Inorg. Chem. **2000,** *39,* 2254−2255

Model for the Cofactor Formation Reaction of *E. Coli* Ribonucleotide Reductase. From a Diiron(II) Precursor to an $Fe^{III}Fe^{IV}$ Species via a Peroxo Intermediate

Vicki L. MacMurdo, Hui Zheng, and Lawrence Que Jr.*

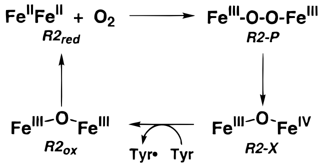
Department of Chemistry and Center for Metals in Biocatalysis, University of Minnesota, Minneapolis, Minnesota 55455

Received December 28, 1999

The formation of the diiron(III) cluster−tyrosyl radical cofactor of the R2 protein of ribonucleotide reductase of *E. coli* (RNR $R2_{ox}$)[1] from its diiron(II) precursor and O_2 has been the subject of intense recent investigation.[2] The reduced diiron center ($R2_{red}$) appears likely to traverse through peroxo (R2-P) and high-valent (R2-X) intermediates en route to $R2_{ox}$ with oxidation states designated in Scheme 1. To date, this redox process has not been modeled in its entirety by one biomimetic system, although synthetic precedents for the individual steps of the cluster assembly reaction have been reported.[3−5] Building on our earlier success in generating Fe(III)Fe(IV) species from the reaction of diiron(III) precursors with H_2O_2,[4] we report herein the first example of a synthetic diiron(II) complex that reacts with dioxygen to generate in sequence an Fe(III)Fe(III)−peroxo species and an Fe(III)Fe(IV) complex.[6]

The anaerobic reaction of equimolar amounts of $Fe(ClO_4)_2$, 6-Me_3-TPA,[7] and NaOH in methanol yields an orange powder **1**, which can be recrystallized from MeCN/Et_2O.[8] X-ray crystallography shows that **1** corresponds to $[Fe_2(\mu\text{-}OH)_2(6\text{-}Me_3\text{-}TPA)_2](ClO_4)_2$ (Figure 1) with a centrosymmetric $Fe_2(OH)_2$ core. The four nitrogen atoms of the tetradentate ligand complete the iron coordination sphere to form a distorted octahedral environment. The $Fe_2(\mu\text{-}OH)_2$ diamond core has C_{2h} symmetry, with Fe−OH bond lengths of 1.973(2) and 2.168(2) Å. The 1.973(2) Å distance associated with Fe1−O1 is trans to the tertiary amine N and is one of the shortest Fe^{II}−OH bonds thus far observed,[9] comparable in length to the Fe^{III}−μ-OH bonds in diiron(III) complexes that range from 1.937 to 2.055 Å.[5b,10] On the other hand, the Fe1−O1* bond length of 2.168(2) Å associated with the Fe−μ-OH trans to a pyridine is one of the longest Fe^{II}−OH bonds observed to date.[11] The two Fe^{II}−OH bonds average 2.07 Å, which is comparable to that found for $[Fe_2(\mu\text{-}OH)_2(Tp^{3,5-iPr2})_2]$ (2.03 Å).[9b] The diiron center of **1** thus appears to result from the weak

Scheme 1



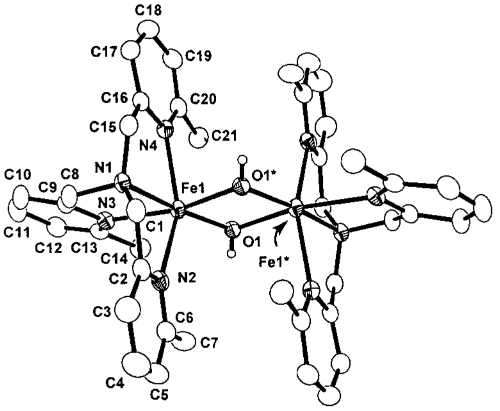


Figure 1. Representation of the crystal structure of $[Fe^{II}_2(\mu\text{-}OH)_2(6\text{-}Me_3\text{-}TPA)_2]^{2+}$. Ellipsoids are drawn at the 50% probability level, and the ligand hydrogen atoms are omitted for clarity. Selected interatomic distances (Å) and angles (deg): Fe1−O1, 1.973(2); Fe1−O1*, 2.168(2); Fe1−N1, 2.222(2); Fe1−N3, 2.285(2); Fe1−N2, 2.326(3); Fe1−N4, 2.290(3); Fe1−Fe1*, 3.187(1); Fe1−O1−Fe1*, 100.55(9).

---

* To whom correspondence should be addressed. Phone: +1-612-625-0389. Fax: +1-612-624-7029. E-mail: que@chem.umn.edu.

