The τ-Nitrogen of D2 Histidine 189 is the Hydrogen Bond Donor to the Tyrosine Radical $Y_D^\bullet$ of Photosystem II

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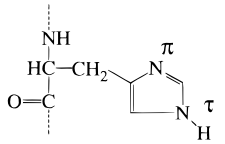
The D1 and D2 membrane-spanning proteins that form the core of photosystem II (PSII) each contain a redox-active tyrosine.[1] The D1 redox-active tyrosine Y_Z (D1-Tyr161, *Synechocystis* notation) mediates fast electron transfer between the photooxidized P_{680} Chl moiety and the Mn cluster of the PSII oxygen-evolving complex. In addition, recent models invoke Y_Z directly in the oxygen evolution chemistry as an abstractor of protons or hydrogen atoms from substrate water bound to the Mn complex.[2] In contrast, the D2 redox-active tyrosine Y_D (D2-Tyr160, *Synechocystis* notation) is bypassed in the fast electron transfer of the oxygen evolution process. The biological function of the Y_D tyrosine, which is stable for hours in its neutral radical form $Y_D^\bullet$ is not well defined. It is of great interest that these two tyrosine radicals, apparently symmetrically positioned within the PSII reaction center about a 2-fold rotation axis,[3] have such different properties in terms of function, redox potential, and electron transfer kinetics. These differences must be induced by the protein environments of the two tyrosines. Both $Y_D^\bullet$ and $Y_Z^\bullet$ are neutral, deprotonated radicals.[4] Therefore, the hydrogen bonding to the phenoxyl oxygens provides protein control of the properties of these radicals. Little is known about the hydrogen bonding to $Y_Z^\bullet$ in intact, oxygen-evolving PSII, but in Mn-depleted preparations, ENDOR[5] and high-field EPR experiments[6] have shown that $Y_Z^\bullet$ is hydrogen bonded, but this hydrogen bonding is highly disordered. In contrast, $Y_D^\bullet$ shows well-ordered hydrogen bonding, giving rise to well-defined ENDOR transitions from a single class of exchangeable hydrogen nuclei (^{1}H or ^{2}H).[5] ^{1}H ENDOR experiments have demonstrated that this hydrogen bonding is disrupted in PSII preparations in which D2-His189 has been mutated to a variety of other residues.[7] The $Y_D^\bullet$ CW EPR line shapes are also altered in such mutants.[7,8] In this work, we demonstrate with ^{15}N electron spin echo-electron nuclear double resonance (ESE-ENDOR) that the imidazole τ-nitrogen of D2-His189 is the direct hydrogen bond donor to $Y_D^\bullet$.

Chart 1



We have performed these ^{15}N ESE-ENDOR experiments using four different *Synechocystis* 6803 PSII core preparations: ^{15}N globally labeled wild type; ^{15}N histidine labeled wild type in which both τ and π imidazole nitrogens of the histidine residues are isotopically labeled (Chart 1);[9] ^{15}N histidine labeled wild type in which only the π imidazole nitrogen is isotopically labeled; and an ^{15}N globally labeled mutant, His189Gln.[10] The ESE-ENDOR spectra were obtained on a laboratory built spectrometer[12] using the Mims pulse sequence,[13] which is sensitive for the detection of the relatively weak couplings to hydrogen-bonded ^{15}N nuclei.[14]

Figure 1 presents the $Y_D^\bullet$ ESE-ENDOR spectra obtained for each *Synechocystis* 6803 preparation. Trace (a) displays the ENDOR transitions of ^{15}N nuclei magnetically coupled to $Y_D^\bullet$ in the globally ^{15}N-labeled sample. The very small peak at 1.57 MHz, the ^{15}N Larmor frequency (ν_N), arises from relatively distant ^{15}N nuclei. Large, complex peaks symmetrically flank the ^{15}N Larmor frequency, with maximum intensity at these 1.17 and 1.97 MHz frequencies. The ENDOR spectrum of the ^{15}N-labeled histidine wild type sample (trace (b)) consists of two isotropic lines at 1.17 and 1.97 MHz, which, using the first-order perturbation theory expression for ENDOR frequencies ($\nu_\pm = \nu_N \pm A_{iso}/2$), arise from a class of ^{15}N nuclei with an isotropic coupling constant, $A_{iso} = 0.8$ MHz. This isotropic hyperfine coupling arises from unpaired spin density (approximately 0.8%)[15] of $Y_D^\bullet$ of delocalized onto a histidine

