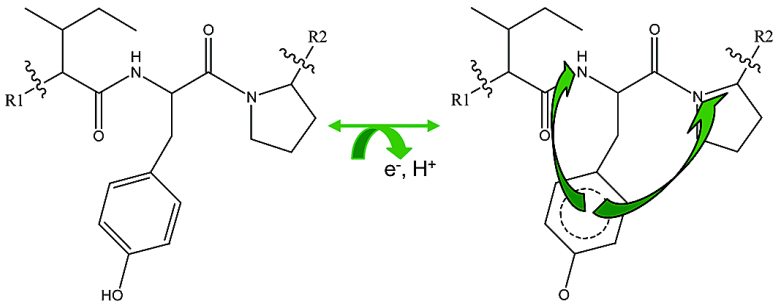
Communication

Redox-Active Tyrosine Residues: Role for the Peptide Bond in Electron Transfer

Idelisa Pujols-Ayala, Colette A. Sacksteder, and Bridgette A. Barry

J. Am. Chem. Soc., **2003**, 125 (25), 7536-7538• DOI: 10.1021/ja035005l • Publication Date (Web): 29 May 2003



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

J|A|C|S
COMMUNICATIONS

Published on Web 05/29/2003

Redox-Active Tyrosine Residues: Role for the Peptide Bond in Electron Transfer

Idelisa Pujols-Ayala,† Colette A. Sacksteder, and Bridgette A. Barry*

Department of Biochemistry, Biophysics, and Molecular Biology, University of Minnesota, 140 Gortner Laboratory, 1479 Gortner Avenue, St. Paul, Minnesota 55108

Received March 5, 2003; E-mail: barry@cbs.umn.edu

In this report, difference FT-IR spectroscopy is used to show that oxidation of a redox-active tyrosine in photosystem II (PSII) causes perturbations of the peptide bond. These perturbations are attributed to spin delocalization, as previously proposed for tryptophan cation radicals[1] and tyrosyl radicals[2] *in vitro*. Migration of the electron hole may be consistent with peptide bond involvement in tyrosyl radical-based electron-transfer reactions.

PSII catalyzes the light-driven oxidation of water and reduction of plastoquinone. Redox-active tyrosines are involved in long-distance electron-transfer reactions in several enzymes.[3] In PSII, tyrosine Z conducts electrons between the primary chl donor and the tetranuclear manganese cluster.[4] In the oxidized form, Z forms a neutral radical, Z•, with a 1, 3, 5 spin density distribution (Figure 1A) over the aromatic ring.[5]

Previous work has provided evidence that a vibrational frequency of the tyrosine Z• radical, attributed to a stretching vibration of the CO bond, is perturbed when compared to the frequencies observed in vitro.[6−10] This band showed ^{13}C isotope shifts when tyrosine was isotopically labeled,[6−10] and this band was observed to decay with kinetics that matched the decay of the Z• EPR signal.[9] The amplitude of this band was sensitive to the addition of hydroxylamine, which blocks Z oxidation.[7,8] Early discrepancies in the literature were resolved by these approaches {see refs 9−12 and references therein}. However, in our previous work, a complete analysis of the Z•/Z spectrum was not performed. Also, recent studies have shown that oxidation of tyrosinate *in vitro* (Figure 1A) perturbs the force constants of the terminal amino and, perhaps, carboxylate groups.[2] Therefore, peptide bond formation (Figure 1B) itself has the potential to influence the oxidation spectrum.[2] To identify potential contributions from the peptide bond, we compare the difference FT-IR spectrum associated with Z photooxidation with model compound data, acquired from tyrosinate *in vitro*.

Model Compound Assignments. In Figure 2A and B, we present difference FT-IR spectra associated with the UV photolysis of natural abundance tyrosinate and $^2H_4$-ring-labeled tyrosinate solutions. Difference spectra were constructed from data acquired before and after photolysis. Spectra were corrected for concentration and path length[2] and were then subtracted on a 1:1 basis to give an isotope-edited spectrum (Figure 2C). ^{2}H shifts are observed for negative bands at 1607, 1499, and 1258 cm^{-1}. These bands shift to 1572, 1421, and 1234 cm^{-1} and are assigned to υ8a (ring stretch), υ19a (ring stretch), and υ7a′ (CO stretch) of the tyrosinate ground state, respectively.[2]

Positive bands at 1552, 1517, 1348, 1323, and 1294 cm^{-1} are also observed (Figure 2C). These bands shift to 1527, 1499, 1334, 1309, and 1271 cm^{-1}. The two highest-frequency bands are assigned to υ8a and υ7a of the radical.[2] Taken together, these spectral assignments support previous Raman studies, which have concluded that tyrosine oxidation perturbs ring stretching vibrations and increases the force constant for the CO bond.[13,14]

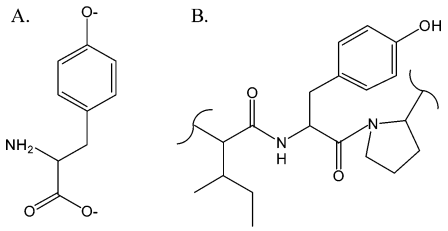


Figure 1. Structure of tyrosinate (A) and a tyrosine-containing tripeptide (B).

