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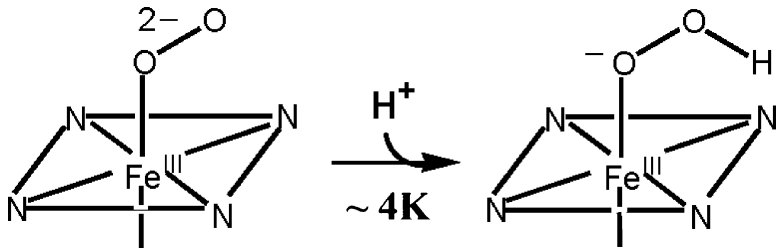
J. Am. Chem. Soc., **2004**, 126 (49), 15960-15961• DOI: 10.1021/ja044646t • Publication Date (Web): 16 November 2004



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Published on Web 11/16/2004

Proton Transfer at Helium Temperatures during Dioxygen Activation by Heme Monooxygenases

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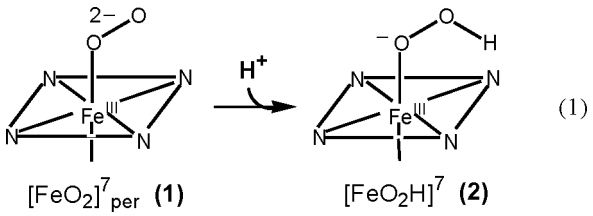
In the hydroxylation of substrate (RH) by heme monooxygenases[1] such as cytochromes P450,[2] heme oxygenase (HO),[3] and nitric oxide synthase (NOS),[4,5] the committed portion of the catalytic cycle involves the one-electron reduction of the enzyme's dioxygen-bound ferroheme (O_2Fe(Por)); with the addition of two protons this leads to the hydroxylation of substrate.[1] The two protons are delivered by an elaborate distal-pocket proton-delivery network connected by H-bonds to the oxy-ferroheme.[6,7]

The physiological reduction and addition of the first proton may well involve proton-coupled electron transfer,[8,9] but radiolytic cryoreduction in general forms a trapped peroxo-ferriheme state ($[FeO_2]^7_{per}$; **1**),[10,11] thereby decoupling the two processes, and allowing us to monitor at all temperatures both the transfer of the "first" proton to generate the hydroperoxo-ferriheme ($[FeO_2H]$;[7] **2**), eq 1, and the subsequent activation of this species by the second



$[FeO_2]^7_{per}$ **(1)** $[FeO_2H]^7$ **(2)** (1)

proton.[12] In the first measurement of enzymatic proton transfer at liquid helium temperatures, we examine protonation of **1** in HO in H_2O and D_2O solvents at ca. 4 K and above, and compare these finding with analogous measurements for oxy-P450cam and for oxy-Mb.

Cryoreduction of oxy-HO frozen in both H_2O and D_2O glycerol/buffer medium at 77 K has been shown to afford a hydroperoxo-ferriheme EPR signal with **g**-tensor components $\mathbf{g}^a = [2.37, 2.180, 1.917]$ (Figure 1, inset).[13] Thus, proton/deuteron 1 is delivered (eq 1) without the need for thermal activation above this temperature.

When oxy-HO frozen in H_2O buffer and situated in the EPR cavity is reduced by an electron beam at ∼4.2 K,[14] a strong EPR signal from **2** (Figure 1) shows that the proton/deuteron has been delivered to the one-electron reduced oxy-heme center even at this temperature. Surprisingly, cryogenic proton transfer is *not* quenched when the ∼4.2 K experiment is repeated with oxy-HO exchanged into D_2O buffer, Figure 1. As shown in Figure 1, the signal remains unchanged upon in situ annealing to ∼77 K, and the signal taken at this temperature within ∼ 20 min of irradiation matches that seen upon 77 K irradiation. Disruption of the distal network through mutation of a critical component in HO(D140X), X = A, F, does

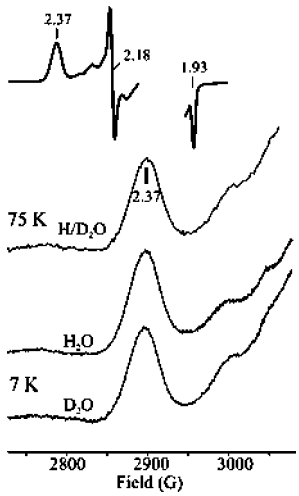


Figure 1. g_1-region X-band EPR spectra of oxy-HO cryoreduced in situ in EPR cavity at ∼4.2 K; spectra collected at ∼7 K. Rise with increasing field is due to intensity from the $m = {}^1/_2$ H-atom line. Small features at ∼3000 G and above are from minority (<5%) oxy-HO substates. In these spectra, differential H/D broadening is not apparent. *Conditions*: microwave frequency, 9.502 GHz; modulation amplitude, 7.5 G. (Inset) Full 35 GHz spectrum of HO intermediate **2** (2 K).

quench helium-temperature proton transfer; as reported, eq 1 only occurs in the mutants at temperatures above ∼170−180 K.[13]

