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Letter

Biomimetic oxidation studies. 10. Cyclohexane oxidation reactions with active site methane monooxygenase enzyme models and *t*-butyl hydroperoxide in aqueous micelles: Mechanistic insights and the role of *t*-butoxy radicals in the C–H functionalization reaction [1]

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Abstract

The oxidation of cyclohexane (CyH) in an aqueous micelle system with *t*-butyl hydroperoxide (TBHP) in the presence of biomimetic methane monooxygenase enzyme complexes, $[Fe_2O(\eta^1\text{-}H_2O)(\eta^1\text{-}OAc)(TPA)_2]^{3+}$, **1**, $[Fe_2O(\eta^1\text{-}H_2O)(\eta^1\text{-}OAc)(BPIA)_2]^{3+}$, **2**, and O_2, was studied and found to provide cyclohexanol (CyOH), cyclohexanone (CyONE), and cyclohexyl-*t*-butyl peroxide (CyOO*t*-Bu). The mechanistic aspects of this oxidation reaction in aqueous micelles were studied and included the effects of the surfactant concentration, cetyltrimethylammonium hydrosulfate; concentration of CyH and TBHP; and a trapping reagent, CCl_4. Several factors allowed us to conclude that a *t*-butoxy radical (*t*-BuO$^{\cdot}$) was generated from the favorable redox chemistry of the biomimetic complexes with TBHP, and was responsible for the free radical initiation process with CyH in the aqueous micelle system.

Keywords: Aqueous micelles; Aqueous biomimetic oxidations; *t*-butoxy radicals; Hydrocarbon oxidation in micelles; MMO biomimics

The unique ability of water soluble methane monooxygenase enzymes (MMO) to oxidize a broad range of hydrocarbons, such as methane to adamantane [1], has led to several applications of the methanotropic bacteria, including bioremediation of land contaminated by oil spills, [2] and the oxidative removal of trichloroethylene from drinking water [3,4]. In view of its environmental implications, synthesis of active site MMO models, which can mimic the catalytic activity of the MMO in aqueous media, would be of significant interest to the discipline of bioinorganic chemistry [5].

Recently, we reported on the first example of the oxidation of water soluble alcohols with

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MMO biomimics at pH 4.2 with *t*-butyl hydroperoxide (TBHP) as the oxidant [6]. In that study, we determined that favorable redox chemistry in H_2O provided homolytic cleavage of the *t*-BuOO–H and *t*-BuO–OH bonds by MMO biomimics, $[Fe_2O(\eta^1\text{-}H_2O)(\eta^1\text{-}OAc)(L)_2]^{3+}/[Fe_2O(\eta^1\text{-}H_2O)_2(L)_2]^{4+}$, to produce *t*-BuO$^{\cdot}$ radicals. Thus, it was the *t*-BuO$^{\cdot}$ radicals that initiated homolytic C–H abstraction from alcohols to produce the aldehyde/ketone product; no putative LFe=O intermediates, as suggested for CH_3CN based oxidations [7,8], were found to be evident in these aqueous oxidation reactions.

However, the catalytic oxidation of water insoluble hydrocarbons, such as cyclohexane, was problematic in aqueous media. The usual observation with two phase systems was that reactions do not occur, since the substrate was only soluble in the organic phase (or forms the organic phase), and the active species (catalyst and oxidant or oxidant only) are partly, or only soluble, in the aqueous phase. By using a surfactant to 'solubilize' the hydrophobic substrate or a phase transfer agent for bringing an anionic reactant from one phase to another in which it was normally insoluble, one can readily overcome these difficulties [9].

Notably, the problem of the very low solubility of hydrocarbon substrates in aqueous media was readily overcome by biological enzyme systems, e.g., cytochrome P450 (Fe^{3+}-porphyrin active site) or methane monooxygenase ($[Fe_2(\mu\text{-}OH)(\eta^1\text{-}H_2O)(L)_x]$ active site), in that these systems situate the active hydroxylase component in a relatively large, hydrophobic pocket that was dispersed within a continuous aqueous phase [10]. Therefore, as stated above, such enzymatic assemblies may be duplicated by dissolving a synthetic biomimetic MMO catalyst within a surfactant, and thus, form aqueous micelles [9].