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(10) Global ^{15}N-labeling of the *Synechocystis* wildtype and D2-His189Gln (see ref 6) strains was carried out in BG-11 (Rippka, R.; Deruelles, J.; Waterbury, J. B.; Herdman, M.; Stanier, R. Y. *J. Gen. Microbiol.* **1979**, *111*, 1−61) growth medium plus 5 mM glucose in which the normal 17.6 mM $^{14}NO_3$ was replaced by 2.6 mM $^{15}NO_3$. Greater than 90% labeling of *Synechocystis* PCC 6803 with histidine (imidazole π- and τ-^{15}N, or π-^{15}N) was carried out using a histidine-tolerant strain (described in Tang, X.-S.; Diner, B. A.; Larsen, B. S.; Gilchrist, M. L.; Lorigan, G. A.; Britt, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 704−708). Photosystem II core complexes were prepared from the wild type and mutant strains of *Synechocystis* 6803 using procedures previously described (see: Tang, X.-S.; Diner, B. A. *Biochemistry* **1994**, *33*, 4594−4603. See also ref 5b). For each sample, the $Y_D^\bullet$ radical was trapped by illumination (250 W/m^2 white light from a tungsten halogen lamp) at 0 °C for 35 s in the presence of 300 μM ferricyanide, followed by a 15 min dark incubation on ice before freezing in liquid N_2 in the dark. Under these conditions we do not produce detectable amounts of other photoaccumulated radicals such as Chl_Z^+[11] (CW EPR spectra not shown).
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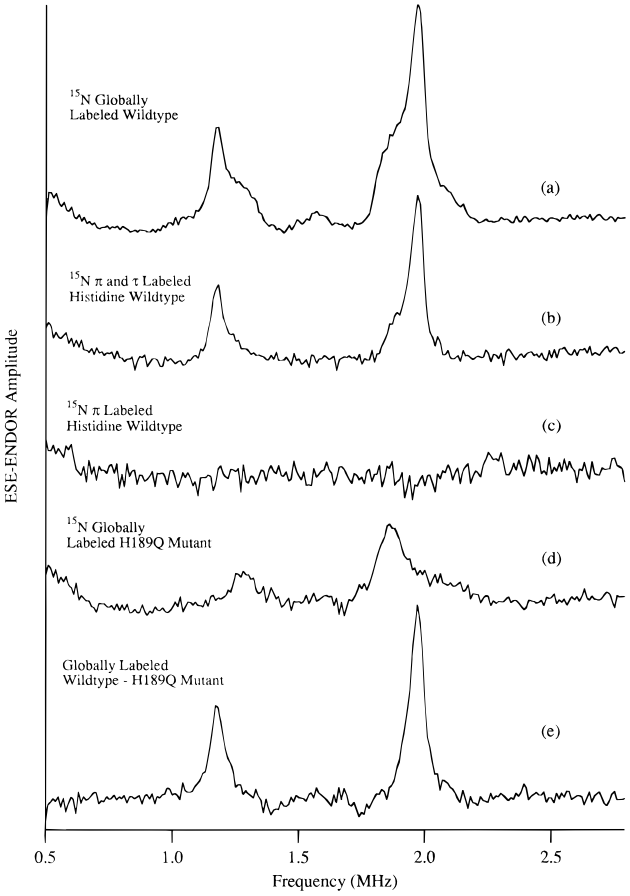

Figure 1. Mims ESE-ENDOR spectra of $Y_D^\bullet$ in *Synechocystis* 6803: (a) globally ^{15}N-labeled wild type; (b) π- and τ-^{15}N-labeled histidine wild type; (c) π-^{15}N-labeled histidine; (d) globally ^{15}N-labeled H189Q mutant; (e) globally ^{15}N-labeled wild type minus ^{15}N-labeled H189Q mutant spectrum (trace (a) to trace (d)). Mims ESE-ENDOR experimental parameters: microwave frequency 10.228 GHz; magnetic field 3646 G; temperature 11 K; $\tau = 476$ ns; rf pulse width 40 μs; microwave pulse widths 15 ns. All data were collected at a field position 4 G lower than the $g = 2.0023$ field position to further eliminate any possible contributions to the ESE-ENDOR spectrum from any trace amounts of photoaccumulated Chl_Z radical.[11] Spectra were also collected for the globally ^{15}N-labeled wild type sample (data not shown) at a field position 10 G lower than the $g = 2.0023$ field position, with identical results to trace (a). The Mims ESE-ENDOR effect is negative; the amplitude of the stimulated echo is reduced by the coupled nuclear spin transitions. Each trace has been normalized at the off resonance frequency of 2.5 MHz, and then inverted to present positive ENDOR peaks. The total data collection time for each spectrum ranged between 15 and 20 min.

nitrogen, almost certainly through a hydrogen bond. ESE-ENDOR experiments on the π-^{15}N-labeled histidine sample (trace (c)) reveal no ^{15}N transitions, demonstrating that it is the τ histidine nitrogen that is the hydrogen bond donor.

The isotropic 1.17 and 1.97 MHz peaks are absent in the ENDOR spectrum of the globally ^{15}N-labeled His189Gln mutant (trace (d)), indicating that it is His189 that provides the coupled τ-nitrogen. However, the two remaining broad peaks at 1.28 and 1.86 MHz align with the inner shoulders of the complex ENDOR peaks of the globally ^{15}N-labeled wild type sample. The difference of the ENDOR spectra of the globally ^{15}N-labeled wild type and H189Q mutant samples (trace (e)) is virtually identical to that of the ^{15}N-labeled histidine wild type spectrum. This allows us to conclude that the mutagenesis did not induce any secondary structural changes around $Y_D^\bullet$ and that there is a second class of ^{15}N nuclei coupled to $Y_D^\bullet$. This second class of ^{15}N nuclei is unlikely to belong to another hydrogen bond donor, since ^{1}H and ^{2}H ESE-ENDOR[5] reveals only one class of hydrogen-bonded proton/deuteron, and recent ^{2}H ESEEM experiments have demonstrated that a single deuteron with the ENDOR-derived hyperfine and quadrupolar couplings accounts for all the observed ESE envelope modulation,[16] and since the proton ENDOR[7] and high-field EPR[6] of $Y_D^\bullet$ in the D2-His189Gln mutant reveal no remaining hydrogen bonds. We are investigating the possibility that this second class of nitrogens corresponds to the peptide nitrogen of the Y_D tyrosine.

In addition to demonstrating that the τ-nitrogen of D2 histidine 189 is the hydrogen bond donor to $Y_D^\bullet$, these ESE-ENDOR results explicitly demonstrate that, in a π -radical molecule such as $Y_D^\bullet$, hydrogen bonding can provide significant unpaired spin density onto a nitrogen hydrogen bond donor to an oxygen group on the radical. Such coupling is particularly notable given the ESEEM spectra reported for the Q_A^- quinone anion radicals in PSII[17] and the bacterial reaction center,[18] which have been interpreted to favor relatively isotropic couplings to peptide and/or histidine nitrogen nuclei.

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