^{2}H ESE-ENDOR Study of Hydrogen Bonding to the Tyrosine Radicals $Y_D^\bullet$ and $Y_Z^\bullet$ of Photosystem II

Dee Ann Force,[†] David W. Randall,[†] R. David Britt,*[,†] Xiao-Song Tang,*[,‡] and Bruce A. Diner*[,‡]

Department of Chemistry, University of California Davis, California 95616-0935
E. I. du Pont de Nemours & Company Central Research and Development Department Wilmington, Delaware 19880-0173

Received September 11, 1995

Photosystem II (PS II) contains two symmetry-related redox-active tyrosine residues designated Y_D (D2-Tyr160) and Y_Z (D1-Tyr161).[1] The conventional view of the function of tyrosine Y_Z is as an electron transfer intermediate between the tetranuclear Mn cluster where water is oxidized and the photooxidized chlorophyll moiety P_{680}^+.[2] Our recent ESE-ENDOR study indicates a 4.5 Å separation between the Mn cluster and Y_Z.[3] This result along with an ESEEM study showing disorder in β-methylene hyperfine (HF) couplings[4] has provided a basis for models in which the $Y_Z^\bullet$ radical is directly involved in water oxidation by acting as a photogenerated base abstracting protons from water bound to the Mn cluster.[3,5] In contrast, Y_D is bypassed in the fast electron transfer between the Mn cluster and P_{680}^+ and is typically present as a dark stable neutral radical ($Y_D^\bullet$). Though functionally distinct, $Y_D^\bullet$ and $Y_Z^\bullet$ have very similar EPR spectra.[4,6,7] To trap the $Y_Z^\bullet$ radical the Mn cluster is removed by chemical treatment, which slows the re-reduction kinetics of $Y_Z^\bullet$. However, the $Y_Z^\bullet$ EPR signal decays faster than that of $Y_D^\bullet$, making observation of a pure $Y_Z^\bullet$ EPR signal impossible in a PS II sample from a wild-type organism. Thus, to observe a pure $Y_Z^\bullet$ EPR signal, Y_D must be selectively mutagenized to a non-redox-active residue such as phenylalanine.[7]

The presence of an exchangeable proton hydrogen bonded to the phenolic oxygen of $Y_D^\bullet$ is well established by ESEEM[8] and ^{1}H ENDOR.[9] Experiments designed to detect equivalent hydrogen bonding to $Y_Z^\bullet$ have been interpreted as providing positive,[7] negative,[10] and indeterminate[4] results. In this study we use ^{2}H ESE-ENDOR to examine the hydrogen-bonding status of $Y_Z^\bullet$. An advantage of performing ^{2}H ENDOR is that no subtraction of spectra is required; exchangeable deuterons give rise to new peaks in a frequency region well below that of nonexchangeable protons.[11] By using *Synechocystis* PCC 6803 PS II core complexes in which Y_D has been mutated to phenylalanine (D2-Tyr160Phe),[7] we are able to perform ^{2}H ESE-ENDOR experiments on $Y_Z^\bullet$ following ^2H_2O exchange without interference from the $Y_Z^\bullet$ signal. ESE-ENDOR experiments are performed using the Davies and Mims ESE-ENDOR sequences[12] implemented on our laboratory-built spectrometer.[13]

Figure 1 displays ESE-ENDOR data and simulations obtained on spinach and *Synechocystis* PS II tyrosine radicals. The