Several aqueous oxidation studies using transition metal complexes as catalysts have been reported in the presence of surfactants [11–13]. For example, a water-soluble ruthenium complex in the presence of a surfactant, and $KHSO_5$ as oxidant, was found to be very effective in the degradation of chlorinated olefins [11]. As well, iron salts and H_2O_2, in microemulsions, were studied with hydrocarbons [12]. As far as we have been able to discern, no such studies with MMO biomimetic complexes have been reported that catalyze the oxidation of hydrophobic substrates, such as cyclohexane, in aqueous media in the presence of a surfactant, and an appropriate oxidant.

Therefore, our approach included the use of the following in situ formed MMO models from their μ-OAc derivatives as catalysts at pH 4.0, [6] $[Fe_2O(\eta^1\text{-}H_2O)(\eta^1\text{-}OAc)(TPA)_2]^{3+}$, **1**, $[Fe_2O(\eta^1\text{-}H_2O)(\eta^1\text{-}OAc)(BPIA)_2]^{3+}$, **2**, where TPA = tris[(2-pyridyl)methyl]amine, and BPIA = bis[(2-pyridyl)methyl][2-(1-methylimidazolyl)methyl]amine, utilizing the oxidant, TBHP, in combination with a surfactant. To clearly demonstrate the need of a surfactant in our oxidation reactions, a preliminary control experiment, *in the absence of a surfactant*, on the functionalization of cyclohexane in water (pH 4.0) using the water soluble catalysts, **1** and **2**, and $TBHP/O_2$ as oxidant, showed that *no functionalization process (cyclohexanol < 1 turnover numbers (TON); cyclohexanone < 1 TON) occurred* either in the biphasic system or in a microemulsion obtained by vigorous stirring. Since the substrate (cyclohexane) was not soluble in water, which contains both the catalyst and the oxidant, the oxidation reaction would occur at the interface of the two phases. These types of reactions tend to be limited not only by the concentration of the reactants at the interface, but also by the interfacial area available and hence the stirring rate. Therefore, under our control reaction conditions, the TBHP decomposition reaction catalyzed by **1** and **2**, in the aqueous phase, was found to be much faster than the cyclohexane oxidation reaction occurring at the interface.

Thus, the choice of an adequate surfactant constituted the first step in our aqueous micelle studies, since the nature of the surfactant would

have a direct influence on this type of phase-transfer-catalysis by its interaction with the biomimetic catalyst. Therefore, we chose cetyltrimethylammonium hydrogensulfate (CTAHS) to create micelles for the oxidation of cyclohexane with TBHP in aqueous solution [11], Fig. 1 clearly demonstrates that the addition of the surfactant, CTAHS, to the biphasic system, provides a more efficient oxidation system. This system (complex **1** or **2**, TBHP/O_2) provided CyOH, CyONE, and CyOOBu-*t*; no other products were formed (GC/MS).

As the CTAHS concentration is increased, the total turnover numbers (TON, mmol of product/mmol of catalyst) increased. At $C_{CTAHS} > 10$ mM, no significant increase in the TON of the products is observed. Moreover, the results at $C_{CTAHS} > 10$ mM are in the same order as those obtained in the homogeneous system using CH_3CN as the solvent (27 TON and 19 TON for the μ-OAc derivatives of **1** and **2**, respectively, in CH_3CN), and show the efficiency of this catalytic system due to the combined presence of the micelles (CTAHS) and the catalytic effects of complex **1** or **2**. The

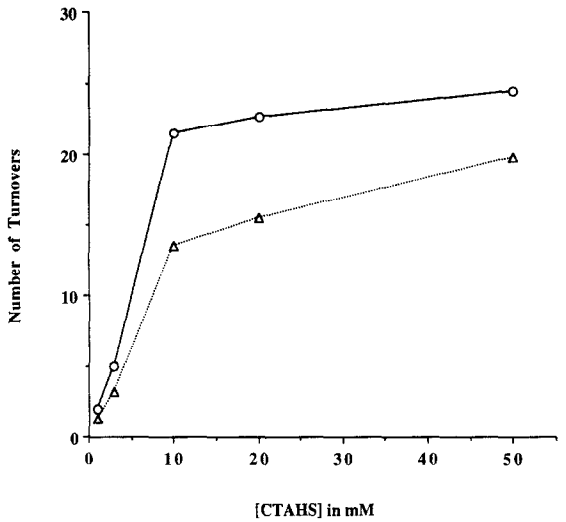

Fig. 1. Turnover numbers (mmol of product/mmol of catalyst) of oxidation products (CyOH and CyONE) versus the CTAHS concentration. Catalysts [**1**, △] and [**2**, ○] (1 μmol); TBHP (150 μmol); cyclohexane (500 μmol) in 1 ml of H_2O at pH 4 for 1 h with **1**, and 5 h with **2**; this represents the time period for complete consumption of TBHP.