(1) (a) Stubbe, J.; Riggs-Gelasco, P. *Trends Biochem. Sci.* **1998**, *23*, 438−443. (b) Wallar, B. J.; Lipscomb, J. D. *Chem. Rev.* **1996**, *96*, 2625−2658. (c) Kurtz, D. M., Jr. *J. Biol. Inorg. Chem.* **1997**, *2*, 159−167.

(2) (a) Bollinger, J. M.; Edmondson, D. E.; Huynh, B. H.; Filley, J.; Norton, J.; Stubbe, J. *Science (Washington, D.C.)* **1991**, *253*, 292−298. (b) Bollinger, J. M., Jr.; Krebs, C.; Vicol, A.; Chen, S.; Ley, B. A.; Edmondson, D. E.; Huynh, B. H. *J. Am. Chem. Soc.* **1998**, *120*, 1094−1095. (c) Sturgeon, B. E.; Burdi, D.; Chen, S.; Huynh, B. H.; Edmondson, D. E.; Stubbe, J.; Hoffman, B. M. *J. Am. Chem. Soc.* **1996**, *118*, 7551−7557. (d) Riggs-Gelasco, P. J.; Shu, L.; Chen, S.; Burdi, D.; Huynh, B. H.; Que, L., Jr.; Stubbe, J. *J. Am. Chem. Soc.* **1998**, *120*, 849−860.

(3) (a) Dong, Y.; Ménage, S.; Brennan, B. A.; Elgren, T. E.; Jang, H. G.; Pearce, L. L.; Que, L., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 1851−1859. (b) Kitajima, N.; Tamura, N.; Amagai, H.; Fukui, H.; Moro-oka, Y.; Mizutani, Y.; Kitagawa, T.; Mathur, R.; Heerwegh, K.; Reed, C. A.; Randall, C. R.; Que, L., Jr.; Tatsumi, K. *J. Am. Chem. Soc.* **1994**, *116*, 9071−9085. (c) Hayashi, Y.; Kayatani, T.; Sugimoto, H.; Suzuki, M.; Inomata, K.; Uehara, A.; Mizutani, Y.; Kitagawa, T.; Maeda, Y. *J. Am. Chem. Soc.* **1995**, *117*, 11220−11229. (d) Hagadorn, J. R.; Que, L., Jr.; Tolman, W. B. *J. Am. Chem. Soc.* **1998**, *120*, 13531−13532.

(4) (a) Dong, Y.; Fujii, H.; Hendrich, M. P.; Leising, R. A.; Pan, G.; Randall, C. R.; Wilkinson, E. C.; Zang, Y.; Que, L., Jr.; Fox, B. G.; Kauffmann, K.; Münck, E. *J. Am. Chem. Soc.* **1995**, *117*, 2778−2792. (b) Hsu, H.-F.; Dong, Y.; Shu, L.; Young, V. G., Jr.; Que, L., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 5230−5237. (c) Dong, Y.; Zang, Y.; Kauffmann, K.; Shu, L.; Wilkinson, E. C.; Münck, E.; Que, L., Jr. *J. Am. Chem. Soc.* **1997**, *119*, 12683−12684. (d) Dong, Y.; Que, L., Jr.; Kauffmann, K.; Münck, E. *J. Am. Chem. Soc.* **1995**, *117*, 11377−11378.

(5) (a) Lee, D.; DuBois, J.; Petasis, D.; Hendrich, M. P.; Krebs, C.; Huynh, B. H.; Lippard, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 9893−9894. (b) Lee, D.; Lippard, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 12153−12154.

(6) Another diiron(II) model system has recently been reported that also reacts with dioxygen to form an Fe(III)Fe(IV) species. However, no peroxo intermediate has been observed (ref 5a).

(7) Abbreviations used: 6-Me_3−TPA, tris(6-methyl-2-pyridylmethyl)amine; $Tp^{3,5-iPr2}$, hydrotris(3,5-diisopropylpyrazol-1-yl)borate; 6-Me-TPA, *N*-(6-methyl-2-pyridylmethyl)-*N*,*N*-bis(2-pyridylmethyl)amine.

(8) *Caution: Complexes with organic ligands and perchlorate anions are potentially explosive!* Elemental analysis for **1** (isolated in 46% yield). Anal. Calcd: C, 49.97; H, 4.99; N, 11.10. Found: C, 49.80; H, 5.02; N, 11.15. Crystal data for **1**·$2CH_3CN$ ($C_{50}H_{62}Cl_2Fe_2N_{12}O_{10}$, fw 1173.72) at 173 K: yellow needles, triclinic, space group $P\bar{1}$, $a = 11.5338(2)$ Å, $b = 11.6035(2)$ Å, $c = 12.0914(1)$ Å, $\alpha = 112.663(1)°$, $\beta = 107.952(1)°$, $\gamma = 96.141(1)°$, $V = 1372.80(4)$ Å^3, $Z = 1$. For 3703 unique observed reflections with $I > 2\sigma(I)$ and 351 parameters, the current discrepancy indices are $R = 0.0475$ and $R_w = 0.1027$.