The assignments of bands between 1340 and 1260 cm^{-1} await a complete normal coordinate analysis, but probable assignments include aromatic and aliphatic CH bending and CC stretching vibrational modes. Aliphatic modes may be coupled to ring vibrations and may be isotope sensitive. Weak, ^{2}H-sensitive bands are observed above 1610 cm^{-1}. DFT calculations predict in-plane bending vibrations of the amino group in this spectral region,[2] which are also coupled to ring stretching vibrations (Range et al., unpublished results). In agreement with the calculations, ^{15}N-labeling of tyrosinate resulted in isotope shifts in model tyrosyl radical.[2]

Z• and Z Assignments in PSII. In Figure 2D, we present the difference FT-IR spectrum associated with the oxidation of tyrosine Z in cyanobacterial PSII.[15] Difference spectra were constructed by methods previously described.[8] In particular, conditions were employed in which the quinone acceptors make only a small contribution to the spectrum.[10] In addition to contributions from Z and Z•, the PSII photooxidation spectrum will also exhibit contributions from the protein matrix. To identify Z and Z• contributions, we have acquired FT-IR spectra on cyanobacterial PSII, containing $^2H_4$-ring-labeled tyrosine (Figure 2E). Spectra were corrected for concentration and path length[8] and then were subtracted on a 1:1 basis to give an isotope-edited spectrum (Figure 2F). Labeling was performed through feedback inhibition of the shikimate pathway.[16] ^{13}C from tyrosine is known to be incorporated in low yield into plastoquinone, a molecule which has overlapping spectral contributions with tyrosine.[10,11] However, previous work, which has recently been confirmed,[10] showed no significant incorporation of ^{2}H from ^{2}H-labeled tyrosine into plastoquinone under the conditions used for labeling.[16] There was also no detectable isotope incorporation into chlorophyll, as assessed by mass spectrometry[17](see Supporting Information and refs 10, 18).

Ring Vibrational Frequencies of Z• and Z Are Similar to Those of Model Compounds. In Figure 2F, Z• and Z bands with similar frequencies, when compared to the model compound data, are observed. Bands at (−) 1602 and (+) 1583 cm^{-1} in the isotope-edited Z•-minus-Z spectrum are assigned to υ8a of Z and its isotopomer, respectively. Bands at (+) 1558 and (−) 1547 cm^{-1}

† Present address: Department of Pharmacology, PO Box 100267, University of Florida, Gainesville, FL 32610.

