Inorg. Chem. Radiochem.* **1970**, *13*, 135–362.

sidered unambiguous experimental evidence for such a transfer of spin density onto the ligands.[26] The absence of observable ^{14}N superhyperfine coupling in the NH_3-bound form of the S_2 state EPR spectrum, however, argues against a large change in the covalency of the Mn site due to ligation of NH_3 molecules. In fact, ligand superhyperfine couplings are rarely resolved in Mn complexes having direct coordination of ^{14}N atoms to Mn ions.[25,27,28]

Alternatively, it is possible to account for a reduction in the observed hyperfine line spacing by invoking a change in the exchange couplings between the Mn ions in the Mn site, since the hyperfine coupling from each Mn ion in an exchange coupled Mn tetramer or Mn dimer complex is scaled by the projection of the individual ion's spin angular momentum onto the total spin angular momentum.[29] In Mn tetramer and also in Mn dimer complexes[28] the largest term of the effective spin Hamiltonian is that of the exchange coupling. A model for the Mn site consisting of a Mn tetramer complex containing two antiferromagnetically exchange-coupled Mn dimers that are coupled together through ferromagnetic exchange[30] is compatible with the known stoichiometry of Mn in the O_2-evolving complex[1] and with the magnetic properties of the S_2 state as previously described.[12] Altering the size of the exchange coupling between the Mn ions can cause large changes in the ^{55}Mn hyperfine coupling constants and in the magnetic properties observed. Work in our laboratory shows that altering the exchange coupling parameters for a $3Mn^{III}$–Mn^{IV} tetramer complex can account for both the reduction of the hyperfine line spacing and the near-Curie law temperature dependence of the S_2 state EPR spectrum in NH_4Cl-treated samples.[30] Thus, we attribute the change in the observed spacing of the hyperfine lines in the EPR signal from the NH_3-bound form of the S_2 state to a significant change in the exchange interactions between the Mn ions caused by the binding of one or more NH_3 molecules.

The finding that the Mn site binds ligands in an EPR-detectable manner raises several new questions and possibilities concerning the structure of the Mn site and its coordination properties. Further studies are in progress to determine the number of exchangeable coordination sites on the Mn site and to determine the steric environment of the ligand-binding site(s). The results of this paper indicate that the S_2 state multiline EPR spectrum will provide a spectroscopic probe sensitive to ligand exchange at the Mn site, which should greatly facilitate studies of the coordination chemistry involved in the mechanism of photosynthetic O_2 evolution.

Conclusions

The effects of NH_3 binding to the O_2-evolving complex are consistent with coordination of one or more NH_3 molecules directly to the Mn site detectable in the S_2 state by EPR spectroscopy. The novel S_2 state EPR spectrum produced in the presence of NH_4Cl with illumination at 0 °C shows a near-Curie law temperature dependence which is significantly different from the temperature dependence of S_2 state EPR signals observed in untreated samples, showing that the binding of NH_3 significantly alters the exchange couplings between the Mn ions. Such a change in exchange couplings between Mn ions also accounts for the more than 20% reduction in hyperfine line spacing in the EPR spectrum of the NH_3-bound form of the S_2 state relative to the hyperfine line spacing in the S_2 state EPR spectrum observed in untreated samples. Because of the likelihood that NH_3 binds in competition with H_2O to the substrate binding site of the O_2 evolving complex, causing inhibition of photosynthetic O_2 evolution activity, our results indicate that the EPR-detectable Mn site is the H_2O-binding site. Since previous work strongly implicates this same Mn site in the process of accumulation of oxidation equivalents, it is probable that a single Mn site exists on the electron donor side of PSII, functioning both in the oxidation of bound H_2O molecules and in the storage of oxidation equivalents. The finding that the Mn site binds ligands in a EPR-detectable manner suggests new possibilities for the study of the coordination chemistry of the Mn site.

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(26) Abragam, A.; Bleaney, B. *Electron Paramagnetic Resonance of Transition Ions*; Clarendon Press: Oxford, 1970; p 777.
(27) Chan, S. I.; Fung, B. M.; Lütje, H. *J. Chem. Phys.* **1967**, *47*, 2121–2130.
(28) Cooper, S. R.; Dismukes, G. C.; Klein, M. P.; Calvin, M. *J. Am. Chem. Soc.* **1978**, *100*, 7248–7252.
(29) Scaringe, R. P.; Hodgson, D. J.; Hatfield, W. E. *Mol. Phys.* **1978**, *35*, 701–713.
(30) de Paula, J. C.; Beck, W. F.; Brudvig, G. W. *J. Am. Chem. Soc.*, in press.

Pyrolytic, Kinetic, and Theoretical Studies on the Isomerization of $Me_2HSiCH_2^{\cdot}$ to $Me_3Si^{\cdot}$

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Abstract: Evidence for the isomerization of $^{\cdot}CH_2SiHMe_2$ to $Me_3Si^{\cdot}$ via a 1,2-migration of hydrogen has been obtained through pyrolytic studies of 4-(dimethylsilyl)-1-butene which decomposes through loss of the allyl radical to produce $^{\cdot}CH_2SiHMe_2$. Rearrangement to $Me_3Si^{\cdot}$ was established by product analysis and trapping with methyl chloride. Kinetic analysis afforded a barrier of ca. 41 kcal/mol in excellent agreement with a calculated barrier of 42.6 kcal/mol achieved with fourth-order perturbation theory corrections (MP4) and addition of zero-point vibrational corrections.

Although silicon-centered radicals are significantly more stable than are nonresonance stabilized carbon-centered radicals,[1] only two examples of rearrangement of R_3Si–$\dot{R}'_2$ to $R_2\dot{S}i$–CR'_2R have been reported, and both of these have silicon as the migrating group, R. The key step in the extensively studied isomerization of hexamethyldisilane[2] involves a 1,2-silyl migration in **1** to produce

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(1) Walsh, R. *Acc. Chem. Res.* **1981**, *14*, 246.

0002-7863/86/1508-4022$01.50/0