Table 1
The influence of cyclohexane and TBHP concentrations on the turnover numbers of cyclohexanol/cyclohexanone/cyclohexyl-*t*-butyl peroxide with catalyst **2**[a,b]

Amounts of reactant (μmol)		Products (TON)			CyOH/CyONE
CyH	TBHP	CyOH	CyONE	CyOO^{t}Bu	
150	112	2.9	6.2	1.2	0.47
375	112	9.6	13.6	4.9	0.70
750	112	12.2	14.8	5.5	0.82
375	56	5.6	7.7	2.3	0.71

[a]CyH = cyclohexane; CyOH = cyclohexanol; CyONE = cyclohexanone; CyOO^{t}Bu = cyclohexyl-*t*-butyl peroxide.
[b]Catalyst **2** (1 μmol) in 1 ml of H_2O at pH 4 for 5 h. This represents the time period for complete consumption of TBHP. The conversion of CyH to products is ~5%, while selectivity is also based on CyH conversion. The efficiency to products from TBHP is ~26%; however, addition of another 112 μmol of TBHP doubles the TON. Reaction were conducted under atmospheric conditions, while purging with N_2 showed no significant difference in the product concentrations. The products were quantified by GC and identified by GC/MS analysis.

alcohol/ketone ratios are similar for both complexes **1** and **2** with values between 0.5 ($C_{CTAHS} < 10$ mM) and 0.7 ($C_{CTAHS} > 10$ mM). For comparison, a ratio of 0.8 was obtained in CH_3CN. The excess of cyclohexanone observed under the aqueous emulsion conditions is in agreement with the concentration of cyclohexane 'solubilized' in the aqueous phase being low and cyclohexanol, which is formed during the oxidation process and is further oxidized to cyclohexanone (confirmed with CyOH and catalyst **2**), becoming a competing substrate within the micelle.

The change in the curve shape in Fig. 1 (found to be more important for complex **2**) at CTAHS concentrations of ~10 mM corresponds to the critical micelle concentration (CMC) in the aqueous system [11]. As we increase the concentration of CTAHS below the CMC, then this results in increasing the volume of the micelles, i.e., the amount of cyclohexane 'solubilized' in the micelles. Alternatively, by increasing the concentration of CTAHS above

the CMC, further provides an increase in the overall number of micelles [13]. As observed in water, complex **1** provides faster oxidation reactions than complex **2**, while complex **2** gives rise to a larger TON of oxidation products. The catalytic system with complex **1** or **2** appears to be stable under these aqueous micelle conditions, since the addition of another 150 mM TBHP solution led to a doubling of the TON of the oxidation products, CyOH, CyONE, and CyOOBu-*t*; this procedure was repeated several times without loss of activity.

Table 1 shows 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 **2**. We also observe that an increase in the cyclohexane concentration from 150 to 750 μmol 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 see a decrease in the amount of oxidation products by a factor ~ 1.8, while the CyOH/CyONE ratio does not change. *These overall results suggest that the cyclohexane oxidation occurs within the aqueous micelles.*

The addition of a competitive substrate, or radical trap, in the cyclohexane oxidation reaction provided insight into the mechanism of the catalyzed cyclohexane functionalization reaction. Therefore, the presence of CCl_4 (13% by volume) in the reaction of **2**/TBHP/CTAHS/cyclohexane afforded 3 TON for chlorocyclohexane formation (GC-MS), which shows the presence of cyclohexyl radicals as intermediates that were then trapped by CCl_4. Several other experiments with complex **2** were performed that evaluated the TBHP decomposition product ratio (R) of $(CH_3)_2C{=}O/t$-BuOH in the presence and/or absence of cyclohexane or CTAHS, and these results were as follows: (1) $R = 3.5$ (in the absence of cyclohexane and CTAHS); (2) $R = 1.3$ (in the absence of cyclohexane and in the presence of CTAHS); and (3) $R = 0.5$ (in the presence of 15 mM CTAHS and 500 mM cyclohexane). The first experiment shows that t-BuO$^{\cdot}$ radicals are present from the $(CH_3)_2C{=}O$ decomposition product [6], while the latter experiment further demonstrates the predominant formation of Cy$^{\cdot}$ radicals.