10.1021/ja035005l CCC: $25.00

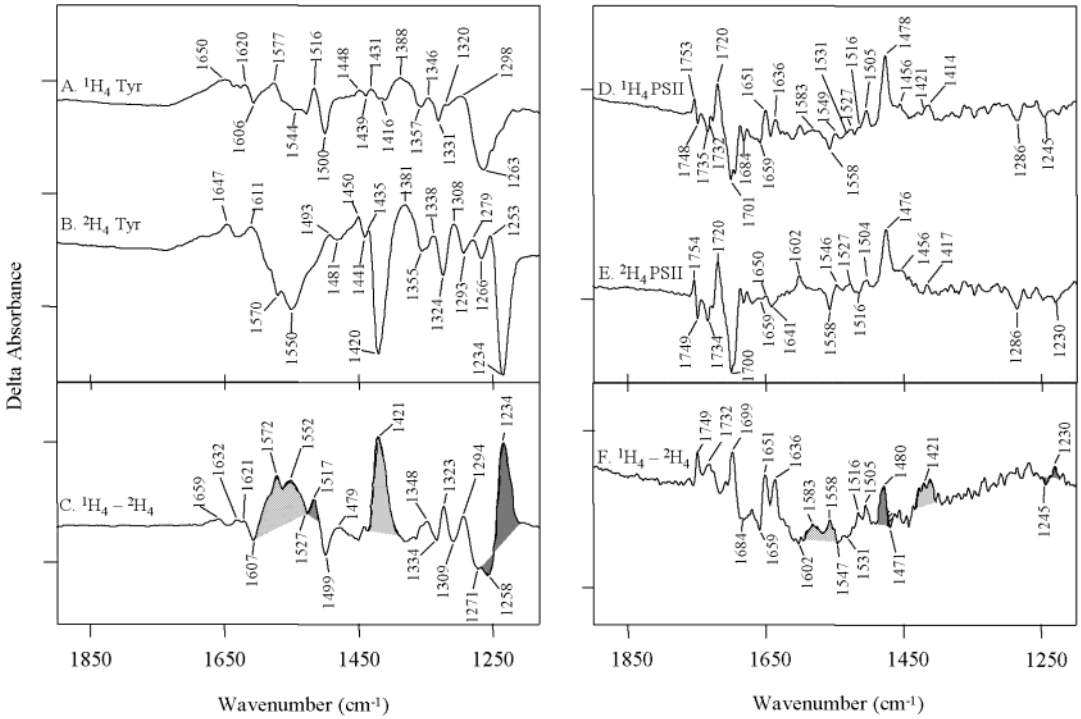


Figure 2. Difference FT-IR spectra associated with the oxidation of tyrosinate at 77 K (A−C) and of tyrosine Z in photosystem II at 263 K (D−F). In (A) and (B), spectra were acquired on natural abundance tyrosinate and $^2H_4$-ring-labeled tyrosinate, respectively, in 10 mM borate−NaOH, pH 11. (C) Isotope-edited spectra (A−B). Spectra were acquired at 4 cm^{-1} resolution and represent the average of two or eight data sets. The *y*-axis tick marks are 1.5×10^{-2} AU for the top panel and 2.0×10^{-2} AU for the bottom panel. In (D) and (E), spectra were acquired on natural abundance Z and $^2H_4$-ring-labeled Z, respectively. (F) shows the isotope-edited spectra (D−E). Spectra were acquired at 4 cm^{-1} resolution and represent the average of 16 or 34 data sets. The *y*-axis tick marks are 5.0×10^{-4} AU for the top panel and 2.0×10^{-4} AU for the bottom panel. $^2H_4$-ring-labeled tyrosine was purchased from Cambridge Isotopes (Andover, MA), and its isotope enrichment was 98%.

are assigned to υ8a of the radical, Z•, and its isotopomer, respectively. These frequencies are similar (within 5−20 cm^{-1}) to the observed frequencies of these bands in model tyrosinate and tyrosyl radical (Figure 2C), and the magnitudes of the isotope shifts are also similar. A positive 1421 cm^{-1} band is observed in Figure 2F. This band corresponds to the isotopically shifted υ19a band, which is the most intense spectral feature in the *in vitro* data (Figure 2C). In the Z•-minus-Z spectrum, υ19a of the unlabeled Z species is most likely a negative spectral feature at 1531 cm^{-1}. The apparent frequency of this band is 30 cm^{-1} higher than the frequency observed in the model compound (Figure 2C), but this may be due solely to changes in band overlap in this spectral region.

The CO Stretch of Z. In Figure 2F, there are no bands observed at (−) 1258 and (+) 1234 cm^{-1}. In the model compound (Figure 2C), these spectral features arise from the CO stretching vibration (υ7a′) of tyrosinate and its isotopomer, respectively. In the PSII data (Figure 2F), weak bands at (−) 1245 and (+) 1230 cm^{-1} may correspond to υ7a′ of Z and its isotopomer, but the signal-to-noise is not sufficient to assign these bands with confidence. We conclude that the amplitude and, possibly, frequency, of υ7a′ is perturbed, when Z is compared to the model tyrosinate compound.

Previous Raman studies have shown that the amplitude and frequency of υ7a′ can be used as a marker of hydrogen bonding to the phenol oxygen of phenol and phenolate.[19] When phenol is protonated and weakly or non-hydrogen-bonded, the frequency of υ7a′ is observed at ∼1255 cm^{-1}.[19] When phenol is protonated and involved in a hydrogen bond as a proton donor, frequencies of 1272−1267 cm^{-1} are expected.[19] When phenol is protonated and involved in a hydrogen bond as a proton acceptor, a frequency of ∼1240 cm^{-1} is observed, and the amplitude of the ν7a′ band is decreased, relative to the proton-donating or the weakly hydrogen-bonded cases.[19] Deprotonation of phenol to form phenolate was observed to result in an upshift of υ7a′ to 1266 cm^{-1}.[19] Therefore, Figure 2F may suggest that Z has a protonated phenolic COH group (Figure 1B) and that Z is involved in one or more hydrogen bonds as the proton acceptor. Future work will address this point.

