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Design of More Powerful Iron-TAML Peroxidase Enzyme Mimics

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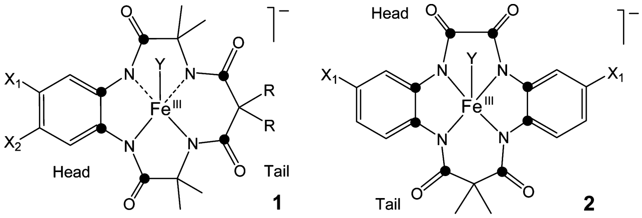
Received October 21, 2009; E-mail: tc1u@andrew.cmu.edu

Peroxidase enzymes activate hydrogen peroxide to perform myriad oxidations in nature[1,2] and have long been targeted by biomimetic chemists.[3,4] Recent mechanistic studies have shown that first generation Fe^{III}-TAMLs (**1**, Figure 1, TAML stands for *t*etra*a*mido *m*acrocyclic *l*igand) rival peroxidases in catalytic performance, functioning efficiently in water under ambient conditions at nM to low μM concentrations.[5] Peroxidase-like activity dominates over wasteful catalase-like activity (H_2O_2 disproportionation) and catalyst inactivation. Fe^{III}-TAML/H_2O_2 systems efficiently purify water of numerous persistent organic pollutants[6−8] and kill hardy pathogens.[9]



(**a**) $X_1 = X_2 = H$, R = CH_3
(**b**) $X_1 = X_2 = Cl$, R = F
(**c**) $X_1 = NO_2$, $X_2 = H$, R = F

(**d**) $X_1 = H$
(**e**) $X_1 = NO_2$

Figure 1. Fe^{III}-TAMLs of ***B**** (**1**) and ***D**** (**2**) generations: sp^2 carbons attached to the N-donor atoms are highlighted. Y is typically water.

In first-generation Fe^{III}-TAMLs (***Fe−B****, prototype is **1a**, Figure 1), adding fluorine to the tail as in **1b, c**[10,11] produces faster peroxidase-like rates, increased oxidative aggression and better medium and operational stabilities.[10−14] Since green chemicals for distributive technologies should be comprised of the common elements of biochemistry,[15] we set out to design a F-free Fe^{III}-TAML with the reactivity advantages of the fluorinated catalysts. Fluorine's singularly high electronegativity (χ) polarizes the iron atom to deliver the reactivity advantages.[11] F-Effects are difficult to mimic. We hypothesized that smaller increases in χ, enacted closer to the metal than the F atoms in **1**, would compensate for the fluorine effect. So, we produced the ***D**** system by swapping the tail F atoms of **1b, c** for methyl groups and countered this by replacing **1**'s two amide α *sp*^{3} carbons (χ, 2.5) with *sp*^{2} carbons (χ, 2.75); the electronegativity at carbon in any bond increases with its *s*-character.[16] Also, the oxalyldiamide fragment designed into **2** places electron-withdrawing carbonyl *sp*^{2} carbons β to two head amide-nitrogen donors. Only moderate aqueous stability was found in **2d**. **2e** was designed as the NO_2 groups were expected to improve this stability.[13] In 0.1 M phosphate at pH 5 and 25 °C, **2d** and **2e** have respective half-lives of 52 and 3500 s.

The synthesis of **2** follows the same basic scheme used to produce **1**[17] but is more straightforward. It includes the (*a*) protection of one amino group of 1,2-diaminobenzene, (*b*) double amination of dimethyl malonyl dichloride, (*b*) HCl deprotection (hydrazine required for **1**), (*c*) macrocyclization with oxalyl dichloride, and (d) base-assisted insertion of iron. An X-ray crystallographic study of **2e** (Figure 2) indicates that the ***D**** system induces square pyramidal iron(III) typical for Fe^{III}-TAMLs.[13,18]

