JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by ISTANBUL TEKNIK UNIV

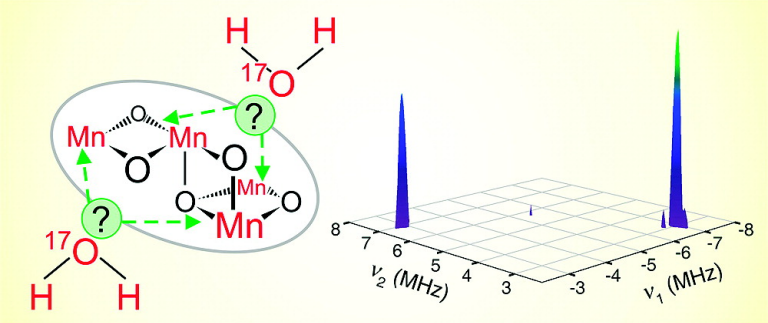
Communication

Probing Mode and Site of Substrate Water Binding to the Oxygen-Evolving Complex in the S State of Photosystem II by O-HYSCORE Spectroscopy

Ji-Hu Su, Wolfgang Lubitz, and Johannes Messinger

J. Am. Chem. Soc., **2008**, 130 (3), 786-787 • DOI: 10.1021/ja076620i

Downloaded from http://pubs.acs.org on February 8, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 5 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S COMMUNICATIONS

Published on Web 12/28/2007

Probing Mode and Site of Substrate Water Binding to the Oxygen-Evolving Complex in the S_2 State of Photosystem II by ^{17}O-HYSCORE Spectroscopy

Ji-Hu Su, Wolfgang Lubitz,* and Johannes Messinger*

Max-Planck-Institut für Bioanorganische Chemie, Stiftstrasse 34-36, D-45470 Mülheim an der Ruhr, Germany

Received September 3, 2007; E-mail: Lubitz@mpi-muelheim.mpg.de; Messinger@mpi-muelheim.mpg.de

In oxygenic photosynthesis light-driven water-splitting is catalyzed by the oxygen-evolving complex (OEC) at the luminal side of photosystem II (PSII). The OEC consists of the inorganic Mn_4O_xCa cluster (Figure 1)[1,2] and its surrounding protein matrix. The latter is still poorly defined, yet highly important for the activity of PSII by facilitating proton-coupled electron transport, regulating substrate entry and product release, and by providing structurally flexible Mn/Ca binding site(s).[1,3−8] During water-splitting, the Mn_4O_xCa cluster steps through a cycle of five distinct redox intermediates. These are known as S_i states, where the index gives the number of stored oxidizing equivalents ($i = 0-4$). O_2 is released during the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition, with the S_4 state being a rapidly ($\leq$1 ms) decaying transient. Despite many efforts it is still an enigma as to precisely when, where, and how the two substrate water molecules bind to the Mn_4O_xCa cluster.[5,6,8] Figure 1 summarizes recent suggestions for substrate "water"[9] binding to the Mn_4O_xCa cluster on the basis of EXAFS model III.[1,10] The principally different possibilities are (i) terminal water or hydroxide ligands to Mn (**1** in Figure 1), (ii) terminal water-derived ligands to Ca (**2** in Figure 1), (iii) μ-oxo bridges between two or three Mn ions (**3**, **4**, **7** in Figure 1), and (iv) water-exchangeable bridges between Mn and Ca (**5**, **6**, **8** in Figure 1). In this report we present the first EPR-based evidence for one water-exchangeable oxygen that is strongly coupled to Mn.