The CO Stretching Frequency of Z• Is Consistent with Spin Delocalization into the Peptide Bond. In Figure 2F, a (+) 1480/(−) 1471 cm^{-1} derivative-shaped feature has a counterpart with a higher frequency in the model compound spectrum (Figure 2C). We assign this band in Figure 2F to υ7a of the Z• radical, which is downshifted (37−28 cm^{-1}) from the observed (+) 1517/(−) 1499 cm^{-1} frequencies in the model compound (Figure 2C). In previous work, ^{13}C shifts of this band were also observed.[6−10] Notice that the magnitude of the isotope effect for this band is one-half the isotope effect observed for υ7a in model tyrosyl radical (Figure 2C). Our interpretation of the small isotope shift for Z• υ7a is decreased coupling between CO and ring vibrational modes; such decreased coupling could be caused by the decrease in frequency. We attribute the downshift of the Z• CO frequency to delocalization of spin density from the phenol oxygen to the amide/imide bonds (see below), because our FT-IR data have shown no significant effect of solvent exchange on this band under the conditions employed.[7,8,12]

Amide and Imide Frequencies Are Observed in the Z•-Minus-Z Spectrum. The isotope-edited Z•-minus-Z spectrum (Figure 2F) also contains bands in the 1650 cm^{-1} region, which are not observed in the photolysis spectrum acquired from polycrystalline tyrosinate (Figure 2C) but are observed in model dipeptides.[2] In this so-called amide I region, contributions from the C═O stretching vibration of amide and imide bonds are observed.[20] In PSII, tyrosine Z is tyrosine 161 of the D1 polypeptide and is contained in the sequence (Figure 1B), ile-tyr(Z)-pro.[21] A previous flash photolysis study of the dipeptides, tyr-pro and ile-

tyr, showed that oxidation of tyrosinate perturbs the imide and amide bonds, respectively.[2] Amide and imide vibrations may be coupled to tyrosine ring stretching motions; therefore, these vibrational bands may show weak sensitivity to ^{2}H-ring labeling.

On the basis of these previous studies,[2] we assign the (−) 1684/(+) 1636 cm^{-1} bands in Figure 2F to the Z-pro imide bond and the (−) 1659/(+) 1651 cm^{-1} bands to the ile-Z amide bond, respectively. The imide frequency correlates well with frequencies previously observed in the tyr-pro dipeptide,[2] in which the oxidation spectrum exhibited bands at (−) 1680/(+) 1636 cm^{-1}. The ile-tyr dipeptide exhibited a weak spectral feature, assigned to the amide C═O vibration, at ∼1620 cm^{-1}.[2] However, the (−) 1659/(+) 1651 cm^{-1} C═O assignment for Figure 2F is more to be expected for α helical proteins and polypeptides, where amide I bands are upshifted and increased in intensity relative to peptides.[20] Our previous work has shown that the 1650 cm^{-1} region of the Z•-minus-Z spectrum is ^{15}N sensitive.[7] This observation is consistent with these assignments because the C═O vibration is coupled to displacements of the amide nitrogen.[20]

In Figure 2F, bands at 1516 (+) and 1505 (+) cm^{-1} are observed. There is no clear $^2H_4$ isotope shift for these bands, suggesting that the normal coordinates are only weakly coupled to ring stretching vibrations. We tentatively assign these bands to the CN stretching vibration of the imide bond and the coupled NH/CN (amide II) vibration of the amide bond. Such low amide II frequencies have been observed previously for some conformers of polyglycine.[20] These vibrational modes are expected to be less intense in the infrared, when compared to the C═O stretching vibration.[2] Supporting this interpretation, a ^{15}N shift has been previously observed in the 1516−1500 cm^{-1} spectral region.[7]

Bands between 1749 and 1699 cm^{-1}. Bands assigned to tyrosyl radical NH bending modes are not observed in Figure 2F, because Z• does not have a free amino group (Figure 1B). Instead, we see isotope-sensitive bands between 1749 and 1699 cm^{-1}. $^2H_4$-ring isotope-sensitive bands are not expected for tyrosyl radical in this spectral region. Chl was not labeled from tyrosine, as discussed above. Therefore, we attribute these bands to a small amount of isotope incorporation into the carboxylic acid side chain of asp or glu residues, which are perturbed when Z is photooxidized. GC−MS analysis does not detect significant ^{2}H label in asp and glu (data not shown), but some amino acids, such as tyr, glu and asp, contain side-chain hydrogens that are exchangeable to acid hydrolysis.[22] Asp and glu may also contribute to the spectrum at 1211 cm^{-1},[23] but the infrared intensity of this C−O band is >2 smaller than the C═O vibrational bands, and is therefore not easily detected.

