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Autoxidation-Product-Initiated Dioxygenases: Vanadium-Based, Record Catalytic Lifetime Catechol Dioxygenase Catalysis

Cindy-Xing Yin, Yoh Sasaki, and Richard G. Finke*

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

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In recent work, it was shown that V-containing polyoxometalates such as $(n\text{-}Bu_4N)_7SiW_9V_3O_{40}$ or $(n\text{-}Bu_4N)_9P_2W_{15}V_3O_{62}$, as well as eight other V-containing precatalysts tested, evolve to a high activity, long catalytic lifetime (≥30 000-100 000 total turnovers) 3,5-di-*tert*-butylcatechol dioxygenase, in which Pierpont's complex $[VO(DBSQ)(DTBC)]_2$ (where DBSQ is 3,5-di-*tert*-butylsemiquinone and DTBC is the 3,5-di-*tert*-butylcatecholate dianion) was identified as a common catalyst or catalyst resting state (Yin, C.-X.; Finke, R. G. Vanadium-Based, Extended Catalytic Lifetime Catechol Dioxygenases: Evidence For a Common Catalyst. *J. Am. Chem. Soc.* **2005**, *127* (25), 9003–9013). Herein, those findings are followed up by studies aimed at answering the following questions about this record catalytic lifetime 3,5-di-*tert*-butylcatechol dioxygenase catalyst: (i) What is the key to how V leaches from, for example, seemingly robust V-containing polyoxometalate precatalysts? (ii) What is the key to the sigmoidal, apparently autocatalytic kinetics observed? (iii) What can be learned about the underlying reactions that form $[VO(DBSQ)(DTBC)]_2$? (iv) Finally, do the answers to (i–iii) lead to any broader insights or concepts? Key findings from the present work include the fact that the reaction involves a novel, autoxidation-product-induced dioxygenase, that is, one in which the *undesired* autoxidation of the 3,5-di-*tert*-butylcatechol substrate to the corresponding benzoquinone and H_2O_2 turns on the *desired* dioxygenase catalysis via a V-leaching process which eventually yields Pierpont's complex, $[VO(DBSQ)(DTBC)]_2$. Plausible reactions en route to $[VO(DBSQ)(DTBC)]_2$ consistent with the kinetic data, the role of H_2O_2, and the relevant literature are provided. The results provide a prototype example of the little observed but likely more general concept of an autoxidation-product-initiated reaction. The results also provide considerable simplification of, and insight into, the previously disparate literature of V-based 3,5-di-*tert*-butylcatechol dioxygenase catalysis.

Introduction

In 1999, we reported that a polyoxometalate vanadium-containing complex serves as an effective precatalyst for the dioxygenase-type oxidative cleavage of 3,5-di-*tert*-butylcatechol (hereafter 3,5-DTBC) with a record number, ≥100 000, of total turnover (TTO) catalytic lifetimes to produce the oxidative ring-cleavage products, **2**−**5**, plus the autoxidation product, **6** (Scheme 1).[1] The new product **4** was also isolated and identified by X-ray crystallography as a part of that study. The above results are of considerable interest, since man-made, highly catalytic, long-lived dioxygenase catalysts are the Holy Grail of oxidation catalysis.[2]

As part of the 1999 study, initial O_2-uptake kinetic studies[1] revealed a novel product and catalyst evolution pathway consisting of an A → B induction period (rate constant k_1) followed by an A + B → 2B autocatalytic step (rate constant k_2), as shown in Figure 1. Note that A + B → 2B is the kinetic definition of autocatalysis, where the reaction product, B, is also a reactant. In this manner, the 2B-product-stoichiometry autocatalytically "turns on" the reaction (and, concomitantly, as we will see, the catalyst formation reaction, vide infra), resulting in sigmoidal-shaped kinetic curves, as seen in Figure 1.

In more recent work, evidence from electron paramagnetic resonance (EPR) spectroscopy, negative ion electrospray ionization mass spectrometry (ESI-MS), and kinetics, as well as evidence from catalytic activity, selectivity, and lifetime studies, revealed that Pierpont's vanadium semiquinone catecholate dimer complex, $[VO(DBSQ)(DTBC)]_2$ (where

* To whom correspondence should be addressed. E-mail: rfinke@lamar.colostate.edu.

(1) Weiner, H.; Finke, R. G. *J. Am. Chem. Soc.* **1999**, *121*, 9831−9842.
(2) Hill, C. L.; Weinstock, I. A. *Nature* **1997**, *388*, 332−333.

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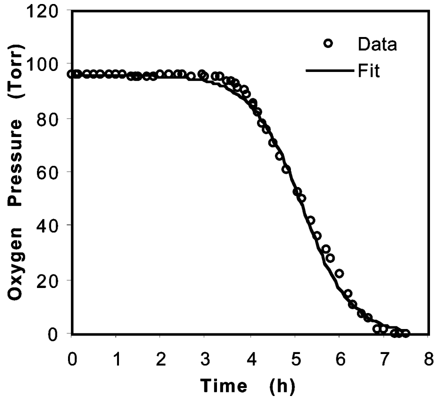
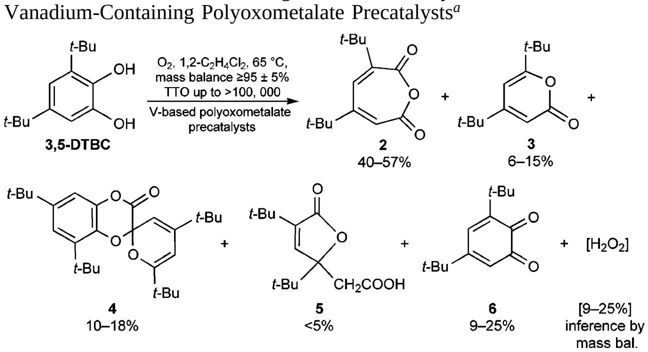