(9) (a) Hikichi, S.; Ogihara, T.; Fujisawa, K.; Kitajima, N.; Akita, M.; Moro-oka, Y. *Inorg. Chem.* **1997**, *36*, 4539−4547. (b) Kitajima, N.; Tamura, N.; Tanaka, M.; Moro-oka, Y. *Inorg. Chem.* **1992**, *31*, 3342−3343.

(10) (a) Kurtz, D. M. *Chem. Rev.* **1990**, *90*, 585−606. (b) Zheng, H.; Zang, Y.; Dong, Y.; Young, V. G., Jr.; Que, L., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 2226−2235.

(11) Reynolds, R. A., III; Yu, W. O.; Dunham, W. R.; Coucouvanis, D. *Inorg. Chem.* **1996**, *35*, 2721−2722.

10.1021/ic991482m CCC: $19.00

association of two Fe(OH)(6-Me_3−TPA) units. This core, however, remains intact in solution, as evidenced by the presence of an X-band EPR feature at $g = 16$ in parallel mode at 2 K that is characteristic of weakly coupled diiron(II) centers.[12]

Bubbling dioxygen into a CH_2Cl_2 solution of **1** at −40 °C generates within 5 min an EPR-silent, metastable green species, **2** (λ_{max} = 490 and 640 nm, ϵ = 1100 M^{-1} cm^{-1} for both bands), which is very similar to that derived from the reaction of $[Fe^{III}_2(\mu\text{-}O)_2(6\text{-}Me_3{-}TPA)_2]^{2+}$ (**3**) with H_2O_2.[4c] A comparison of absorption features indicates that **1** is quantitatively converted to **2**. Electrospray mass spectral data of **2** reveal a positive ion with *m/z* 923 with a mass and isotope pattern corresponding to the peroxo species $\{[Fe_2(\mu\text{-}O)(O_2)(6\text{-}Me_3{-}TPA)_2](ClO_4)\}^+$. The resonance Raman spectrum of **2** shows features at 848 and 462 cm^{-1}, both of which downshift with $^{18}O_2$ (Figure 2).[13] The 848 cm^{-1} peak ($\Delta^{18}O$ = 46 cm^{-1}) and the Fermi doublet centered at 462 cm^{-1} ($\Delta^{18}O$ = 21 cm^{-1}) are respectively assigned to ν(O−O) and ν_s(Fe−O), by analogy to features found for other (μ-1,2-peroxo)diiron complexes.[3a−c] Unlike the latter compounds, **2** also shows an intense feature at 531 cm^{-1} with an ^{18}O shift of 21 cm^{-1}, which is consistent with another ν(Fe−O) mode. We assign this feature to the ν_{as}(Fe−O) mode, recently found for the peroxo intermediates of W48F/D84E RNR R2, stearoyl-ACP Δ^9 desaturase, and ferritin.[14] Thus, **2** serves as the first model diiron(III)−peroxo species to contain the three prominent oxygen-sensitive vibrations seen for these enzyme peroxo intermediates.[15]

Intermediate **2** decays slowly at −30 °C in the presence of 1 equiv of $HClO_4$ to a brown species, following first-order kinetics over at least seven half-lives (Figure 3, $k = 1.2 \times 10^{-3}$ s^{-1}). When this decay is monitored by EPR, a species with an isotropic signal at $g = 1.999$, **4**, is observed to grow with a time course commensurate with the decay of **2** (Figure 3). This signal is identical to that seen for an Fe(III)Fe(IV) species in the reaction of **3** with H_2O_2.[4c] At 20 K, this signal exhibits a peak-to-peak width of 2.9 mT that broadens by 0.3 mT when $H_2{}^{17}O$ is added to the solution of **1** prior to the addition of O_2 (Figure 3, inset), a broadening comparable to that seen for $[Fe^{III}Fe^{IV}(O)_2(6\text{-}Me\text{-}TPA)_2]^{3+}$ and R2-X.[4d,16] These observations support the notion that the isotropic signal arises from a high-valent metal complex that has solvent exchangeable oxygen atoms.