Summary. The Z•-minus-Z spectrum exhibits bands assignable to the ile-Z amide and Z-pro imide bonds. To contribute to the oxidation spectrum, the force constants of these bonds must be perturbed by photooxidation. A possible mechanism for this perturbation is spin delocalization.[1,2] Although the amount of delocalization may be small,[1,2] such delocalization could be consistent with a role for the peptide bond in tyrosyl radical-based electron-transfer reactions.

Acknowledgment. Supported by GM43273 (B.A.B.) and GM19541 (I. P.-A). We thank Professor S. Kim for experimental assistance, and Professor Kim, Professor D. York, and K. Range for discussions.

Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Walden, S. E.; Wheeler, R. A. *J. Am. Chem. Soc.* **1997**, *119*, 3175−3176.
(2) Ayala, I.; Range, K.; York, D.; Barry, B. A. *J. Am. Chem. Soc.* **2002**, *124*, 5496−5505.
(3) Pesavento, R. P.; van der Donk, W. A. *Adv. Protein Chem.* **2001**, *58*, 317−385.
(4) Gerken, S.; Brettel, K.; Schlodder, E.; Witt, H. T. *FEBS Lett.* **1988**, *237*, 69−75.
(5) Boerner, R. J.; Barry, B. A. *J. Biol. Chem.* **1993**, *268*, 17151−17154.
(6) MacDonald, G. M.; Bixby, K. A.; Barry, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11024−11028.
(7) Kim, S.; Barry, B. A. *Biophys. J.* **1998**, *74*, 2588−2600.
(8) Kim, S.; Ayala, I.; Steenhuis, J. J.; Gonzalez, E. T.; Razeghifard, M. R.; Barry, B. A. *Biochim. Biophys. Acta* **1998**, *1364*, 337−360.
(9) Ayala, I.; Kim, S.; Barry, B. A. *Biophys. J.* **1999**, *77*, 2137−2144.
(10) Kim, S.; Patzlaff, J. S.; Krick, T.; Ayala, I.; Sachs, R. K.; Barry, B. A. *J. Phys. Chem. B* **2000**, *104*, 9720−9727.
(11) Razeghifard, M. R.; Kim, S.; Patzlaff, J. S.; Hutchison, R. S.; Krick, T.; Ayala, I.; Steenhuis, J. J.; Boesch, S. E.; Wheeler, R. A.; Barry, B. A. *J. Phys. Chem. B* **1999**, *103*, 9790−9800.
(12) Pujols-Ayala, I.; Barry, B. A. *Biochemistry* **2002**, *41*, 11456−11465.
(13) Tripathi, G. N. R.; Schuler, R. H. *J. Chem. Phys.* **1984**, *81*, 113−121.
(14) Johnson, C. R.; Ludwig, M.; Asher, S. A. *J. Am. Chem. Soc.* **1986**, *108*, 905−912.
(15) Noren, G. H.; Boerner, R. J.; Barry, B. A. *Biochemistry* **1991**, *30*, 3943−3950.
(16) Barry, B. A.; Babcock, G. T. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7099−7103.
(17) Kim, S.; Barry, B. A. *J. Am. Chem. Soc.* **2000**, *122*, 4980−4981.
(18) MacDonald, G. M.; Steenhuis, J. J.; Barry, B. A. *J. Biol. Chem.* **1995**, *270*, 8420−8428.
(19) Takeuchi, H.; Watanabe, N.; Satoh, Y.; Harada, I. *J. Raman Spectrosc.* **1989**, *20*, 233−237.
(20) Krimm, S.; Bandekar, J. In *Advances in Protein Chemistry*; Anfinsen, C. B., Edsall, J. T., Richards, F. M., Eds.; Academic Press: New York, 1986; Vol. 38, pp 181−364.
(21) Barry, B. A.; Boerner, R. J.; de Paula, J. C. In *The Molecular Biology of the Cyanobacteria*; Bryant, D., Ed.; Kluwer Academic Publishers: Dordrecht, 1994; Vol. 1, pp 215−257.
(22) Manning, J. *J. Am. Chem. Soc.* **1970**, *92*, 7449−7454.
(23) Hutchison, R. S.; Steenhuis, J. J.; Yocum, C. F.; Razeghifard, M. R.; Barry, B. A. *J. Biol. Chem.* **1999**, *274*, 31987−31995.

JA035005L