Figure 1. Autocatalytic O_2-uptake kinetic curve and curve fit of a 3,5-DTBC plus precatalyst $(n\text{-}Bu_4N)_7SiW_9V_3O_{40}$ and O_2 reaction. The conditions are as follows: 1.8 mmol of 3,5-DTBC, 5.6 μmol of precatalyst, 8.4 mL of 1,2-$C_2H_4Cl_2$, 40 °C, and 0.8 atm O_2; note that for the purposes of the curve fitting the final pressure has been subtracted from the initial pressure to "zero" the *y* axis so that the *net pressure change* is shown here and in analogous curves hereafter. The curve is fit to the analytic kinetic equations for the two-step mechanism A → B (k_1) and A + B → 2B (k_2), as detailed elsewhere.[3,4] The rate constants obtained from the fit are $k_1 = (1.8 \pm 0.3) \times 10^{-4}$ h^{-1} and $k_2 = (1.9 \pm 0.1) \times 10^{-2}$ Torr^{-1} h^{-1}; these rate constants are the same within experimental error as those obtained previously by an independent researcher in our lab,[1,5] $k_1 = (2.8 \pm 0.9) \times 10^{-4}$ h^{-1} and $k_2 = (1.6 \pm 0.1) \times 10^{-2}$ Torr^{-1} h^{-1}. The sigmoidal form of the curve and its A → B and A + B → 2B curve fit are the key results here, for reasons discussed in the Results and Discussion section.

Scheme 1. Oxidative Cleavage of 3,5-DTBC by Vanadium-Containing Polyoxometalate Precatalysts[a]



[a] The products are **2**, 3,5-di-*tert*-butyl-1-oxacyclohepta-3,5-diene-2,7-dione; **3**, 4,6-di-*tert*-butyl-*2H*-pyran-2-one; **4**, spiro[1,4-benzodioxin-2(*3H*),2′(*2H*)-pyran]-3-one-4′,6,6′,8-tetrakis(1,1-dimethylethyl); **5**, 3,5-di-*tert*-butyl-5-(carboxymethyl)-2-furanone; and **6**, 3,5-di-*tert*-butyl-1,2-benzoquinone.

DBSQ is 3,5-di-*tert*-butylsemiquinone and DTBC is the 3,5-di-*tert*-butylcatecholate dianion), is a common component—and apparently the catalyst resting state—produced from a wide variety of V-containing precatalysts,[6] notably the above V-containing polyoxometalate or nine other vanadium-containing precatalysts, including simple $VO(acac)_2$.[6] Those studies show that virtually any V-based precatalyst appears to leach vanadium under the dioxygenase reaction conditions to produce $[VO(DBSQ)(DTBC)]_2$.

However, several important questions remained unanswered, including (i) what is the reaction product, B, which turns on the V-leaching and sigmoidal, A + B → 2B, autocatalytic evolution of the true catalyst? The most likely candidates are H_2O_2 derived from the initial DTBC autoxidation[7] (H_2O_2 being a precedented[8] product required by mass balance when the benzoquinone autoxidation product, **6**, is produced, as in Scheme 1); possibly the semiquinone anion; benzoquinone, **6**; or perhaps H_2O_2 in combination with the benzoquinone product, **6**. If H_2O_2 is B, then we have discovered an example of the seemingly improbable situation where *undesired autoxidation* turns on the *desired dioxygenase chemistry*, a novel *autoxidation-initiated dioxygenase*.[9] Another important question is (ii) can H_2O_2 be detected among the reaction products, or is it as rapidly consumed as it is made? Additionally, (iii) can any insights be obtained into the more detailed reactions of how virtually any V-containing precatalyst evolves into the apparent catalyst resting state, $[VO(DBSQ)(DTBC)]_2$? Also, (iv) what more general insights, principles, or concepts emerge from addressing questions (i-iii)? It is just the above questions and issues, for the most highly catalytically active and long-lived, man-made dioxygenase presently known, that are the focus of the present paper.

Experimental Section

Reagents. 3,5-DTBC (Aldrich, 99%) was recrystallized three times using *n*-pentane (Fisher Scientific, 98%, pesticide grade) under argon (melting point 99-100 °C, lit. mp 96-99 °C) and was stored in a Vacuum Atmospheres drybox (O_2 level ≤ 5 ppm). (Note: it is important to recrystallize the 3,5-DTBC substrate more than one time to remove impurities such as 3,5-di-*tert*-butylsemiquinone, due to effects such as those shown in Figure 2.) 3,5-Di-*tert*-butylbenzoquinone (Aldrich, 98%) was recrystallized twice from *n*-pentane and stored in the drybox (melting point 114-115 °C, lit. mp 113-115 °C). All the polyoxometalate precursors were synthesized according to the most recent literature procedures.[10,11] The Na/Hg amalgam (Strem, 20% Na of 99.9+% purity) was stored in the drybox and used as received. High-performance liquid chromatography (HPLC) grade solvents (1,2-dichloroethane and acetonitrile) were purchased from Aldrich and stored in the drybox; each of the above solvents was dried by standing for at least 48 h over ~5 vol % 3- or 4-Å molecular sieves, which were preactivated by heating at 170 °C under vacuum for at least 12 h and, then, cooling under dry N_2 in the drybox. Tetrahydrofuran (THF) (Fisher Scientific)

