Biomimetic Oxidation Studies: Alkane Functionalization in Aqueous Solution Utilizing Methane Monooxygenase Model Enzyme Assemblies

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Introduction

Methane monooxygenase (MMO) and cytochrome P-450 enzymes, although active in aqueous media, have a structure wherein the diiron non-porphyrin and iron porphyrin active sites, respectively, are embedded within a hydrophobic pocket, which enables the uptake and subsequent functionalization (hydroxylation) of alkanes. In the continuing search for biomimetic models for these metalloenzyme oxidation catalysts, the emphasis is usually placed on the structure of the active site, and if this biomimic can perform alkane functionalization chemistry. Generally, the macroenvironment and the efficacy of the oxidation process in aqueous media with the various monooxygenase model complexes has not been a focus of the bioinorganic catalysis community. Nevertheless, for metalloporphyrins, some significant research has been directed toward alkene epoxidation and alkane functionalization in aqueous media, by embedding the active site model compounds within lipid bilayers, thermotropic liquid crystals, and micelles. However, in the more nascent field of MMO biomimetic catalysis, the question of the macroenvironment and the possibility of alkane functionalization in aqueous media has not been extensively studied.[1]

In order to study the functionalization of hydrophobic alkanes in aqueous media using a biomimetic MMO enzyme assembly, including the active site and a hydrophobic pocket, we have developed both a derivatized amorphous silica, which allows one to balance the hydrophilicity and the hydrophobicity of a silicate surface, and a classically formed aqueous micelle system utilizing a cationic surfactant, cetyltrimethylammonium hydrogensulfate.[1] In our derivatized amorphous silica technique, hydrophilic poly(ethylene) oxide (PEO) and/or hydrophobic poly(propylene) oxide (PPO) were covalently attached to siloxane monomers. The siloxane monomers were then polymerized using sol-gel synthesis that yields an amorphous silicate with PEO and/or PPO anchored to the silica surface. The PEO/PPO phases are to be considered comparable to anchored solvents, where the anchoring of the solvent is somewhat analogous to the use of bonded phase columns in gas chromatography in place of simple, physically adsorbed phases. Therefore, active MMO biomimetic complexes, such as *in situ* formed $[Fe_2O(\eta^1\text{-}H_2O)(\ (\eta^1\text{-}OAc)(TPA)_2]^{3+}$, **1**, TPA = tris[(2-pyridyl)methyl]amine), from the $[Fe_2O(\mu\text{-}OAc)(TPA)_2]^{3+}$ analog, **2**, at pH = 4.2, can then be embedded into the PEO and PPO tethered silica (Figure 1). Thus, the placement of the precatalyst into the anchored solvents allows one to potentially functionalize alkanes in aqueous solution with *t*-butyl hydroperoxide (TBHP) in the presence of oxygen gas (O_2) and, in this paper, we compare this former MMO model enzyme system to the latter mentioned technique, where the precatalyst, alkane, and oxidants were dissolved within the hydrophobic layers of the aqueous micelle dispersions (Figure 2).[1]

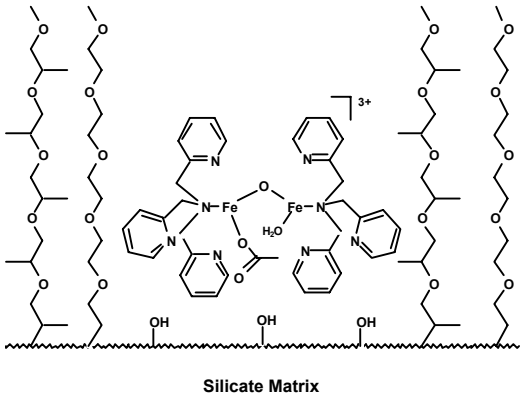

Figure 1: A schematic representation of *in situ* formed $[Fe_2O(\eta^1\text{-}H_2O)(\ (\eta^1\text{-}OAc)(TPA)_2]^{3+}$, **1**, embedded in the PEO, PPO derivatized silica, for alkane functionalization in water at pH 4.2.

