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A Highly Reactive P450 Model Compound I

Seth R. Bell and John T. Groves*

Department of Chemistry, Princeton University, Princeton, New Jersey 08544

Received April 27, 2009; E-mail: jtgroves@princeton.edu

The substrate oxygenation reactions mediated by cytochrome P450 proteins comprise many key transformations in steroid biosynthesis and drug metabolism. Significant advances in heme-protein enzymology,[1] the characterization of synthetic model compounds,[2] and computational approaches[3] have pointed to a reactive oxoiron(IV)−porphyrin π-radical cation in the consensus mechanism. Compound I model compounds, such as the thoroughly characterized $[OFe^{IV}-TMP]^+$,[4,5] have exhibited a full range of oxygen transfer reactions.[6] However, the low kinetic reactivity of $[OFe^{IV}-TMP]^+$ has left unanswered just how the protein manages to generate a sufficiently reactive intermediate and what that reactive species is.[7−10] We have shown that cationic oxomanganese(V)−porphyrin complexes such as OMn^{V}−4-TMPyP display very high rates for oxygen transfer reactions in aqueous solution.[11,12] Here we report the detection and kinetic characterization of $[OFe^{IV}$−4-TMPyP$]^+$, which shows extraordinary rates for C−H hydroxylations.

The reaction of Fe^{III}−4-TMPyP with *m*CPBA was analyzed by stopped-flow spectrophotometry, as we have previously described.[11,13] As can be seen in the UV−vis spectral time course (Figure 1), the starting iron(III)−porphyrin spectrum (λ_{max} 421 nm) was completely transformed within 20 ms into that of a new intermediate, **1**, displaying a weak, blue-shifted Soret band at 402 nm and an absorbance at 673 nm, typical of a porphyrin π-radical cation.[4,14] Accordingly, we assigned the $[OFe^{IV}$−4-TMPyP$]^+$ formulation to **1**. Global analysis afforded a second-order rate constant $k_1 = (1.59 \pm 0.06) \times 10^7$ M^{-1} s^{-1} for the appearance of **1**. In a slower, subsequent phase of the reaction (Scheme 1), **1** was transformed into the well-characterized OFe^{IV}−4-TMPyP (λ_{max} 427 nm; $k_2 = 8.8 \pm 0.1$ s^{-1}).[15−17] Isosbestic points for the conversion of **1** to OFe^{IV}−4-TMPyP were apparent at 413 and 570 nm. This transformation was more rapid at higher pH, in contrast to the behavior of the corresponding OMn^{V}−4-TMPyP complex.[11,18]

Intermediate **1** was found to be very highly reactive toward C−H bond hydroxylations. Slow addition of *m*CPBA to a solution of 5 mM xanthene and 50 μM Fe^{III}−4-TMPyP was monitored by ^{1}H and ^{13}C NMR spectroscopy. The major product, 9-xanthydrol (H9, δ 5.75; C9, δ 62), was formed in 90% yield based on added oxidant (Figure S1 in the Supporting Information). A small amount of xanthone was also formed. The Fe^{III} catalyst was recovered with negligible decomposition. Significantly, when the oxidation was carried out in 47.5% $^{18}OH_2$, electrospray ionization mass spectrometry of the 9-xanthydrol product indicated 21% ^{18}O incorporation (m/z 199/197 = 0.27). This partial exchange is consistent with an oxygen-rebound reaction scenario with ∼50% dilution of the ^{16}O-peroxyacid-derived oxygen with ^{18}O water occupying the second axial ligation site.[19,20] Xanthene/xanthene-d_2 (1:1) revealed a modest kinetic isotope effect, $k_H/k_D = 2.1$ (m/z 197/198).

Since the oxidation of Fe^{III}−4-TMPyP by *m*CPBA was so fast, we could conveniently monitor the kinetics of the reaction of **1** with xanthene using a single-mixing stopped-flow experiment (Figure 2A). Global analysis of the spectral data produced a rate constant $k_3 = (3.6 \pm 0.3) \times 10^6$ M^{-1} s^{-1}. A similar value was obtained by monitoring at 673 nm (Figure 2A). Control experiments showed that OFe^{IV}−4-TMPyP had negligible reactivity toward xanthene over this time course.

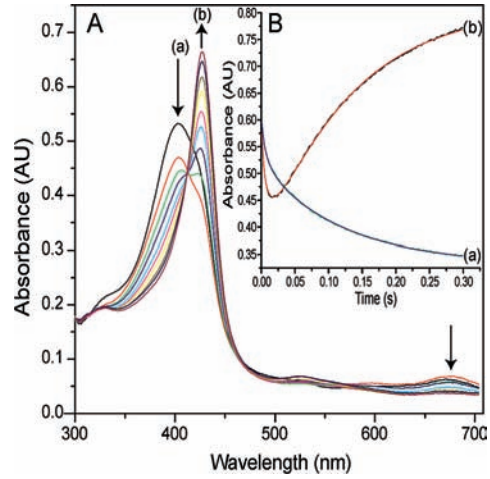
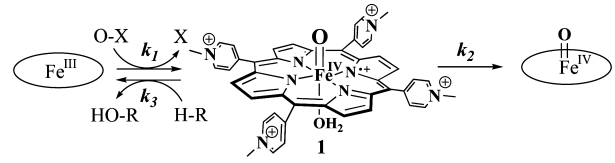


Figure 1. (A) UV−vis transients observed upon mixing of 10 μM Fe^{III}−4-TMPyP with 3 equiv of *m*CPBA at pH 4.7, 10.0 °C. (B) Data obtained at (a) 402 and (b) 427 nm and the superimposed global fits.