(3) Watzky, M. A.; Finke, R. G. *J. Am. Chem. Soc.* **1997**, *119*, 10382−10400.
(4) Widegren, J. A.; Aiken, J. D., III; Özkar, S.; Finke, R. G. *Chem. Mater.* **2001**, *13*, 312−324.
(5) The units of k_1 and k_2 in the captions of Figures 4 and 5 in our previous studies[1] should have been h^{-1} and Torr^{-1} h^{-1}, respectively; the numerical values of k_1 and k_2 are correct, however.
(6) Yin, C.-X.; Finke, R. G. *J. Am. Chem. Soc.* **2005**, *127* (25), 9003−9013.
(7) Tyson, C. A.; Martell, A. E. *J. Phys. Chem.* **1970**, *74*, 2601−2610.
(8) (a) Bianchini, C.; Frediani, P.; Laschi, F.; Meli, A.; Vizza, F.; Zanello, P. *Inorg. Chem.* **1990**, *29*, 3402−3409. Bianchini and co-workers' studies show that H_2O_2 is the primary product when DTBC is oxidized to the benzoquinone, at least for Rh(III) and Ir(III) and under their mildly catalytic reaction conditions (ca. 30 total turnovers). (b) Barbaro, P.; Bianchini, C.; Mealli, C.; Meli, A. *J. Am. Chem. Soc.* **1991**, *113*, 3181−3183. (c) Barbaro, P.; Bianchini, C.; Frediani, P.; Meli, A.; Vizza, F. *Inorg. Chem.* **1992**, *31*, 1523−1529. (d) Barbaro, P.; Bianchini, C.; Linn, K.; Mealli, C.; Meli, A.; Vizza, F.; Laschi, F.; Zanello, P. *Inorg. Chim. Acta* **1992**, *198−200*, 31−56. (e) For an earlier work by others see: Muto, S.; Tasaka, K.; Kamiya, Y. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 2493−2494.
(9) We define an "autoxidation-product-initiated reaction" as one where an ROOH or other product of an (typically unintended) autoxidation initiates and facilitates an otherwise slow reaction.
(10) Finke, R. G.; Rapko, B.; Saxton, R. J.; Domaille, P. J. *J. Am. Chem. Soc.* **1986**, *108*, 2947−2960.
(11) Hornstein, B. J.; Finke, R. G. *Inorg. Chem.* **2002**, *41*, 2720−2730.

was distilled over $LiAlH_4$ under Ar and stored under Ar. HBF_4 (Aldrich) was purchased as a 54 wt% diethyl ether solution and stored in the drybox. The sodium salt of 3,5-di-*tert*-butylsemiquinone (Na^+DBSQ^-) was prepared via the literature procedure[12] by reacting 1 equiv of Na amalgam with 3,5-di-*tert*-butylbenzoquinone. Its UV-visible absorbance in *t*-BuOH is at 728 nm (580 $\pm$ 20 M^{-1} cm^{-1}, measured under N_2), literature[13] 730 nm (680 M^{-1} cm^{-1}, no error bar was reported). An $Na_2S_2O_3$ aqueous solution (0.05 M) was prepared from $Na_2S_2O_3 \cdot 5H_2O$ (Fisher Chemicals, certified ACS grade), and a pellet of KOH (Fisher Chemicals, certified ACS grade) was added for stabilization. KIO_3 (Mallinckrodt Powder, analytical grade), H_2SO_4 (Mallinckrodt, analytical reagent), KI, starch (Fisher chemicals, certified ACS grade), and H_2O_2 (Fisher Scientific, ca. 30 wt % in H_2O) were used as received.

Instrumentation. ^1H, ^{31}P, and ^{51}V NMR were recorded in 5-mm o.d. tubes on a Varian Inova (JS-300) NMR spectrometer. ^1H NMR was referenced to the residual proton impurity in the deuterated solvent, ^{31}P NMR was referenced to 85% H_3PO_4 in H_2O using the external substitution method, and ^{51}V NMR was referenced to neat $VOCl_3$ using the external substitution method. Spectral parameters for ^{31}P NMR include a tip angle of 60° (pulse width 10 μs), an acquisition time of 1.6 s, and a sweep width of 10 000 Hz. Spectral parameters for ^{51}V NMR include a ^{51}V tip angle of 90° (pulse width 3.1 μs), an acquisition time of 0.096 s, and a sweep width of 83 682.0 Hz. Gas chromatography (GC) analyses were performed on an HP (Hewlett-Packard) 5890 Series II gas chromatograph equipped with a flame ionization detector (FID) and a SPB-1 capillary column (30 m, 0.25 mm i.d.) with the following temperature program: initial temperature, 200 °C (initial time, 2 min); heating rate, 2 °C min^{-1}; final temperature, 240 °C (final time, 3 min); FID temperature, 250 °C; injector temperature, 250 °C. An injection volume of 1 μL was used. Negative ion ESI-MS experiments were performed on a Thermo Finnigan LCQ Advantage Duo mass spectrometer which was directly coupled with a syringe pump (feeding speed, 5 μL min^{-1}; spray voltage, -4.5 kV; capillary voltage, -38 to -42 V; capillary temperature, 180 °C) using CH_3CN as the solvent.

Impurities in Crude DTBC. GC accomplished under the above-noted conditions detected ~4% 3,5-di-*tert*-butyl-1,2-benzoquinone (3,5-DBQuinone) impurity in crude DTBC, while liquid secondary ion mass spectroscopy (LSI-MS), performed on a Fisons VG AutoSpec mass spectrometer at 27 kV with Cs^+ ion, *m*-nitrobenzyl alcohol as the matrix, failed to detect any impurities (a 3,5-DBQuinone parent ion peak was not detected by LSI-MS, perhaps due to its ready fragmentation).

Preparation of Precatalysts. $(n\text{-}Bu_4N)_7SiW_9V_3O_{40}$ and $(n\text{-}Bu_4N)_9P_2W_{15}V_3O_{62}$ were prepared and characterized as described in the Supporting Information of ref 6. $[Et_3NH]_2[VO(DTBC)_2] \cdot nCH_3OH$ ($n \sim 1-2$) and $[VO(DBSQ)(DTBC)]_2$ were prepared and characterized by UV-visible spectroscopy, elemental analysis, and negative ion ESI-MS, as previously detailed.[6]

Procedure of the Oxygen-Uptake Experiments. All the experiments were carried out on a volume-calibrated oxygen-uptake line as detailed in the Supporting Information of ref 1 (the total, calibrated volume, determined using three different-volume reaction flasks, is in the range of 246-257 mL). To ensure that O_2 gas-to-solution mass transfer is not influencing the observed kinetics, an O_2 rate vs catalyst concentration plot was generated as a control (Figure S7 of the Supporting Information).