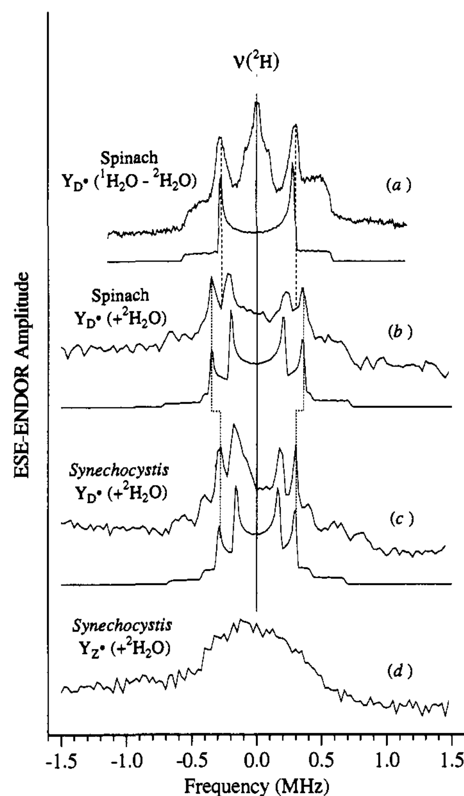

Figure 1. ESE-ENDOR spectra and simulations of $Y_Z^\bullet$ and $Y_D^\bullet$. Simulation parameters are given in Table 1. (a) $g_N(^2H)/g_N(^1H)$ frequency-scaled Davies ^{1}H ENDOR $^1H_2O - {}^2H_2O$ difference spectrum of $Y_D^\bullet$ in Mn-depleted spinach PS II particles. The spinach ^2H_2O PS II particles were prepared by incubating ^1H_2O particles for 3 h in ^2H_2O buffers as discussed in footnote 14. (b) Mims ^{2}H ENDOR spectrum of $Y_D^\bullet$ in Mn-depleted spinach PS II particles incubated as in a. (c) Mims ^{2}H ENDOR spectrum of $Y_D^\bullet$ in Mn-depleted *Synechocystis* PS II core preparations incubated for 14 h in ^2H_2O buffer (pD = 7.5). (d) Mims ^{2}H ENDOR spectrum of $Y_Z^\bullet$ of Mn-depleted *Synechocystis* PS II core preparations from the Y_D-less mutant (D2-Tyr160Phe) incubated in ^2H_2O as in c. The $Y_Z^\bullet$ radical signal was cryotrapped after illumination above liquid nitrogen. There was no evidence in either CW or ESE-detected EPR spectra for any radicals other than $Y_Z^\bullet$ trapped by this procedure (data not shown). Additionally, ESE-ENDOR spectra were collected 4 G upfield from the $g = 2.0023$ field position to further minimize ENDOR contributions from any trace quantities of narrow radical species. Spectra are plotted as $\delta\nu = \nu_{RF} - \nu_D$. Experimental parameters: $\nu_{MW} = 10.106$ GHz; $B = 3609$ G ($\nu_H = 15.35$ MHz; $\nu_D = 2.35$ MHz). Trace a: $T = 10.0$ K. Traces b–d: $T = 4.2$ K.

† University of California.
‡ E. I. du Pont de Nemours & Company.

(1) (a) Barry, A.; Babcock, G. T. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7099–7103. (b) Debus, R. J.; Barry, B. A.; Babcock, G. T.; McIntosh, L. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 427–430. (c) Vermaas, W. F. J.; Rutherford, A. W.; Hansson, Ö. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 8477–8481. (d) Debus, R. J.; Barry, B. A.; Sithole, I.; Babcock, G. T.; McIntosh, L. *Biochemistry* **1988**, *27*, 9071–9074. (e) Metz, J. G.; Nixon, P. J.; Rögner, M.; Brudvig, G. W.; Diner, B. A. *Biochemistry* **1989**, *28*, 6960–6969. (f) Koulougliotis, D.; Tang, X.-S.; Diner, B. A.; Brudvig, B. W. *Biochemistry* **1995**, *34*, 2850–2856.
(2) Debus, R. J. *Biochim. Biophys. Acta* **1992**, *1102*, 269–352.
(3) Gilchrist, M. L.; Ball, J. A.; Randall, D. W.; Britt, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9545–9549.
(4) Tommos, C.; Tang, X.-S.; Warncke, K.; Hoganson, C. W.; Styring, S.; McCracken, J.; Diner, B. A.; Babcock, G. T. *J. Am. Chem. Soc.* **1995**, *117*, 10325–10335.
(5) Hoganson, C. W.; Lyadkis-Simantiris, N.; Tang, X.-S.; Tommos, C.; Warncke, K.; Babcock, G. T.; Diner, B. A.; McCracken, J.; Styring, S. *Photosynth. Res.*, in press.
(6) Mino, H.; Kawamori, A. *Biochim. Biophys. Acta* **1994**, *1185*, 213–220.
(7) Tang, X.-S.; Zheng, M.; Chisholm, D. A.; Dismukes, G. C.; Diner, B. A. *Biochemistry*, submitted.
(8) Evelo, R. G.; Hoff, A. J.; Dikanov, S. A.; Tyryshkin, A. M. *Chem. Phys. Lett.* **1989**, *161*, 479–484.
(9) (a) Rodriguez, I. D.; Chandrashekar, T. K.; Babcock, G. T. In *Progress in Photosynthesis Research*; Biggins, J., Ed.; Martinus Nijhoff: Dordrecht, 1987; pp 471–474. (b) Mino, H.; Satoh, J.-I.; Kawamori, A.; Toriyama, K.; Zimmerman, J.-L. *Biochim. Biophys. Acta* **1993**, *1144*, 426–433. (c) Tang, X.-S.; Chisholm, D. A.; Dismukes, G. C.; Brudvig, G. W.; Diner, B. A. *Biochemistry* **1993**, *32*, 13742–13748.
(10) Bernard, M. T.; MacDonald, G. M.; Nguyen, A. P.; Debus, R. J.; Barry, B. A. *J. Biol. Chem.* **1995**, *270*, 1589–1594.
(11) Fan, C.; Kennedy, M. C.; Beinert, H.; Hoffman, B. M. *J. Am. Chem. Soc.* **1992**, *114*, 374–375.
(12) (a) Davies, E. R. *Phys. Lett.* **1974**, *47A*, 1–2. (b) Mims, W. B. *Proc. R. Soc. London, Ser. A* **1965**, *283*, 452–457.
(13) Sturgeon, B. E.; Ball, J. A.; Randall, D. W.; Britt, R. D. *J. Phys. Chem.* **1994**, *98*, 12871–12883. [A 1 kW rf amplifier (ENI A1000) has been utilized in this study.]