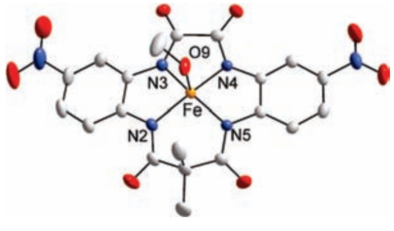
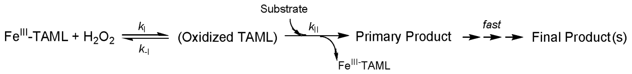


Figure 2. An ORTEP diagram of the anionic part of **2e** (Y = H_3COH) (H atoms are not shown for clarity; thermal ellipsoids are at 50% probability level). Selected bond distances: Fe−N2 1.9165(20), Fe−N3 1.8612(17), Fe−N4 1.8831(17), Fe−N5 1.8992(17), Fe−O9 2.0624(18) Å.

To evaluate these design ideas for improving oxidation catalysts, we have studied in detail the **2e**/H_2O_2 oxidizing activity toward the dye, Orange II ([4-[(2-hydroxynaphthyl)azo]-benzenesulfonic acid], Na^+ salt) at pH 7, 9, and 11 and compared the results with studies for **1a**[19] and **1b** (SI, Figure 2S). The data in Figure 3A−C are consistent with the previously proposed catalysis mechanism, which leads to the kinetic eq 1 under steady state conditions.[10,11]



$$-\frac{d[\text{Substrate}]}{dt} = \frac{k_I k_{II}[\text{Fe}^{III}][H_2O_2][\text{Substrate}]}{k_{-I} + k_I[H_2O_2] + k_{II}[\text{Substrate}]} \qquad (1)$$

The corresponding effective rate constants, k_I and k_{II}, were calculated by fitting the experimental data to eq 1 as shown in the colored surfaces of Figure 3 under the assumption that k_{-I} is negligible (Table 1).[10,11]

The greatest **2e** reactivity is found at pH 9, whereas the maximum reactivities for **1a** and **1b** occur at pH 11 and 10.2, respectively.[10,11] The values of k_I and k_{II} define **2e** as an exceptional peroxidase mimic. The k_I at pH 9 of 1.6×10^4 M^{-1} s^{-1} falls around the lower limit for peroxide-activating enzymes,[10] and the k_{II} value of ca. 10^6 M^{-1} s^{-1} is in the midrange of enzymatic catalytic activity.[1] At pH 7, **2e** is the most reactive Fe^{III}-TAML activator to date bleaching Orange II 5 times faster than **1a** and 2 times faster than **1b** (SI, Figure 1S).

Catalysts **1a**, **b** are known to undergo slow self-destruction while catalyzing peroxide oxidation reactions.[12] We therefore emphasize the significance of the inset in Figure 4 which shows (at pH 11 and where $k_I[H_2O_2] \gg k_{II}$[Substrate]) that an exponential kinetic curve holds for ca. 10 half-lives for the disappearance of Orange

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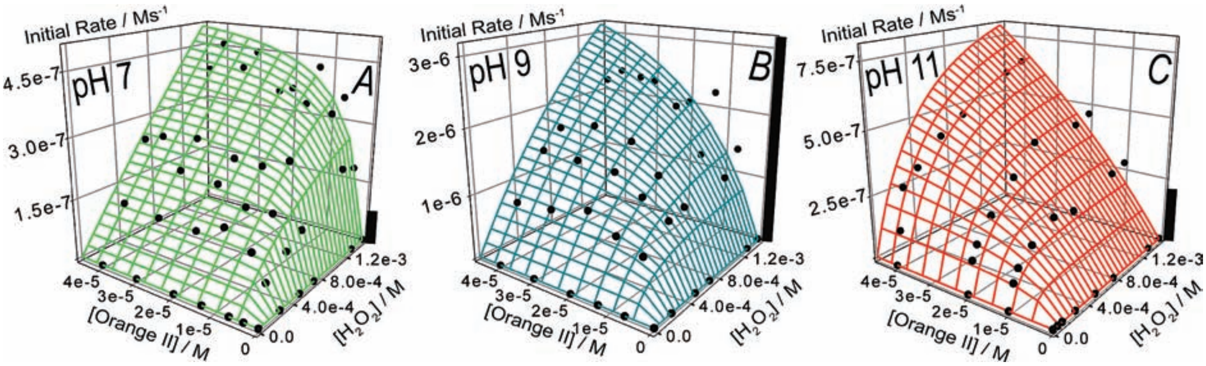