Our standard procedure is as follows: 400 $\pm$ 5 mg (ca. 1.8 mmol) of three-times-recrystallized 3,5-DTBC was weighed in the drybox into a 50-mL round-bottom reaction flask equipped with a septum, a sidearm, and an egg-shaped, 3/8-in. $\times$ 3/16-in., Teflon-coated magnetic stir bar. Approximately 8 mL of predried, HPLC grade 1,2-dichloroethane were transferred into the flask using a 10-mL glass syringe; the flask was sealed with a Teflon stopcock and taken out of the drybox. The flask was then connected to the oxygen-uptake line through an O-ring joint, and the reaction solution was frozen in a dry ice/ethanol bath (-76 °C) for 10 min. Two pump-and-fill cycles were performed (with O_2). Next, the dry ice bath was replaced with a temperature-controlled oil bath. The flask was brought up to 40 $\pm$ 0.7 °C and allowed to equilibrate with stirring for 25 min under O_2. In the drybox, 5.7 ($\pm$0.4) $\times$ 10^{-6} mol of a precatalyst was weighed into a 5-mL glass vial and dissolved in ca. 0.2 mL of 1,2-dichloroethane. The catalyst solution was drawn into a 1-mL gastight syringe and brought out of the drybox protected from the air by insertion of the syringe needle into a septum-capped vial. The catalyst was injected through the sidearm of the reaction flask, and $t = 0$ was set. Pressure readings from the manometer were used to follow the reaction ($\pm$1 Torr or ca. $\pm$1% precision over a pressure loss of ca. 80-100 Torr). The reaction was stopped when no oxygen loss was observed for 1-2 h.

Oxygen-Uptake Experiments with Preselected Additives. The following substances were tested as candidates for the product, B, which is able to remove the induction period and speed up the reactions (Table 1 provides the specific amounts added): a 30% H_2O_2-water solution; H_2O; product **6** (3,5-DBQuinone); product **5** (3,5-di-*tert*-butyl-5-(carboxymethyl)-2-furanone); 54% HBF_4 in diethyl ether; a free radical initiator, 2,2′-azobisisobutyronitrile (AIBN); a mixture of 30% H_2O_2 and product **6**; and the sodium salt of 3,5-di-*tert*-butylsemiquinone (NaDBSQ). *o*-Dichlorobenzene and H_2O-saturated 1,2-dichloroethane were also examined as tests of the effects of solvent and trace water. The effect of each additive was monitored by comparing the induction period with the additive to the induction period under standard conditions without the additive. These experiments were set up exactly as described in the previous section, Procedure of the Oxygen-Uptake Experiments, except that the additive was injected at $t = 2$ min after the injection of the precatalyst.

H_2O_2 Detection Experiment Procedure. Because H_2O_2 was shown to eliminate the induction period for two vanadium-containing polyoxometalates and a vanadium catecholate precursor and since the known stoichiometry is 1 H_2O_2 molecule formed for every 1 equiv of the 18-25% benzoquinone formed,[8] attempts were made to detect H_2O_2 in the product mixture at various times. Specifically, a 0.05 M $Na_2S_2O_3$ solution was first standardized by titration with a primary standard 0.01 M KIO_3 solution (10 mL), 5 mL of a 10% w/v KI solution, and 4 mL of 9 M H_2SO_4. This results in a color change from dark brown to pale yellow. Next, an indicator starch solution was added to detect the presence of I_3^-; the titration reached its end with a color change from violet to colorless. A blank titration was also performed to account for the effects of any possible oxidizing reagents in the background. Our H_2O_2 detection limit (set as 3σ of the signal seen as the background) is ~7.5 $\times$ 10^{-6} mol (0.4 mol % of the reaction substrate).

Attempts to detect H_2O_2 were carried out at three different reaction times during an oxygen-uptake experiment: at 2 h (i.e., during the induction period), at 5 h (after the induction period and during the main active period of the reaction), and at 8 h (after the reaction). The H_2O_2 test was accomplished as follows: the product solution was transferred into a separation funnel, and the reaction flask was washed three times with fresh 1,2-dichloroethane and

(12) White, L. S.; Hellman, E. J.; Que, L., Jr. *J. Org. Chem.* **1982**, *47*, 3766−3769.

(13) Muto, S.; Bruice, T. C. *J. Am. Chem. Soc.* **1980**, *102*, 4472−4480.

four times with distilled water. All of the washes were added to the separation funnel and shaken vigorously to extract any possible H_2O_2 into the aqueous layer. Next, 5 mL of a 10 w/v % KI solution and 4 mL of 9 M H_2SO_4 were added to the water layer and the I_3^- was titrated by the previously standardized 0.05 M $Na_2S_2O_3$ solution. The results are described in the Results and Discussion section and Figure 5.