0002-7863/95/1517-12643$09.00/0 © 1995 American Chemical Society

Table 1. ENDOR Simulation Parameters and Calculated Hydrogen-Bond Lengths for $Y_D^•$

tyrosine radical		A_{iso} (kHz)	A_{dip} (kHz)	e^2qQ^a (kHz)	r (Å)
$Y_D^•$ (spinach)	^1H	0	+3700		1.67
$Y_D^•$ (spinach)	^2H	0	+570	+200	1.67
$Y_D^•$ (*Synechocystis*)	^2H	−50	+470	+170	1.87

[a] $\eta = 0$ for all quadrupolar simulations.

presence of an exchangeable proton hydrogen-bonded to $Y_D^•$ is confirmed upon subtraction of a Davies ^1H ENDOR spectrum of ^2H_2O-exchanged Mn-depleted spinach PS II membranes from the ^1H ENDOR spectrum of a natural abundance ^1H_2O sample[14] (trace a). The frequency axis of trace a has been scaled by the ratio $g_N(^2H)/g_N(^1H)$ to allow direct comparison with ^2H ENDOR spectra. The Mims ^2H ENDOR spectrum obtained on $Y_D^•$ of a Mn-depleted ^2H_2O-exchanged spinach sample is presented in trace b. The symmetric splitting of the ^2H ENDOR perpendicular turning points around the frequency-scaled ^1H peaks in trace a demonstrates that this splitting arises from the nuclear quadrupolar (NQ) interaction for the $I = 1$ deuterium nucleus. Trace c shows the Mims ^2H ENDOR spectrum of $Y_D^•$ in Mn-depleted *Synechocystis* PS II particles.[15] The slightly different HF and NQ couplings (Table 1) used to simulate the ^2H ESE-ENDOR spectra of the hydrogen-bonded deuterons in traces b and c show that there are minor differences in the hydrogen-bonding environments of the two species. However, the sharp and well-resolved transitions exhibited by both species indicate well-defined hydrogen bonds with little variation between individual PS II reaction centers. Radial distances between the electron spin and an exchangeable proton or deuteron can be calculated from the dipolar HF couplings using the expression $A_{dip} = \rho_O g_e g_N \beta_e \beta_N / r^3$, where ρ_O is the unpaired spin density on the tyrosyl oxygen, g_e and β_e are the electronic g-factor and Bohr magneton, and g_N and β_N are the nuclear g-factor and nuclear magneton. Utilizing literature values for the combined C_4 and tyrosyl oxygen spin density[16] in the two species and assuming $\rho_{C_4} = 0$, we obtain $r = 1.67$ Å for spinach ($\rho_{C_4+O} = 0.22$) and $r = 1.87$ Å for *Synechocystis* ($\rho_{C_4+O} = 0.25$).