For the study of substrate water binding the use of isotopically labeled water is essential. Time-resolved $H_2{}^{16}O/H_2{}^{18}O$-exchange measurements with membrane-inlet mass spectrometric (MIMS) detection of flash-induced O_2-release demonstrate that the two substrate water molecules exchange with different rates and activation energies in the S_3 state.[11,12] Both substrate waters are also bound in the S_2 state, and at least the slower exchanging one is present in the S_1 and S_0 states.[5] In another approach $^1H_2O/^2H_2O$-exchange has been utilized for $^1H/^2H$ pulse electron paramagnetic resonance (EPR) and $^1H/^2H$ continuous-wave electron nuclear double resonance (*cw* ENDOR) experiments. Such experiments can be performed in the S_0 and S_2 states of the OEC,[13−15] which have ground spin states of $S = {}^1/_2$. However, the poor resolution of the ^2H ($I = 1$) signals and problems in proving that the exchangeable protons stem from water molecules or hydroxide ions directly ligated to Mn make it difficult to draw solid conclusions from such data. Furthermore, fully deprotonated substrate water molecules bound as terminal oxo or as μ-oxo bridges would escape this method of detection. Therefore, $H_2{}^{17}O$ was employed in electron spin−echo envelope modulation (ESEEM) studies, but the reported ^{17}O ($I = {}^5/_2$) signals were not well resolved.[16,17] In the present study spinach PSII membranes enriched with $H_2{}^{17}O$ are studied in the S_2 state by a two-dimensional pulse EPR technique known as hyperfine sublevel correlation (HYSCORE) spectroscopy. Several different time intervals τ (pulse sequence given in legend of Figure 2) were tested to avoid blind spots and to establish optimal conditions.[24,25]

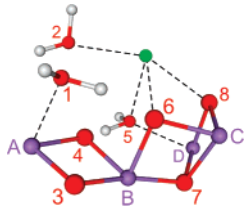


Figure 1. Recent model for the Mn_4O_xCa cluster in photosystem II with suggested substrate "water"[9] binding sites. O−O bond formation has been proposed to occur in the S_4 state between oxygen's **1** and **2**,[6,7,11,18,19] **3** and **4**,[20,21] **4** and **5**,[10] **1** and **6**,[5,19,22] or **6** and **8**.[23] All suggestions (see also ref 8) are adapted to EXAFS model III.[1] The positions of the water molecules were optimized by DFT calculations.[10] Purple (Mn), red (O), green (Ca), and gray (H).

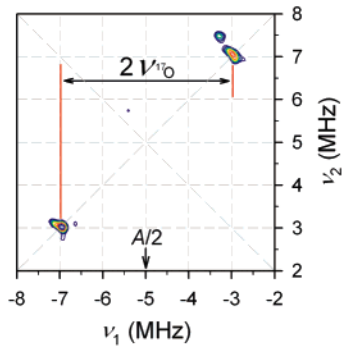


Figure 2. (− +) quadrant of the Fourier-Transform (FT) HYSCORE spectrum of PSII membranes (S_2 state) in $H_2{}^{17}O$-enriched (~75%) buffer. The pulse sequence $\pi/2$-τ-$\pi/2$-t_1-π-t_2-$\pi/2$-τ-echo was employed with $\pi/2 = 24$ ns, $\tau = 196$ ns, and t_1 and t_2 were varied between 60 and 6720 ns in 24 ns-steps. Four-step phase cycling was used with 50 shots per point and a shot repetition time of 5 ms. Other conditions: $B_0 = 355$ mT, temperature 4.2 K, microwave frequency 9.70 GHz. $\nu_{^{17}O}$ and A are the ^{17}O-Larmor frequency (2.05 MHz) and the hyperfine coupling, respectively.