Catalyst Evolution Stoichiometry Experiments. The experiments that follow were done to test the catalyst evolution steps postulated in Scheme 1. The experiments immediately below were performed using a high vacuum line equipped with a high precision Baratron pressure transducer (±0.1 Torr up to 1000 Torr), which resides in the lab of Professor S. H. Strauss at Colorado State University. The volume of the line was calibrated to be 96-97 mL via a calibration flask of known volume. Due to the necessity of using a larger amount of the precatalyst and a smaller amount of the substrate (i.e., so as to see a measurable amount of O_2 uptake), the reaction procedure was changed to the following: 210 ± 10 mg (ca. 0.05 mmol) of $(n\text{-}Bu_4N)_7SiW_9V_3O_{40}$ was weighed in the drybox into a 50-mL round-bottom reaction flask equipped with a septum, a sidearm, and an egg-shaped, 3/8-in. × 3/16-in., Teflon-coated magnetic stir bar. Approximately 7.6 mL of predried, HPLC grade 1,2-dichloroethane was transferred into the flask using a 10-mL glass syringe; the flask was sealed with a Teflon stopcock and taken out of the drybox. The flask was then connected to the oxygen-uptake line through an O-ring joint, and the reaction solution was frozen in a dry ice/ethanol bath (-76 °C) for 10 min. Two pump-and-fill cycles were performed (with ca. 500 Torr O_2). Next, the dry ice bath was replaced with a temperature-controlled oil bath. The flask was brought up to 40 ± 0.5 °C and allowed to equilibrate with stirring for 2 h under O_2. In the drybox, 44-70 mg (ca. 0.2-0.3 mmol) of three-times-recrystallized 3,5-DTBC was weighed into a 5-mL glass vial and dissolved in ca. 0.4 mL of 1,2-dichloroethane. The substrate solution was drawn into a 1-mL gastight syringe and brought out of the drybox protected from the air by insertion of the syringe needle into a septum-capped vial. The substrate was injected through the sidearm of the reaction flask, and $t = 0$ was set (H_2O_2 was injected immediately after the addition of the substrate in one separate run). Pressure readings from the transducer were used to follow the reaction. The reaction was stopped when no oxygen loss was observed for 10-15 min. The results are described in the Results and Discussion section (Plausible Stoichiometries for the Conversion of $SiW_9V_3O_{40}^{7-}$ to the Active Catalyst, $[V^V(O)(DBSQ)(DTBC)]_2$, Plus Initial Experimental Tests of Those Stoichiometries).

Confirmed Formation of $[VO(DBSQ)(DTBC)]_2$ from $[V(DTBC)_3]^-$ under Catalytic Conditions by EPR. The predicted conversion of $[V(DTBC)_3]^-$ to $[VO(DBSQ)(DTBC)]_2$ under catalytic conditions was also tested in experiments in toluene that are detailed elsewhere[6] and in the Results and Discussion which follow.

Kinetic Curve-Fitting: Using Origin. OriginLab's nonlinear least-squares curve-fitting program, Origin, and the analytic equation shown below, corresponding to the reaction steps A → B and A + B → 2B, were used to fit the kinetic data in Figure 1, as was done in our prior work.[3]

$$[A]_t = \frac{\dfrac{k_1}{k_2} + [A]_0}{1 + \dfrac{k_1}{k_2[A]_0} \exp(k_1 + k_2[A]_0)t}$$

Results and Discussion

Oxygen-Uptake Kinetics of Triply Recrystallized vs Crude DTBC. We began with a control experiment testing whether our normal precaution of triply recrystallizing the DTBC substrate under argon and storing it under N_2 was necessary. The red 3,5-DBQuinone, **6**, is present in crude, brownish DTBC at an ∼4% level, as detected by GC.

Figure 2a shows a normal DTBC oxygenation run using precatalyst $(n\text{-}Bu_4N)_7SiW_9V_3O_{40}$ and triply recrystallized DTBC (mp 99-100 °C) under our standard reaction conditions, as detailed in the Experimental Section. Note the typical shape of Figure 2a with an induction period of ca. 4 h. Once recrystallized DTBC (mp 98-99 °C) behaves similarly, as shown in Figure 2b. However, *crude, as-received DTBC* (Aldrich, labeled mp 96-99 °C; mp 92-95 °C in our hands) *behaves much differently*, exhibiting a 10-15 min induction period, as shown in Figure 2c, which is a repeatable result throughout different batches of crude DTBC and by two different experimentalists. Gas-liquid chromatography (GC) analysis shows that the product selectivity for the crude DTBC is the same (within experimental error) as the selectivity for triply recrystallized DTBC. These results (i) emphasize that the continued use of triply recrystallized DTBC is crucial for the kinetic studies herein and (ii) show that the crude DTBC contains a contaminant that, along with molecular O_2, is able to dramatically shorten the induction period.

Oxygen-Uptake Experiments with Selected Additives: Which Reaction Products Shorten the Observed Induction Period? Our previous kinetic studies[1] and our more recent studies[6] both reveal that vanadium-containing polyoxometalates act as only precatalysts, displaying a significant induction period, ca. 4 h in the case of $SiW_9V_3O_{40}^{7-}$, before the oxygen uptake starts; for examples see Figures 1 and 2. Hence, in 12 independent experiments, we added each of the following key products or other additives at the beginning of the reaction to determine which additive(s) can turn on the reaction and eliminate the long induction period: H_2O_2; 3,5-DBQuinone (both of these are DTBC autoxidation products); a mixed solution of H_2O_2 and 3,5-DBQuinone; H_2O; the carboxylic acid product, **5**; the strong acid HBF_4; a free radical initiator (AIBN); or the semiquinone, NaDBSQ. The results from these 12 experiments are summarized in Table 1.

The most informative results came when examining 30% H_2O_2 in H_2O. The amount of H_2O_2 added all at once (18% relative to DTBC) corresponds to the maximum amount produced in the reaction, ∼18% (i.e., 1 equiv of H_2O_2 was produced for each equiv of the 18% benzoquinone yield). The addition of this level of H_2O_2 reduced the induction periods of two different polyoxometalate precatalysts by a factor of 7-14, representing a complete removal of the induction period when the effects of the H_2O added with the H_2O_2 are taken into account, vide infra. H_2O_2 also reduced the induction period of the precatalyst $[VO(DTBC)_2]^{2-}$ by a factor of 4. A control experiment of adding the same amount of water as was present in the 70% H_2O/30% H_2O_2 solution

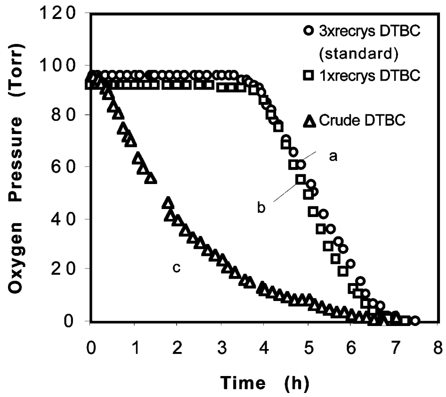


Figure 2. Kinetic O_2-uptake experiments for DTBC plus precatalyst (n-$Bu_4N)_7SiW_9V_3O_{40}$ and O_2 with (a, circles) three-times-recrystallized DTBC; (b, squares) once-recrystallized DTBC; and (c, triangles) crude DTBC. The conditions were as follows: 1.8 mmol of 3,5-DTBC, 5.6-5.7 μmol of precatalyst, 8.2-8.4 mL of 1,2-$C_2H_4Cl_2$, 40 °C, and 0.8 atm O_2; note that the *y* axis has been "zeroed" to show the net pressure change, as described in the caption of Figure 1.