Figure 3. 3D-plots showing the initial rates of **2e**-catalyzed bleaching of Orange II by H_2O_2 as a function of $[H_2O_2]$ and [Orange II] at pH 7 (A), 9 (B), and 11 (C) at [**2e**] 2×10^{-7} M, 0.01 M phosphate, 25 °C. The bars on the right of each plot compare catalytic activity referred to the highest activity at pH 9. Each data point is a mean value of three determinations. The meshes are calculated using the rate constants in Table 1.

II to indicate that first-order kinetics in the dye prevail and that no catalyst degradation occurs while the process is ongoing. The calculated pseudofirst-order rate constant k_{obs} could thus be used for the calculation of k_{II} simply by dividing the $k_{\text{obs}} = 0.029 \pm 0.001\ \text{s}^{-1}$ by the **2e** concentration. The resulting value of $1.45 \times 10^5\ \text{M}^{-1}\ \text{s}^{-1}$ agrees well with that reported in Table 1 at pH 11 obtained from *all* the data points in Figure 3C.

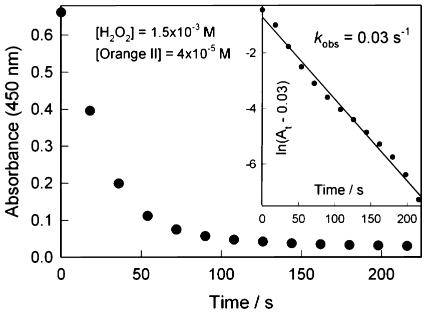

Figure 4. Catalytic bleaching by **2e** at pH 11: Inset shows that the bleaching follows exponential kinetics over approximately 10 half-lives.

Table 1. Rate Constants ($\text{M}^{-1}\ \text{s}^{-1}$) for the Fe^{III}-TAML Catalyzed Bleaching of Orange II by $[H_2O_2]$ at 25 °C in 0.01 M Phosphate

pH	Fe-TAML	k_{I}	k_{II}
7	**2e**	$(1.9 \pm 0.1) \times 10^3$	$(5.2 \pm 0.7) \times 10^5$
9	**2e**	$(16 \pm 2) \times 10^3$	$(10 \pm 1) \times 10^5$
11	**2e**	$(10 \pm 1) \times 10^3$	$(1.2 \pm 0.1) \times 10^5$
11	**1a**[5]	3.5×10^3	1.5×10^4
11	1**b** (SI)	$(8.4 \pm 0.6) \times 10^3$	$(1.1 \pm 0.1) \times 10^5$

In conclusion, by focusing on the electronegativity of groups within the TAML framework, we have been able to iteratively design a new type of iron-TAML activator, called ***Fe-D****, that is comprised exclusively of the common elements of biochemistry[15] and that exhibits the highest reactivity and stability, especially at pH 7, found to date. So this is a case where the singular electronic effects of fluorine's high electronegativity have been mimicked by the type of detailed iterative design that green chemists are working to develop. Future work will quantify the relationship between pH, stability, and reactivity in the ***Fe-D**** system.

Acknowledgment. Dedicated to Prof. W. F. Boldridge on his 92nd birthday. Support (T.J.C.): Heinz Endowments, C. E. Kaufman Foundation; HHMI (C.T.). We thank R. Gil for NMR assistance and the NSF for NMR Instrumentation (CHE-0130903).