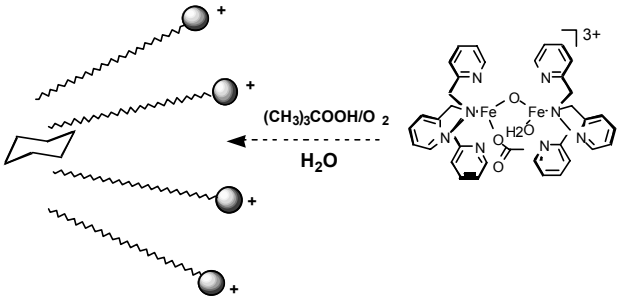

Figure 2: A schematic representation of *in situ* formed **1** diffusing, along with the oxidants, TBHP/O_2, into aqueous micelles formed with the surfactant, cetyltrimethylammonium hydrogensulfate, for alkane functionalization in water at pH 4.2.

Results and Discussion

Alkane Functionalization Studies with Surface Derivatized Silicates

As an initial example of alkane functionalization using various surface derivatized silicates, the oxidation of cyclooctane was carried out with *in situ* formed (pH = 4.2) $[Fe_2O((\eta^1\text{-}H_2O)(\eta^1\text{-}OAc)(TPA)_2]^{3+}$, **1**, as the precatalyst, in the presence of TBHP/O_2. From the results presented in Table 1, one can clearly observe that silicates with both hydrophobic PPO and hydrophilic PEO tethers provided a significantly improved reaction medium, compared to either PEO or PPO alone, with ~140 turnovers (TON, mmol of product/mmol of catalyst) being observed with 10 mol% PPO and 10 mol% PEO–SiO_2, while approximately 25 TON were observed with PPO–SiO_2 or PEO–SiO_2. It is interesting to note that $Fe(ClO_4)_3$ showed no alkane functionalization activity when impregnated in 10 mol% PPO and 10 mol% PEO–SiO_2, which indicates that the TPA ligands might be necessary for solubilization in the anchored polyether solvents, and therefore, for catalysis to occur.

The reaction selectivity also differed as a function of the silicate support. For example, with PPO–SiO_2 or PEO–SiO_2, the major product was cyclooctanone and the minor product was cyclooctene, formed consistently in a ratio of ~1.2:1. Futhermore, very minor amounts (<5%) of cyclooctanol and the mixed dialkylperoxide, cyclooctyl-*t*butylperoxide, were also formed. For 10 mol% PPO and 10 mol% PEO–SiO_2, about 25% of the products formed was the dehydrogenated cyclooctene, while the remaining 75% of oxygenated products, cyclooctanol, cyclooctanone, and cyclooctyl-*t*-butylperoxide, were formed in approximately a 1:3:2 ratio. The reaction was carried out at pH = 4.2 and, as in previous studies, the solution turned colorless from its original yellow color with formation of the proposed $[(TPA)Fe^{2+}(\mu\text{-}OH)Fe^{3+}(TPA)]$ complex that designates a Haber-Weiss process in the decomposition of TBHP. This observation is further supportive of the Haber-Weiss process in that after the TBHP oxidant was completely decomposed, the yellow color returned.

Table 1. **Oxidation of Cyclooctane with Complex 1 Embedded in Surface Derivatized Silica.**[a]

Silica Support	Total TON (hr^{-1})	Products[b]
10% PEO–SiO_2	19 (6.3)	43%, 55%, 1%, 1%
20% PEO–SiO_2	25 (8.3)	42%, 56%, 1%, 1%
10% PPO,10% PEO–SiO_2	142 (47.3)	26%, 35%, 13%, 25%
10% PPO–SiO_2	24 (8.0)	40%, 55%, 0%, 5%
20% PPO–SiO_2	21 (7.0)	37%, 49%, 0%, 4%
none	< 1	na

[a]Reactions were carried out by mixing 0.38 mmol cyclooctane, 3.8 mmol TBHP in 5 mL water at pH 4.2 and 0.38 µmol of *in situ* formed (as 1% wt. $[Fe_2O((\eta^1\text{-}H_2O)(\eta^1\text{-}OAc)(TPA)_2]^{3+}$ on derivatized silica) for 3 h at room temperature. [b]Mol% total products for cyclooctene, cyclooctanone, cyclooctanol, and cyclooctyl-*t*-butyl peroxide, respectively. Yields based on TBHP were 80-90%.