Scheme 1. Kinetic Partitioning of $[OFe^{IV}$−4-TMPyP$]^+$ (**1**) between Spontaneous Decay to OFe^{IV}−4-TMPyP (k_2) and Substrate Hydroxylation Resulting in the Formation of Fe^{III}−4-TMPyP (k_3)



Reactions of fluorene-4-carboxylic acid and 4-isopropyl- and 4-ethylbenzoic acid with **1** also gave very high rates for C−H hydroxylation, with second-order rate constants of $(1.80 \pm 0.06) \times 10^5$, $(9.0 \pm 0.2) \times 10^3$, and $(7.5 \pm 0.1) \times 10^3$ M^{-1} s^{-1}, respectively. The corresponding alcohol products were formed in each case. These reactions are remarkably fast, and as can be seen in Figure 2B, the rates correlated well with the scissile C−H bond dissociation energy (BDE), indicating a homolytic hydrogen abstraction transition state.[21] Reaction of **1** with bromide ion afforded hypobromite with $k_3 = (3.14 \pm 0.04) \times 10^5$ M^{-1} s^{-1}.[13]

These results show that $[OFe^{IV}$−4-TMPyP$]^+$ (**1**), which is accessed chemically from a peroxide precursor, is *orders of magnitude* more reactive than the well-studied tetramesityl analogues.[4,6,7,9] Mapping the rate constants observed here for C−H bond cleavage by **1** onto the Brønsted−Evans−Polanyi relationship[21,22] for similar substrates (as shown in the Table of Contents graphic) yielded an estimate of ∼100 kcal/mol for the BDE of H−OFe^{IV}−4-TMPyP; this is much larger than the values estimated for H−OFe^{IV}−TMP (92 kcal/mol)[7] and H−OFe^{III}−TPFPP (∼86

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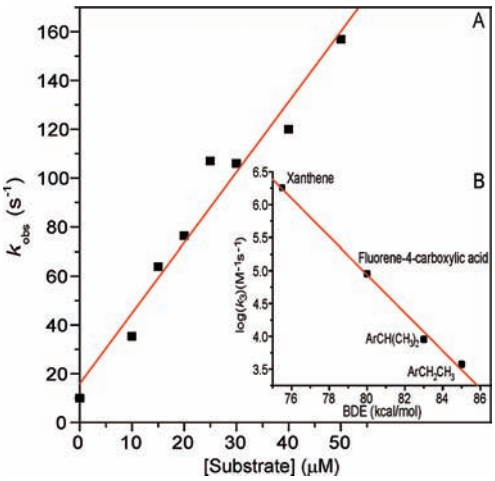

Figure 2. (A) Observed rate constant k_{obs} at 673 nm vs [xanthene] for its oxidation by **1** at pH 4.7, 14.5 °C, yielding $k_3 = (2.8 \pm 0.3) \times 10^6$ M^{-1} s^{-1}. (B) Correlation of k_3 with C−H bond dissociation energy.

kcal/mol).[23] Indeed, **1** showed a rate constant for ethylbenzoic acid hydroxylation similar to that reported recently by Newcomb and co-workers[24] for ethylbenzene with CYP119 and chloroperoxidase compound I.

The interesting question is *why* synthetic OFe^{IV}−porphyrin π-radical cations should have such a great variability in their intrinsic reactivities. We have shown that electron-withdrawing meso substituents dramatically *decrease* the reactivity of $oxoMn^{V}$−porphyrins.[11] The effects have been traced to stabilization of the singlet d^2 ground state with respect to the triplet and quintet manifolds, stabilization of the porphyrin HOMO, and the unique centrosymmetric *trans*-dioxo ligand arrangement.[25] Clearly, the situation for iron described here is markedly different. A relatively high redox potential for porphyrin ring oxidation is expected for Fe^{III}−4-TMPyP. Further, efficient transmission of the electron deficit to the metal core is assured by the large orbital coefficients on the meso carbon and the pyrrole nitrogens in the a_{2u} porphyrin HOMO. Indeed, the pK_a of the axial water in $diaquaFe^{III}$−4-TMPyP is only 5.5, while the corresponding acidity of $diaquaFe^{III}$−TMPS is 7.5.[26] Density functional theory calculations have shown that reaction barriers in oxo transfer reactions may be lowered by decreasing the energy gaps to higher-spin configurations for both manganese[25,27] and iron.[22,28] A decrease in the σ-donor properties of the coordinating pyrrole nitrogens due to the meso substituent would be expected to *decrease* the energy of the iron $d_{x^2-y^2}$ orbital. The high-spin state of an unsubstituted $oxoFe^{IV}$−porphyrin radical cation, with the iron $d_{x^2-y^2}$ orbital singly occupied, has been calculated to be only 14 kcal/mol above the nearly degenerate doublet and quartet ground-state structures.[29] We suggest that the high kinetic reactivity observed here experimentally for **1** may result from both a low-lying a_{2u} porphyrin HOMO and facilitated spin-state crossing phenomena in the course of the reaction. More generally, the results suggest that even subtle charge modulation at the heme active site of P450 to accomplish a similar tuning of state-crossing energies or lower stability of the porphyrin π-radical cation could result in high reactivity of a cytochrome P450 compound I.

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Supporting Information Available: Experimental details and NMR data (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) Abbreviations: TMP, 5,10,15,20-tetramesitylporphyrin; 4-TMPyP, 5,10,15,20-tetrakis(*N*-methyl-4-pyridinium)porphyrin; *m*CPBA, *m*-chloroperoxybenzoic acid; TMPS, 5,10,15,20-tetramesityloctasulfonate.
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