The prompt delivery of "proton 1" at ∼4−7 K is not seen in P450cam, even though it too has a distal-pocket proton-delivery network.[15] As reported, when the camphor complex of oxy-P450cam is cryoreduced at 4−7 K, the major product is **1**; as the sample temperature is raised in situ, substantial proton delivery to generate **2** occurs by ∼55 K and above,[17] a process which is slowed in D_2O buffer glass.[18,19] As with HO, perturbation of the proton-delivering network in P450cam by mutation D252N disrupts the ready proton transfer (eq 1), which occurs only at temperatures above ∼170 K in the mutant.[17]

The behavior of the HO-1(D140X) and P450cam(D251N) mutants in fact is similar to that of the O_2-carrying proteins, Hb and Mb. Cryoreduction of oxy-Mb and oxy-Hb at 77 K affords **1**, and it is stable at this temperature for years; for completeness, we reduced oxy-Mb in glycerol/buffer at ∼4.2 K and confirmed that there is no proton transfer at this temperature or upon annealing to ∼77 K. The oxy-Mb (and oxy-Hb) intermediates **1** do not convert to **2** at temperatures less than 170 K;[20] by 200 K, the reaction, eq **1**, is too fast to measure by progressive annealing with either H_2O or D_2O solvents, $\tau \ll 1$ min. We determined the solvent kinetic isotope effect (*solv*-KIE) for eq 1 in oxy-Mb at 180 K, through measurements in H_2O and D_2O glycerol/buffer,[12] Figure 2. At this temperature the decay of **1** is roughly biphasic, as has been seen

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10.1021/ja044646t CCC: $27.50

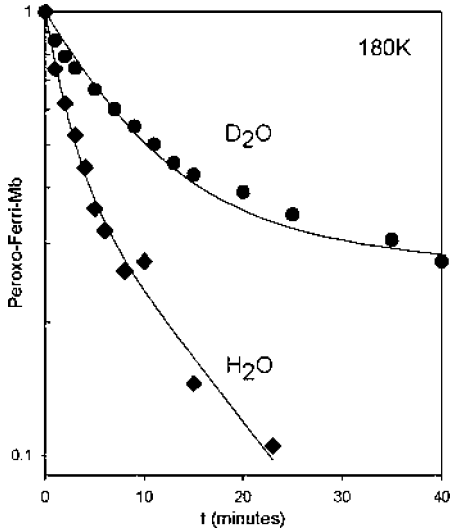

Figure 2. EPR signal intensity of Mb intermediate **1** in H_2O (◆) and D_2O buffers (●) during stepwise annealing to 180 K and recooling to 77 K for analysis; intensities are peak−trough heights of g_2 feature.

for transfer of proton 1 in P450cam,[19] presumably corresponding to two conformational substates. For the majority (∼60%) rapid phase, the decay constants are $\tau(H_2O) = 2.2$ min, $\tau(D_2O) = 15$ min, giving *solv*-KIE $= \tau(D_2O)/(H_2O) = 3.8$; for the slow phase, *solv*-KIE ≈ 20.

We have made an unprecedented observation of enzymatic proton transfer in HO (eq 1) at liquid helium temperatures. Such a process would be suppressed at these temperatures if it were described by barrier-crossing proton transfer, and thus we infer that it occurs via through-barrier, quantum proton tunneling. A useful "umbrella" under which to discuss our observations is the picture of environmentally coupled tunneling.[21−23] This model links proton transfer to two classes of protein motions: environmental reorganization (λ in Marcus-like[24] equations),[23,25] protein fluctuations ("active dynamics"; gating) which modulate the distance of proton transfer. The helium-temperature results show that HO has an active-site structure fully organized to support proton tunneling, with a relatively short proton-transfer distance (narrow barrier) and no need for dynamic modulation of the distance. As proton transfer in P450cam can occur at ∼ 50 K and above, where "gating" fluctuations still remain frozen,[26,27] proton transfer in P450cam likely is analogous to that in HO, but with an increased environmental reorganization energy (λ) which slows eq 1 and introduces the temperature dependence. For Mb/Hb, the rapid onset of eq 1 at $T > 170$ K is attributable to a combination of two factors: (i) relaxation of the heme pocket to accommodate the reduced oxyheme, reducing the width of the static barrier for proton transfer; (ii) the onset of dynamical modulation of the width of the tunneling barrier as the protein undergoes a "glass transition"[26,27] and gating fluctuations become possible. The HO(D140X) and P450cam-(D251N) mutations disrupt the distal pocket, converting the proton-delivery process into one like that in Mb. We anticipate that detailed studies of the temperature/pH dependence of proton transfer in these proteins and their mutants will offer deeper insights into protein control of proton delivery.

Acknowledgment. This work has been supported by the NIH (HL 13531, B.M.H.), by the Grants-in-Aid from the Ministry of Education, Science, Culture and Sports, Japan (1214702 and 16370056 to M.I.S. and 147403 to T.M.), and by Takeda Science Foundation (M.I.S.). We thank Prof. H. Halpern (U. of Chicago) for access to a Gammacell 220 ^{60}Co irradiator.