Table 1. Effects of Products or Other Additives on the Induction Period of DTBC Oxygenation Beginning with Three Different Vanadium-Containing Precatalysts

additive(s)	induction period (h)	$\Delta n_{O_2}/n_{DTBC}$[a]	Figure
With Precatalyst (n-$Bu_4N)_7SiW_9V_3O_{40}$ (5.3−5.7 μmol)			
none[b]	3.5	0.75	2a
H_2O_2 (0.33 mmol)	0.3−0.5[c]	0.71	3
H_2O (1.3 mmol)	4.8	0.79	3
H_2O_2 (0.31 mmol) and 3,5-DBQuinone (0.32 mmol)	0.3	0.70	S1
3,5-DBQuinone (0.32 mmol)	2.5	0.77	4
product **5** (RCOOH, 0.090 mmol)	3.8	0.76	4
HBF_4 (7.2 μmol)	1.0	0.79	4
AIBN (0.018 and 0.054 mmol)	3.8−3.9	0.73	S2
NaDBSQ (0.33 mmol)[d]	∼0	0.53[d]	S3
H_2O-saturated 1,2-$C_2H_4Cl_2$	4.6	0.76	S4
o-dichlorobenzene	3.3	0.75	S5
With Precatalyst (n-$Bu_4N)_9P_2W_{15}V_3O_{62}$ (5.7 μmol)			
none[b]	11.0	0.65	3
H_2O_2 (0.31 mmol)	1.0	0.69	3
H_2O (1.3 mmol)	14.6	0.66	3
With Precatalyst $[Et_3NH]_2[VO(DTBC)_2]\cdot nCH_3OH$ (4.4−4.7 μmol)			
none[b]	2.0	0.73	3
H_2O_2 (0.33 mmol)	0.5	0.74	3
H_2O (1.3 mmol)	2.5	0.70	3

[a] This ratio includes the autoxidation product, 3,5-di-*tert*-butylbenzoquinone (i.e., the dioxygenase *only* stoichiometry calculated elsewehere[1] does not include this product). [b] Standard reaction conditions are as follows: 8 mL of 1,2-$C_2H_4Cl_2$ solvent, 400 mg of three-times-recrystallized DTBC, 40 °C, and 0.8 atm O_2. [c] Two runs were carried out using once-recystallized DTBC. [d] The stoichiometry and the final product, 100 ± 6% 3,5-DBQuinone (<0.5% yield of products **2-5**), show that this is an autoxidation reaction (i.e., not an autoxidation-initiated catechol oxygenation reaction).

has the *opposite effect*, lengthening the induction period,[14] as shown in Figure 3. Hence, it follows that the 18% H_2O_2 effectively eliminates the induction period when the masking effect of the added H_2O is removed. The added H_2O_2 (plus H_2O) did not change the catalyst performance, as is shown by the number of moles of oxygen consumed; importantly, *the product yields are the same* except that of benzoquinone increases ∼10% (from ∼18% to ∼27% with the added H_2O_2). Adding H_2O_2 and the benzoquinone, **6**, together has no increased effect over adding the H_2O_2 alone (Table 1).

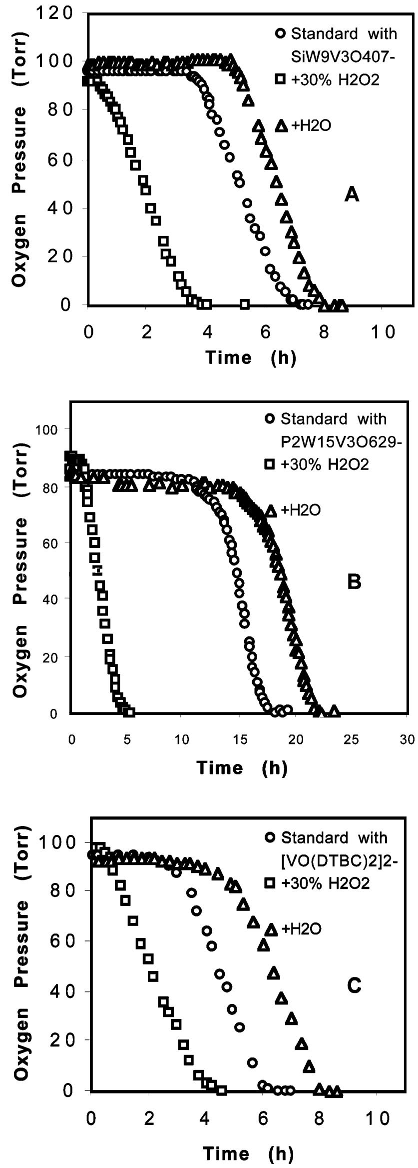


Figure 3. Kinetic O_2-uptake experiments for DTBC plus precatalyst alone, precatalyst with 30% H_2O_2 (0.33 mmol), or only H_2O (1.3 mmol) as a control: (A) precatalyst (n-$Bu_4N)_7SiW_9V_3O_{40}$; (B) precatalyst (n-$Bu_4N)_9P_2W_{15}V_3O_{62}$; or (C) precatalyst $[Et_3NH]_2[VO(DTBC)_2]\cdot nCH_3OH$. The other conditions were as follows: 1.8 mmol of 3,5-DTBC, 4.4-5.7 μmol of precatalyst, ca. 8 mL of 1,2-$C_2H_4Cl_2$, 40 °C, and 0.8 atm O_2; note that the *y* axis has been "zeroed" to show the net pressure change.