Figure 2 displays the (− +) quadrant of the HYSCORE spectrum of a PSII sample that was first enriched with $H_2{}^{17}O$ in the dark-stable S_1 state and then transferred into the S_2 state by illumination at 200 K.[26] The spectrum shows a clearly resolved signal with a hyperfine coupling of $A \approx 10$ MHz (center of signal equals $A/2$ when $\nu_{^{17}O} < A/2$) and a splitting of approximately 4.1 MHz. The latter corresponds to 2 times the ^{17}O-Larmor frequency ($\nu_{^{17}O} = 2.05$ MHz at $B_0 = 355$ mT). This is consistent with the idea that the signal originates from the coupling of ^{17}O with a paramagnetic center.[27] This coupling appears to be highly isotropic since no ridges are observed. The signal was absent (i) in samples in which all Mn was released from the OEC into the $H_2{}^{17}O$-buffer as Mn^{2+} by reduction with NH_2OH, and (ii) in samples suspended in $H_2{}^{16}O$-buffer. The first control indicates that the HYSCORE signal is specific to the intact Mn_4O_xCa cluster (and does not, e.g., originate from cyt b559), while the second experiment confirms that it stems from ^{17}O (for spectra see Figures S1 and S2 in the Supporting

10.1021/ja076620i CCC: $40.75 © 2008 American Chemical Society

Information). The strong ($A \approx 10$ MHz) ^{17}O hyperfine coupling was observed throughout the spectral width of the S_2 multiline signal, which was established by measurements at $B_0 = 270$, 305, 330, 360, 390, and 420 mT (not shown). We therefore propose that the HYSCORE signal arises from the magnetic interaction between one solvent-exchangeable ^{17}O that is directly ligated to one or more Mn ions of the Mn_4O_xCa cluster in the S_1 and S_2 states of PSII. In contrast to a previous report employing the less direct approach of $H_2{}^{17}O$-minus-$H_2{}^{16}O$ 3-pulse ESEEM spectroscopy,[16,17] we cannot detect a $^{17}O-Mn$ hyperfine coupling of $A \approx 5$ MHz.

Weak couplings ($\nu_{^{17}O} > A/2$) between ^{17}O and Mn arising from through-space dipole−dipole interactions between matrix water molecules and Mn are expected to give rise to signals in the (++) quadrant that are centered at the ^{17}O Larmor frequency ($\nu_{^{17}O}$). Such a signal was not detected for the intact PSII samples (Figure S2a in Supporting Information). In principle the absence of this signal indicates that there are no matrix water molecules in the vicinity of the Mn_4O_xCa cluster; however, at the present signal-to-noise level these signals may have escaped detection.

For a firm assignment of the $A \approx 10$ MHz coupling to substrate water a rapid $H_2{}^{17}O$ mix-freeze experiment is required that relates this signal to known substrate exchange rates.[5] However, the fact that only one ^{17}O coupling was found for the Mn_4O_xCa cluster in the ^{17}O-HYSCORE experiments appears to agree with the MIMS-based reports of one or two bound substrate water molecules in the S_1 and S_2 states of the OEC.[28,29] We therefore tentatively assign the present HYSCORE signal to the binding of a substrate water molecule to the Mn_4O_xCa cluster. A full DFT-based interpretation of the data will eventually allow identifying the ^{17}O binding site within the Mn_4O_xCa cluster (Figure 1). At present we can only qualitatively compare the measured coupling to the few known examples from the literature (see Table S1 in Supporting Information). The closest match appears to be to the coupling of 12.8 MHz determined recently for an exchangeable μ-oxo bridge in a bis-μ-oxo bridged $Mn^{III}Mn^{IV}$ dimer.[30] This value is slightly larger than the one determined here, but taking into account the different nuclearities of the Mn-clusters (two vs four Mn ions) a μ-oxo bridge (e.g., oxygen **3** or **4** in Figure 1) is an interesting possibility. Terminal ligands generally appear to have weaker hyperfine couplings.

In the S_1 state and the S_2 state (200 K illumination) the second substrate water may then either be absent or bound to a place where it does not couple to Mn, for example to Ca^{2+} or to the protein matrix.[6,11] However, it is also possible that other experimental conditions are required for its detection by EPR-related methods. Future experiments with site-directed mutants performed in the S_2 and S_0 states of PSII together with detailed theoretical analysis[10,31] are expected to give a wealth of new information about the mode and site(s) of substrate water binding to the Mn_4O_xCa cluster, and thereby provide vital clues toward understanding the mechanism of photosynthetic water-splitting.