More importantly, experiments using a 1:1 mixture of cyclohexane and cyclohexane-d_{12} (GC; GC/MS analysis) as substrates afforded substantial primary kinetic isotope effects for the products, CyOH, CyONE and cyclohexyl-*t*-butyl peroxide (CyOOBu-*t*), indicating that the C–H abstraction reaction was an important component in the mechanism of formation of CyOH, CyONE, and CyOOBu-*t* [6]. Complexes **1** and **2** provided the same k_H/k_D value, 7.8 ± 0.3, for CyOOBu-*t* formation, 6.4 ± 0.3 and 7 ± 0.3, respectively, for CyOH formation, and 11.0 ± 0.4 for CyONE formation with **1**. For comparison, in CH_3CN, the μ-OAc derivatives of complexes **1** and **2** provided k_H/k_D values of 4.6 ± 0.3 and 4.8 ± 0.3, respectively, for CyOH formation, and 7.7 ± 0.5 (complex **1**) for CyOOBu-*t* formation.

It is interesting to note that we have proven in CH_3CN with MMO biomimics $[Fe_2O(\mu\text{-}OAc)(L)_2]^{3+}/[Fe_2O(\eta^1\text{-}H_2O)_2(L)_2]^{4+}$ and the oxidant, TBHP, that O_2 trapping of the formed carbon radical occurs rather than any rebound reaction ($Fe{-}OH + {}^{\cdot}CHR_2 \rightarrow Fe + HOCH_2R_2$) [7]. In the latter study, we proposed on the basis of k_H/k_D values that a putative LFe=O intermediate formed the carbon radicals ($LFe{=}O + H_2CR_2 \Rightarrow Fe{-}OH + {}^{\cdot}CHR_2$). In contrast, a more recent report provided data concerning the decomposition of TBHP in CH_3CN, in the presence of a mononuclear complex, $[FeCl_2(TPA)]^+$, that produced t-BuO$^{\cdot}$ radicals, and not a putative LFe=O intermediate [14]. Thus, in CH_3CN, and with TBHP as oxidant, both LFe=O and t-BuO$^{\cdot}$ intermediates appear possible to generate carbon radicals, but is an on-going controversy.

To reiterate, the mechanism of CyOH oxidation in water with complexes **1** and **2** as catalysts, and TBHP as the oxidant, was a conse-

quence of a facile redox process ($Fe^{3+}Fe^{3+}$ to $Fe^{2+}Fe^{3+}$) for the homolytic decomposition of TBHP at pH 4.2 [6]. Furthermore, in the latter study, we also observed for catalysts **1** and **2**, in their Uv–vis spectra, that the addition of TBHP was followed by a rapid change of color from yellow to colorless (the yellow color returns after ~ 20 min). Notably, the observation of a colorless solution was indicative of the presence of an $LFe^{2+}(\mu\text{-}OH)Fe^{3+}L$ intermediate (Haber–Weiss process) [6]. In fact, we see similar Uv–vis color changes, yellow to colorless, as noted above for **1**, in the presence of TBHP and CTAHS, and this observation seems to further strengthen the argument for a homolytic decomposition mechanism for TBHP in the aqueous micelle system to form *t*-BuO· radicals.

In conclusion, we have observed the first example of the oxidation of a hydrocarbon substrate with MMO biomimetic complexes in an aqueous micelle system using TBHP as the oxidant. These free radical reactions were presumably initiated by the favorable redox chemistry of complexes **1** and **2** in the aqueous micelle system that provided *t*-BuO· radicals (Haber–Weiss process). The *t*-BuO· radicals, we speculate, initiate cyclohexyl radical formation, which was then trapped by O_2 to provide alcohol and ketone products [14]. Finally, we have also observed that cyclohexene, **2**, CTAHS, and TBHP/O_2 predominately provide cyclohexenyl radicals, and the products emanating from this radical, 2-cyclohexen-1-ol and its ketone, along with a substantial amount of coupling product, 3-(*t*-butylperoxy)cyclohexene; this result further corroborates our postulated mechanism of *t*-BuO· radical formation and initiation of the C–H abstraction reaction in an aqueous micelle system [15]. We will report on other hydrocarbon substrates in a future manuscript to demonstrate the scope of these aqueous micelle oxidation reactions.

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