References

(1) Sono, M.; Roach, M. P.; Coulter, E. D.; Dawson, J. H. *Chem. Rev.* **1996**, *96*, 2841−2887.
(2) Ortiz de Montellano, P. R., Ed. *Cytochrome P450*, 2nd ed.; Plenum Press: New York, 1995.
(3) Ortiz de Montellano, P. R. *Curr. Opin. Chem. Biol.* **2000**, *4*, 221−227.
(4) Rosen, G. M.; Tsai, P.; Pou, S. *Chem. Rev.* **2002**, *102*, 1191−1199.
(5) Alderton, W. K.; Cooper, C. E.; Knowles, R. G. *Biochem. J.* **2001**, *357*, 593−615.
(6) Schlichting, I.; Berendzen, J.; Chu, K.; Stock, A. M.; Maves, S. A.; Benson, D. E.; Sweet, B. M.; Ringe, D.; Petsko, G. A.; Sligar, S. G. *Science* **2000**, *287*, 1615−1622.
(7) Unno, M.; Matsui, T.; Chu, G. C.; Couture, M.; Yoshida, T.; Rousseau, D. L.; Olson, J. S.; Ikeda-Saito, M. *J. Biol. Chem.* **2004**, *279*, 21055−21061.
(8) Cukier, R. I.; Nocera, D. G. *Annu. Rev. Phys. Chem.* **1998**, *49*, 337−369.
(9) Mayer, J. M. *Annu. Rev. Phys. Chem.* **2004**, *55*, 363−390.
(10) In certain cases a superoxo-ferriheme is trapped.
(11) Davydov, R.; Satterlee, J. D.; Fujii, H.; Sauer-Masarwa, A.; Busch, D. H.; Hoffman, B. M. *J. Am. Chem. Soc.* **2003**, *125*, 16340−16346.
(12) Davydov, R.; Matsui, T.; Fujii, H.; Ikeda-Saito, M.; Hoffman, B. M. *J. Am. Chem. Soc.* **2003**, *125*, 16208−16209.
(13) In certain cases a superoxo-ferroheme is trapped.
(14) Davydov, R.; Kofman, V.; Fujii, H.; Yoshida, T.; Ikeda-Saito, M.; Hoffman, B. *J. Am. Chem. Soc.* **2002**, *124*, 1798−1808.
(15) Oxyferrous rat HO-[14] and Mb[16] were prepared in H_2O and D_2O buffer (0.02 M Kpi, pH 7) which contained 20% (v/v) glycerol or d_3-glycerol, respectively. Cryoreduction of samples held in a helium-flow cryostat within the X-band cavity of an EPR spectrometer was achieved at 4−6 K by in situ irradiation with 3 MeV electrons from a van de Graaf accelerator.[17] Samples were irradiated for ∼10 min at 4.2 K.; in one set of experiments the temperature was rapidly raised to ∼6 K to increase spectrometer stability and EPR spectra were promptly collected in 5 min; in a second set, the temperature was increased to 9 K before data collection.
(16) Davydov, R.; Kofman, V.; Nocek, J.; Noble, R. W.; Hui, H.; Hoffman, B. M. *Biochemistry* **2004**, *43*, 6330−6338.
(17) Davydov, R.; Makris, T. M.; Kofman, V.; Werst, D. W.; Sligar, S. G.; Hoffman, B. M. *J. Am. Chem. Soc.* **2001**, *123*, 1403−1415.
(18) A *solv*-KIE also is seen for this reaction within P450cam ethylene-glycol/buffer, but the process is complicated by the appearance of multiple oxy-P450cam substates in this medium.
(19) Davydov, R.; Perera, R.; Jin, S.; Yang, T.-C.; Bryson, T. A.; Sono, M.; Dawson, J. H.; Hoffman, B. M. *J. Am. Chem. Soc.* Submitted.
(20) Kappl, R.; Höhn-Berlage, M.; Hüttermann, J.; Bartlett, N.; Symons, M. C. R. *Biochim. Biophys. Acta* **1985**, *827*, 327−343.
(21) Knapp, M. J.; Klinman, J. P. *Eur. J. Biochem.* **2002**, *269*, 3113−3121.
(22) Kohen, A.; Klinman, J. P. *Acc. Chem. Res.* **1998**, *31*, 397−404.
(23) Scrutton, N. S.; Basran, J.; Sutcliffe, M. J. *Eur. J. Biochem.* **1999**, *264*, 666−671.
(24) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265−322.
(25) Hammes-Schiffer, S. *Acc. Chem. Res.* **2001**, *34*, 273−281.
(26) Austin, R. H.; Beeson, K. W.; Eisenstein, L.; Frauenfelder, H.; Gunsalus, I. C. *Biochemistry* **1975**, *14*, 5355−5373.
(27) Ringe, D.; Petsko, G. A. *Biophys. Chem.* **2003**, *105*, 667−680.

JA044646T