Trace d displays the Mims ^2H ESE-ENDOR spectrum of $Y_Z^•$ cryotrapped after illumination in the Y_D-less *Synechocystis* mutant (D2-Tyr160Phe). There is a dramatic absence of resolved structure in the $Y_Z^•$ ^2H ENDOR spectrum compared to the ^2H ENDOR spectrum of $Y_D^•$.[17] However, the overall widths of the ^2H ENDOR spectra are comparable for both tyrosine radical species, indicating similar magnitudes of dipolar HF couplings. The ^2H ENDOR data thus indicate that there is a hydrogen bond to the phenolic oxygen of the Y_Z tyrosyl radical. However there is sufficient site-to-site disorder in the hydrogen bonding to lead to a loss of resolved structure in the ^2H ESE-ENDOR spectrum of $Y_Z^•$. The dominant term describing the ENDOR powder pattern of $Y_D^•$ is the dipolar HF coupling which depends on the distance between the tyrosyl oxygen and the deuteron exchanged into the hydrogen-bond site. The loss of resolution in the $Y_Z^•$ spectrum is thus likely due to a distribution of O···^{2}H bond lengths. The e^2qQ NQ parameter of a hydrogen-bonded ^2H is also dependent on the hydrogen-bond length,[18] and a distribution of bond lengths will therefore also lead to a distribution of the NQ coupling, further reducing resolved structure in the ^2H ESE-ENDOR powder pattern. It is important to again note that these spectra are obtained in Mn-depleted PS II particles. We have previously determined that the Mn cluster is in close proximity to Y_Z,[3] so the removal of the Mn cluster may disrupt the normal hydrogen bonding of this tyrosyl radical, causing the disorder observed in the ^2H ESE-ENDOR spectrum. One possible catalytic site geometry suggested by the proton or hydrogen atom extraction models[3,5] would have the Y_Z tyrosine hydrogen bonded to Mn-bound water. In this case, removal of the Mn cluster would destroy the well-ordered hydrogen bonding to waters positioned by ligation to the cluster, replacing it with heterogeneous hydrogen bonding to waters and/or amino acid residues remaining in the disrupted Mn binding site. The disorder observed via ESEEM[4] in the Y_Z dihedral bond angle between the tyrosine ring normal and the β-methylene C−H bond of Mn-depleted PS II particles is likely due to the disorder in hydrogen bonding, because a well-defined hydrogen bond at an angle well off the phenolic O−C bond axis provides a steep energy barrier for rotation of the ring from its minimum energy position. The rotational flexibility of Y_Z in the Mn-depleted PS II particles is crucial for the proton or hydrogen atom abstraction models. In the intact system the Y_Z orientation would have to be sufficiently flexible for it to move between two well-defined orientations, one of which allows it to donate protons to a proximal base upon its oxidation by P_{680}^+, another of which allows it to abstract protons or hydrogen atoms from waters bound to Mn during the S-state transitions. In either configuration the Y_Z/$Y_Z^•$ hydrogen bonding may be quite well-defined, but the oxidation and subsequent re-reduction drives orientational switching between the two sites.

Acknowledgment. A grant from the National Institutes of Health to R.D.B. (BMT 1 R29 GM48242-01) and NRICGP grants from the United States Department of Agriculture to R.D.B. (92-01413) and B.A.D. (94-01692) are gratefully acknowledged.

JA953122D

(14) PS II enriched membranes (Kim, D. H.; Britt, R. D.; Klein, M. P.; Sauer, K. *Biochemistry* **1992**, *31*, 541−547) were Mn-depleted according to a modified method of Tamura and Cheniae (*Biochim. Biophys. Acta* **1987**, *890*, 179−194). Mn-depletion was performed in 0.8 M Tris (pH 8.5) for 30 min under room light at 277 K. Following centrifugation and two washes (20 mM Tris (pH 8.5), 400 mM sucrose; 400 mM MES (pH 6.0), 400 mM sucrose, 15 mM NaCl, 5 mM $MgCl_2$, 5 mM $CaCl_2$), ferricyanide was added to a final concentration of 0.5 mM and the pellet was loaded into EPR tubes. ^2H_2O-exchanged PS II particles were prepared by performing the Tris-wash procedure as above in ^2H_2O-enriched buffers (pD 8.5 and 6.0).

(15) Tang, X.-S.; Diner, B. A. *Biochemistry* **1994**, *33*, 4594−4603.

(16) (a) Warncke, K.; Babcock, G. T.; McCracken, J. *J. Am. Chem. Soc.* **1994**, *116*, 7332−7340. (b) Rigby, S. E. J.; Nugent, J. H. A.; O'Malley, P. J. *Biochemistry* **1994**, *33*, 1734−1742.

(17) The comparable amplitudes of ENDOR spectra for traces c and d indicate that the broad spectrum of d is not just a small residual underlying component of spectrum c.

(18) Hunt, M. J.; Mackay, A. L. *J. Magn. Reson.* **1976**, *22*, 295−301.