Supporting Information Available: Synthetic procedures, details of X-ray experiment and kinetic measurements. This material is available free of charge via the Internet at http://pubs.acs.org

References

(1) Dunford, H. B. *Heme Peroxidases*; Wiley-VCH: New York, 1999.
(2) Meunier, B. Heme-peroxidases. In *Comprehensive Coordination Chemistry II*; Elsevier: Oxford, 2004; Vol. 8, pp 261−280.
(3) Meunier, B. *Biomimetic Oxidations Catalyzed by Transition Metal Complexes*; Imperial College Press: London, 2000.
(4) Meunier, B. *Models of heme peroxidases and catalases*; Imperial College Press: London, 2000; pp 171−214.
(5) Ryabov, A. D.; Collins, T. J. *Adv. Inorg. Chem.* **2009**, *61*, 471.
(6) Chanda, A.; Khetan, S. K.; Banerjee, D.; Ghosh, A.; Collins, T. J. *J. Am. Chem. Soc.* **2006**, *128*, 12058.
(7) Sen Gupta, S.; Stadler, M.; Noser, C. A.; Ghosh, A.; Steinhoff, B.; Lenoir, D.; Horwitz, C. P.; Schramm, K.-W.; Collins, T. J. *Science* **2002**, *296*, 326.
(8) Shappell, N. W.; Vrabel, M. A.; Madsen, P. J.; Harrington, G.; Billey, L. O.; Hakk, H.; Larsen, G. L.; Beach, E. S.; Horwitz, C. P.; Ro, K.; Hunt, P. G.; Collins, T. J. *Environ. Sci. Technol.* **2008**, *42*, 1296.
(9) Banerjee, D.; Markley, A. L.; Yano, T.; Ghosh, A.; Berget, P. B.; Minkley, E. G., Jr.; Khetan, S. K.; Collins, T. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 3974.
(10) Ghosh, A.; Mitchell, D. A.; Chanda, A.; Ryabov, A. D.; Popescu, D. L.; Upham, E.; Collins, G. J.; Collins, T. J. *J. Am. Chem. Soc.* **2008**, *130*, 15116.
(11) Popescu, D.-L.; Chanda, A.; Stadler, M. J.; Mondal, S.; Tehranchi, J.; Ryabov, A. D.; Collins, T. J. *J. Am. Chem. Soc.* **2008**, *130*, 12260.
(12) Chanda, A.; Ryabov, A. D.; Mondal, S.; Alexandrova, L.; Ghosh, A.; Hangun-Balkir, Y.; Horwitz, C. P.; Collins, T. J. *Chem.—Eur. J.* **2006**, *12*, 9336.
(13) Ghosh, A.; Ryabov, A. D.; Mayer, S. M.; Horner, D. C.; Prasuhn, D. E., Jr.; Sen Gupta, S.; Vuocolo, L.; Culver, C.; Hendrich, M. P.; Rickard, C. E. F.; Norman, R. E.; Horwitz, C. P.; Collins, T. J. *J. Am. Chem. Soc.* **2003**, *125*, 12378.
(14) Polshin, V.; Popescu, D.-L.; Fischer, A.; Chanda, A.; Horner, D. C.; Beach, E. S.; Henry, J.; Qian, Y.-L.; Horwitz, C. P.; Lente, G.; Fabian, I.; Münck, E.; Bominaar, E. L.; Ryabov, A. D.; Collins, T. J. *J. Am. Chem. Soc.* **2008**, *130*, 4497.
(15) Collins, T. *Science* **2001**, *291*, 48.
(16) Bent, H. A. *Chem. Rev.* **1961**, *61*, 275.
(17) Ghosh, A., PhD Dissertation, Carnegie Mellon, Pittsburgh, 2004.
(18) Bartos, M. J.; Gordon-Wylie, S. W.; Fox, B. G.; Wright, L. J.; Weintraub, S. T.; Kauffmann, K. E.; Münck, E.; Kostka, K. L.; Uffelman, E. S.; Rickard, C. E. F.; Noon, K. R.; Collins, T. J. *Coord. Chem. Rev.* **1998**, *174*, 361.
(19) Chahbane, N.; Popescu, D.-L.; Mitchell, D. A.; Chanda, A.; Lenoir, D.; Ryabov, A. D.; Schramm, K.-W.; Collins, T. J. *Green Chem.* **2007**, *9*, 49.

JA9086837