Other products or additives have smaller or no effects on the induction period: the autoxidation product 3,5-DBQuinone, **6**, alone shortens the induction period slightly from 3.5 to 2.5 h, so that the 0.25 h induction period seen for crude DTBC (Figure 2c, vide supra) cannot be accounted for solely by the ∼4% 3,5-DBQuinone impurity in unrecrystallized DTBC. Noteworthy here is that it is known from the literature,[15] as well as from our earlier work,[16] that the benzoquinone generally is *not* an intermediate (i.e., does not

(14) Weiner's preliminary results indicating that added H_2O decreased the induction period[1] did not prove repeatable and are updated by the repeatable results reported herein showing that H_2O in fact increases the induction period. Two other findings different from the initial experimental results provided earlier[1] are that the pressure drop in the early part of Figure 5A of ref 1 is an artifact (of no consequence then or now) and that higher TTOs than previously obtained in Figure 6 (line c) of ref 1 using $VO(acac)_2$ have now been achieved, as described elsewhere.[6]

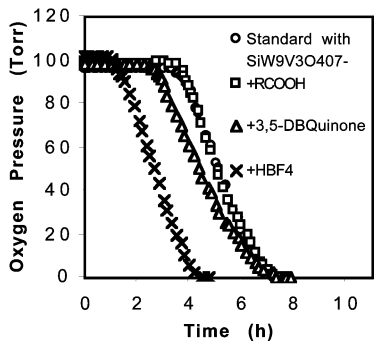


Figure 4. Kinetic O_2-uptake experiments for DTBC plus precatalyst ($(n\text{-}Bu_4N)_7SiW_9V_3O_{40}$ with the addition of product **5** (0.090 mmol), 3,5-DBQuinone (0.32 mmol), or HBF_4 (7.2 μmol). The other conditions were as follows: 1.8 mmol of 3,5-DTBC, 4.4−5.7 μmol of precatalyst, ca. 8 mL of 1,2-$C_2H_4Cl_2$, 40 °C, and 0.8 atm O_2; note that the *y* axis has been "zeroed" to show the net pressure change.

lead to the observed dioxygenase products). In the present V-based system, the almost linear GLC curve for the 3,5-DBQuinone product vs time graph in Figure 1 of ref 1 confirms that it is a stable product and does not go on to yield dioxygenase products.

Product **5** (3,5-di-*tert*-butyl-5-(carboxymethyl)-2-furanone, i.e., an RCOOH) has almost no effect on the induction period (see Table 1). The strong acid HBF_4 does reduce the induction period somewhat, but a significant, 1-h induction period is still present, as shown in Figure 4 and Table 1.

The radical initiator AIBN has no effect on the oxygen-uptake kinetics under the reaction conditions, which argues against initiation of the dioxygenase reaction by adventitious free radicals. We also did control experiments to ensure that the solvent 1,2-$C_2H_4Cl_2$ (C−H BDE of ca. 101 kcal mol^{-1} (ref 17)) is not involved in the DTBC oxidation chemistry by showing that the same kinetics and O_2 uptake within experimental error are obtained in a more oxidation-resistant solvent, *o*-dichlorobenzene (C−H BDE of ca. 111 kcal/mol[17]), as shown in Table 1 and Figure S5.

Autoxidation of 3,5-DTBC is initiated by the addition of $Na^+DBSQ^{\bullet-}$, but the resultant sole product is the benzoquinone, with <0.5% of products **2**−**5** being detected (Table 1), results which are confirmed by our recent study on Na_2(DTBC) (which found benzoquinone, **6**, as the sole product (30%), with <1% of products **2**−**5**[6]).[18] This result is consistent with our mechanistic finding (vide infra) of vanadium-bound semiquinone, but not free $DBSQ^{\bullet-}$, being a component of the DTBC dioxygenase catalyst.

The key finding is that H_2O_2 does effectivly remove the induction periods of three different vanadium-containing catechol oxygenation precatalysts (Figure 3A-C). These kinetic results, the fact that H_2O_2 is an 18% product along with the benzoquinone, **6**, and the fact that an A → B followed by A + B → 2B kinetic scheme fits the parent kinetic curves (e.g., Figure 1), all require that a reaction product, B, is formed, which then turns on the catalyst formation. *The data strongly implicate H_2O_2 as that key reaction product, B.*

We also know from our recent work that Pierpont's complex, $[VO(DBSQ)(DTBC)]_2$,[6] is detectable in solution from a range of different V-containing precatalysts and appears to be the catalyst resting state. It both is precedented and makes chemical sense that H_2O_2 is the autoxidation product that initiates the dioxygenase chemistry by forming $[VO(DBSQ)(DTBC)]_2$, since O_2^{2-} is a powerful σ- and π-donor, one well-established to tightly bind and leach V and other transition metals out of even polyoxometalate structures.[19−23] Precedent for this statement includes literature showing that $V_{10}O_{28}^{6-}$ plus H_2O_2 gives[20] $V(O)O_2^+$ and that the peroxy complexes of vanadium $V(O)(O_2)^+$, $V(O)(O_2)_2^-$, $V(O)(O_2)_4^{3-}$, and $V(O_2)_3^-$ are all known.[21] Overall, the results provide excellent evidence for a previously little known H_2O_2 autoxidation-product-initiated dioxygenase catalysis.

Attempted Detection of H_2O_2 During Oxygen-Uptake Experiments. The finding that H_2O_2 is highly effective at turning on the catalysis raises the question of whether free H_2O_2 might be detectable at any time during the reaction. Alternatively, is H_2O_2 consumed as rapidly as it is formed?