Double integration of the isotropic signal arising from the decay of **2** in the presence of 1 equiv of $HClO_4$ reveals that **4** is generated in 30% yield from **1**. Addition of 2,4,6-tri-*tert*-butylphenol to this solution results in the formation of the phenoxy radical in 34% yield from **1**, showing that **4** is capable of oxidizing phenols in a manner similar to that seen for R2-X. At present, the source of the electron needed for the conversion of **2** to **4** is not established. One possible candidate is H_2O_2, which could dissociate from **2**, but more work is needed to confirm this hypothesis. In summary **1** represents the first synthetic diiron(II) complex that can access each step of the cluster assembly process proposed for RNR R2.[2] Because all of these species can be identified spectroscopically, this system can provide valuable insight into the oxygen activation mechanisms of RNR R2 and other nonheme diiron enzymes.[1]

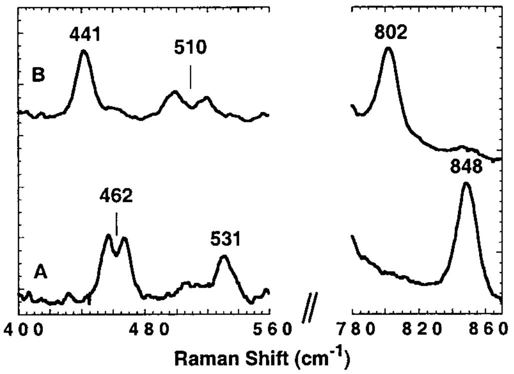


Figure 2. Resonance Raman spectra of frozen solutions of **2** generated in CH_2Cl_2 at −40 °C with (A) $^{16}O_2$ and (B) $^{18}O_2$; excitation wavelength 632.8 nm.

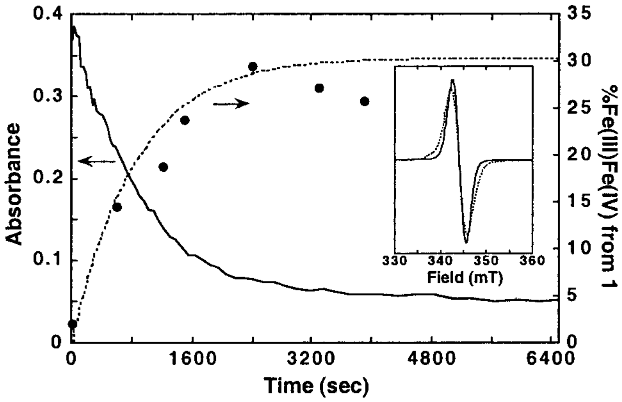


Figure 3. Comparison of decay of the peroxo species **2** and the formation of **4**. The solid line follows the UV−vis decay of **2** at 640 nm. The dotted line represents the predicted amount of **4** formed from the decay of **2**, assuming a 30% conversion of **1** to **4**. The solid circles are the actual amounts of **4** generated as determined by EPR. The inset is of the EPR spectra of **4** with (dotted) and without (solid) enrichment in ^{17}O from $H_2{}^{17}O$. Conditions: T = 20 K, 0.1008 mW.

Acknowledgment. This work was supported by the National Institutes of Health (GM-38767). We are grateful to Victor G. Young, Jr. and the X-ray Crystallographic Laboratory at the University of Minnesota for assistance and expertise in the X-ray diffraction experiments. We also thank Dr. Raymond Y. N. Ho for assistance with the resonance Raman studies.

Supporting Information Available: X-ray crystallographic file, in CIF format, for complex **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

IC991482M

---

(12) (a) Hendrich, M. P.; Münck, E.; Fox, B. G.; Lipscomb, J. D. *J. Am. Chem. Soc.* **1990**, *112*, 5861−5865. (b) Hendrich, M. P.; Pearce, L. L.; Que, L., Jr.; Chasteen, N. D.; Day, E. P. *J. Am. Chem. Soc.* **1991**, *113*, 3039−3044. (c) Jang, H. G.; Hendrich, M. P.; Que, L., Jr. *Inorg. Chem.* **1993**, *32*, 911−918.

(13) Although a similar spectrum has been reported for the peroxo intermediate derived from the reaction of **3** with H_2O_2, the region around 530 cm^{-1} was obscured by unassigned contaminating features (ref 4c).

(14) (a) Moënne-Loccoz, P.; Baldwin, J.; Ley, B. A.; Loehr, T. M.; Bollinger, J. M., Jr. *Biochemistry* **1998**, *37*, 14659−14663. (b) Broadwater, J. A.; Ai, J.; Loehr, T. M.; Sanders-Loehr, J.; Fox, B. G. *Biochemistry* **1998**, *37*, 14664−14671. (c) Moënne-Loccoz, P.; Krebs, C.; Herlihy, K.; Edmondson, D. E.; Theil, E. C.; Huynh, B. H.; Loehr, T. M. *Biochemistry* **1999**, *38*, 5290−5295.

(15) Hayashi et al. reported two Fe−O vibrations that they assigned as the symmetric and asymmetric stretches. However, the much smaller ^{18}O shifts observed suggest that other assignments should be considered (ref 3c).

(16) Ravi, N.; Bollinger, J. M., Jr.; Huynh, B. H.; Edmondson, D. E.; Stubbe, J. *J. Am. Chem. Soc.* **1994**, *116*, 8007−8014.