Thus, it is clearly evident that alkane functionalization reactions with TBHP/O_2 as oxidants, and in the presence of MMO biomimetic complexes, such as **1**, as well as other metal complexes, are initiated by formation of *t*-BuO• and *t*-BuOO• radicals.[1] In this case, by invoking the Haber-Weiss mechanism, the intermediate cyclooctyl radical can react with either dioxygen leading to the formation of cyclooctanone and cyclooctanol, via the proposed cyclooctyl hydroperoxide, or with a *t*-BuOO• radical to form the mixed dialkyl peroxide. Interestingly, and uniquely for cyclooctane, cyclooctene was formed as a significant product, plausibly by an intramolecular oxidative dehydrogenation pathway from the cyclooctyl radical. Moreover, cyclooctene is *not* formed by dehydration of cyclooctanol as its use as a substrate, under typical reaction conditions, yielded no cyclooctene, but was converted to cyclooctanone.

The scope of the alkane functionalization reaction in aqueous solution with TBHP/O_2 and complex **1** in 10 mol% PPO and 10 mol% PEO–SiO_2 was extended to additional substrates (Table 2). For cycloalkanes, it was found that the activity sequence was: cyclohexane > cycloheptane ~ cyclooctane. However, in contrast to the reaction with cyclooctane, no alkene dehydrogenation products were observed, but the product ratios, ketone:alcohol:dialkyl peroxide, were similar. Alkyl aromatic compounds were also oxidized to benzylic ketones and alcohols. Furthermore, a representative linear alkane, nonane, yielded ketones and secondary alcohols in a 2:1 ratio (Table 2).

Table 2. **The Oxidation of Alkanes with 1% wt. Complex 1, 10 % PPO, 10% PEO–SiO_2.**[a]

Substrates	Total TON	Products[b,c]
cyclohexane	238	cyclohexanone 51% cyclohexanol 16% cyclohexyl-*t*-butyl peroxide 33%
cycloheptane	146	cycloheptanone 55% cycloheptanol 19% cycloheptyl-*t*-butyl peroxide 26%
tetrahydronaphthalene	141	α–tetralone 94% α–tetralol 6%
ethylbenzene	157	acetophenone 72% 1-phenylethanol 28%
n-nonane	31	2-, 3-, 4-nonanone 68% 2-, 3-, 4-nonanol 32%

a) Reactions were carried out by mixing 0.38 mmol alkane, 3.8 mmol TBHP in 5 mL water at pH 4.2 with 0.38 µmol precatalyst (as 1% wt. **1** on 10% PPO, 10% PEO–SiO_2) for 3 h at room temeperature. b) products as mol% total products. c) Yields based on TBHP were 80-90%.

Alkane Functionalization Studies with Aqueous Micelles

We chose cetyltrimethylammonium hydrogensulfate (CTAHS) to create micelles for the oxidation of cyclohexane with TBHP/O_2 in aqueous solution with precatalyst, $[Fe_2O((\eta^1\text{-}H_2O)(\eta^1\text{-}OAc)(BPIA)_2]^{3+}$, **3**, (BPIA = bis[(2-pyridyl)methyl][2-(1-methylimidazolyl)methyl]amine) (Figure 2). The results show that as the concentration of cyclohexane and TBHP were varied, a dependence on the TON of the products for each reactant was observed with catalyst **3**. We also observe that an increase in the cyclohexane concentration from 150 to 750 mmols concomitantly increases the amount of oxidation products from 11 TON to 32 TON and, more importantly, an increase in the CyOH/CyONE ratio from 0.47 to 0.82. By halving the amount of TBHP, we observe a decrease in the amount of oxidation products by a factor ~1.8, while the CyOH/CyONE ratio does not change. *These overall results strongly suggest that the cyclohexane oxidation occurs within the micelles.* We also evaluated other substrates, such as toluene (500 mmol) and octanol (500 mmol), in our aqueous micelle system, under similar conditions. We found that toluene provided only benzyl alcohol and benzaldehyde in a ratio of 1.5 with a total TON of 7, while octanol provided only octanal with a total TON of 3. It is intertesting to note, in the latter result, that in the absence of the surfactant (CTAHS) that ~ 1 TON was observed..

In conclusion, we have demonstrated the first example of the functionalization of hydrocarbon substrates with MMO biomimetic complexes embedded in a derivatized surface silica system and compared this synthesized macroenvironment with an aqueous micelle system using TBHP/O_2 as the oxidants. These free radical, alkane functionalization reactions were presumably initiated by the favorable redox chemistry of complexes **1** and **3** in both of the above-mentioned macroenvironments that provided *t*-BuO• and *t*-BuOO• radicals (Haber-Weiss process).

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Reference

Neimann, K.; Neumann, R.; Rabion, A.; Buchanan, R. M.; Fish, R. H. *Inorg. Chem.* **1999**, *38*, 3575 and references therein.