Acknowledgment. We thank Dr. Maurice van Gastel and Dr. Leonid Kulik for discussions and advice. Financial support was provided by the DFG (Me1629/2-4), the EU (Solar-H), and the Max-Planck-Gesellschaft.

Supporting Information Available: Echo-detected and *cw* X-band EPR spectra of the ^{17}O-enriched sample (S_2 state); control HYSCORE spectra obtained under various conditions ($H_2{}^{16}O$; 10 K; NH_2OH treated); and Table with reported $^{17}O-$metal hyperfine couplings. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Yano, J.; Kern, J.; Sauer, K.; Latimer, M. J.; Pushkar, Y.; Biesiadka, J.; Loll, B.; Saenger, W.; Messinger, J.; Zouni, A.; Yachandra, V. K. *Science* **2006**, *314*, 821−825.
(2) O_x symbolizes the number of μ-oxo bridges that varies between the four recent EXAFS models because of the different bridging to Ca ($x \geq 5$).
(3) Ferreira, K. N.; Iverson, T. M.; Maghlaoui, K.; Barber, J.; Iwata, S. *Science* **2004**, *303*, 1831−1838.
(4) Loll, B.; Kern, J.; Saenger, W.; Zouni, A.; Biesiadka, J. *Nature* **2005**, *438*, 1040−1044.
(5) Hillier, W.; Messinger, J. Mechanism of photosynthetic oxygen production. In *Photosystem II. The Light-Driven, Water:Plastoquinone Oxidoredutase*; Wydrzynski, T., Satoh, K., Eds.; Advances in Photosynthesis and Respiration, Vol. 22; Springer: Dordrecht, 2005; pp 567−608.
(6) McEvoy, J. P.; Brudvig, G. W. *Chem. Rev.* **2006**, *106*, 4455−4483.
(7) Meyer, T. J.; Huynh, M. H. V.; Thorp, H. H. *Angew. Chem., Int. Ed.* **2007**, *46*, 5284−5304.
(8) Messinger, J.; Renger, G. Photosynthetic Water Splitting. In *Primary Processes of Photosynthesis: Basic Principles and Apparatus*; Renger, G., Ed.; Comprehensive Series in Photochemical and Photobiological Sciences; Royal Society of Chemistry: Cambridge, UK, 2007; pp 291−349.
(9) "Water" includes all protonation states.
(10) Zein, S.; Kulik, L. V.; Yano, J.; Kern, J.; Pushkar, Y.; Zouni, A.; Yachandra, V. K.; Lubitz, W.; Neese, F.; Messinger, *J. Phil. Trans. Royal Soc. B* **2008**, doi:10.1098/rstb.2007.2212.
(11) Messinger, J.; Badger, M.; Wydrzynski, T. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3209−3213.
(12) Hillier, W.; Messinger, J.; Wydrzynski, T. *Biochemistry* **1998**, *37*, 16908−16914.
(13) Fiege, R.; Zweygart, W.; Bittl, R.; Adir, N.; Renger, G.; Lubitz, W. *Photosynth. Res.* **1996**, *42*, 227−244.
(14) Yamada, H.; Mino, H.; Itoh, S. *Biochim. Biophys. Acta Bioenerg.* **2007**, *1767*, 197−203.
(15) Britt, R. D.; Campbell, K. A.; Peloquin, J. M.; Gilchrist, M. L.; Aznar, C. P.; Dicus, M. M.; Robblee, J.; Messinger, J. *Biochim. Biophys. Acta Bioenerg.* **2004**, *1655*, 158−171.
(16) Turconi, S.; MacLachlan, D. J.; Bratt, P. J.; Nugent, J. H. A.; Evans, M. C. W. *Biochemistry* **1997**, *36*, 879−885.
(17) Evans, M. C. W.; Nugent, J. H. A.; Ball, R. J.; Muhiuddin, I.; Pace, R. J. *Biochemistry* **2004**, *43*, 989−994.
(18) Pecoraro, V. L.; Baldwin, M. J.; Caudle, M. T.; Hsieh, W.-Y.; Law, N. A. *Pure Appl. Chem.* **1998**, *70*, 925−929.
(19) Messinger, J. *Phys. Chem. Chem. Phys.* **2004**, *6*, 4764−4771.
(20) Yachandra, V. K.; Sauer, K.; Klein, M. P. *Chem. Rev.* **1996**, *96*, 2927−2950.
(21) Messinger, J.; Robblee, J. H.; Bergmann, U.; Fernandez, C.; Glatzel, P.; Visser, H.; Cinco, R. M.; McFarlane, K. L.; Bellacchio, E.; Pizarro, S. A.; Cramer, S. P.; Sauer, K.; Klein, M. P.; Yachandra, V. K. *J. Am. Chem. Soc.* **2001**, *123*, 7804−7820.
(22) Siegbahn, P. E. M. *Chem. Eur. J.* **2006**, *12*, 9217−9227.
(23) Dasgupta, J.; van Willigen, R. T.; Dismukes, G. C. *Phys. Chem. Chem. Phys.* **2004**, *6*, 4793−4802.
(24) Deligiannakis, Y.; Louloudi, M.; Hadjiliadis, N. *Coord. Chem. Rev.* **2000**, *204*, 1−112.
(25) Stoll, S.; Calle, C.; Mitrikas, G.; Schweiger, A. *J. Magn. Reson.* **2005**, *177*, 93−101.
(26) The HYSCORE measurements were performed at a Bruker ESP-580 X-band pulse EPR spectrometer in a EN4118X cavity equipped with the super XFT bridge and an Oxford-900 liquid helium cryostat with ITC-503 temperature controller. Pellets of dark-adapted PSII membranes (Messinger, J.; Robblee, J. H.; Yu, W. O.; Sauer, K.; Yachandra, V. K.; Klein, M. P. *J. Am. Chem. Soc.* **1997**, *119*, 11349−11350) were washed twice with either $H_2{}^{16}O$- or $H_2{}^{17}O$ (90% enrichment)-buffer containing 0.4 M sucrose, 25 mM MES-NaOH (pH 6.1), 15 mM NaCl, 3 mM $MgCl_2$, 1 mM EDTA, 0.5 mM PPBQ and 3 % methanol. The samples were then concentrated to 20−30 mg chlorophyll/mL in 4 mm X-band EPR tubes by centrifugation. The final enrichment in $H_2{}^{17}O$ was ∼75%. S_2-state samples were prepared by illumination at 200 K. $H_2{}^{17}O$-buffer was prepared by drying aliquots of $H_2{}^{16}O$-buffer in a vacuum centrifuge at 60 °C, and then adding the same volume of $H_2{}^{17}O$.
(27) We did not resolve a nuclear quadrupole-splitting of our ^{17}O-HYSCORE signal. This may have two reasons: (i) the nuclear quadrupole splitting caused by ^{17}O is small because the oxygen is in a symmetric environment, (ii) we only observe the $m_I = -1/2 \leftrightarrow +1/2$ transition of ^{17}O: the line-width of this transition is affected only in second order by the quadrupole interaction. The signals of the other transitions are too broad.
(28) Hillier, W.; Wydrzynski, T. *Biochemistry* **2000**, *39*, 4399−4405.
(29) Hendry, G.; Wydrzynski, T. *Biochemistry* **2002**, *41*, 13328−13334.
(30) Usov, O. M.; Grigoryants, V. M.; Tagore, R.; Brudvig, G. W.; Scholes, C. P. *J. Am. Chem. Soc.* **2007**, *129*, 11886−11887.
(31) Kulik, L. V.; Epel, B.; Lubitz, W.; Messinger, J. *J. Am. Chem. Soc.* **2007**, *129*, 13421−13435.

JA076620I