Details of the experiments performed and several important controls are provided in the Experimental Section. Not surprisingly, H_2O_2 could *not* be detected at any stage of catechol oxygenation: not during the induction period (t = 2 h), not in the middle of the oxygen-uptake reaction (5 h), and not after the reaction (8 h), even given our sensitive detection limit of H_2O_2 (0.4 mol % relative to the DTBC substrate). To confirm that the H_2O_2 generated in the reaction (Scheme 1, vide supra) is fairly rapidly consumed, four independent experiments were performed in which authentic, exogenous H_2O_2 (18-26% vs 3,5-DTBC, i.e., ca. 1-1.5 times what is generated in the reaction) was added: (i) to pure solvent (1,2-$C_2H_4Cl_2$); (ii) to a substrate solution (3,5-DTBC in 1,2-$C_2H_4Cl_2$); (iii) to a precatalyst solution ((n-$Bu_4N)_7$-

(15) (a) Funabiki, T.; Mizoguchi, A.; Sugimoto, T.; Yoshida, S. *Chem. Lett.* **1983**, 917−920. (b) Nishida, Y.; Shimo, H.; Kida, S. *J. Chem. Soc., Chem. Commun.* **1984**, 1611−1612. (c) Funabiki, T.; Mizoguchi, A.; Sugimoto, T.; Tada, S.; Mitsuji, T.; Sakamoto, H.; Yoshida, S. *J. Am. Chem. Soc.* **1986**, *108*, 2921−2932. (d) An exceptional case is $RuCl_2(PPh_3)_3$-catalyzed DTBC oxygenation in which benzoquinone disappears in the early stages according to GLC analysis: Matsumoto, M.; Kuroda, K. *J. Am. Chem. Soc.* **1982**, *104*, 1433−1434.

(16) Weiner, H.; Hayashi, Y.; Finke, R. G. *Inorg. Chim. Acta* **1999**, *291*, 426−437.

(17) (a) Lide, D. R. *CRC Handbook of Chemistry and Physics*, 85th ed.; CRC Press: Boca Raton, FL, 2004−2005. (b) McMillen, D. F.; Golden, D. M. *Annu. Rev. Phys. Chem.* **1982**, *33*, 493−532.

(18) The products do show a solvent dependence: Na_2(DTBC) plus O_2 in diethyl ether yields 20−60% **5**. Speier, G.; Tyeklár, Z. *J. Mol. Catal.* **1990**, *57*, L17−L19.

(19) See the use of H_2O_2 to solubilize $Nb_6O_{19}^{8-}$ into monomeric Nb-peroxy species useful in subsequent syntheses: Finke, R. G.; Droege, M. W. *J. Am. Chem. Soc.* **1984**, *106*, 7274 and ref 11 therein.

(20) Connor, J. A.; Ebsworth, E. A. V. Peroxy Compounds of the Transition Metals. *Adv. Inorg. Chem. Radiochem.* **1964**, *6*, 279−381.

(21) Butler, A.; Clague, M. J.; Meister, G. E. *Chem. Rev.* **1994**, *94*, 625.

(22) Evidence for polyoxometalate degradation in H_2O_2: (a) Aubry, C.; Chottard, G.; Platzer, N.; Brégeault, J.-M.; Thouvenot, R.; Chauveau, F.; Huet, C.; Ledon, H. *Inorg. Chem.* **1991**, *30*, 4409. (b) Brégeault, J.-M.; Aubry, C.; Chottard, G.; Platzer, N.; Chauveau, F.; Huet, C.; Ledon, H. In *Dioxygen Activation and Homogeneous Catalytic Oxidation*; Simándi, L. I., Ed.; Elsevier Publishers: Amsterdam, 1991; pp 521−529.

(23) Lead references to Venturello's epoxidation catalyst, $\{PO_4[W(O)(O_2)_2]_4\}^{3-}$, utilizing H_2O_2 and which can be formed in situ from phosphotungstates: (a) Venturello, C.; Alneri, E.; Ricci, M. *J. Org. Chem.* **1983**, *48*, 3831. (b) Venturello, C.; D'Aloisio, R.; Bart, J. C. J.; Ricci, M. *J. Mol. Catal.* **1985**, *32*, 107.

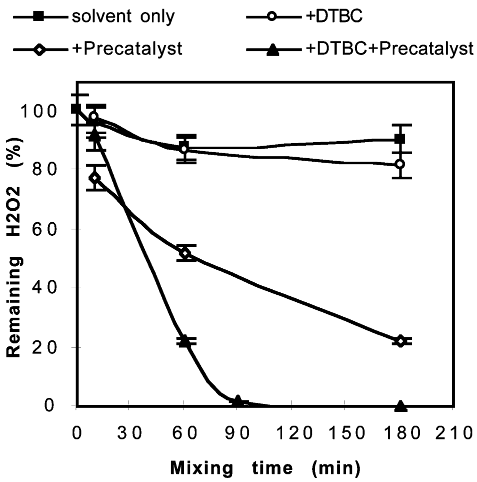


Figure 5. Percentages of exogenous H_2O_2 (0.3−0.5 mmol) remaining: in the 1,2-$C_2H_4Cl_2$ (8 mL) solvent alone; in a DTBC substrate solution (1.8 mmol of DTBC in 8 mL of 1,2-$C_2H_4Cl_2$); in a (n-$Bu_4N)_7SiW_9V_3O_{40}$ precatalyst solution (5.6 μmol of precatalyst in 8 mL of 1,2-$C_2H_4Cl_2$); and in a mixed substrate plus (n-$Bu_4N)_7SiW_9V_3O_{40}$ precatalyst solution (1.8 mmol of DTBC and 5.6 μmol of precatalyst in 8 mL of 1,2-$C_2H_4Cl_2$).

$SiW_9V_3O_{40}$ in 1,2-$C_2H_4Cl_2$); or (iv) to a mixed solution of substrate and precatalyst. Figure 5 presents the results of the iodometric titration of the remaining, detectable H_2O_2 in these four experiments. As Figure 5 reveals, H_2O_2 added to the reaction mixture is consumed fairly rapidly when just the (n-$Bu_4N)_7SiW_9V_3O_{40}$ precatalyst is present, and even more rapidly when DTBC and the V-polyoxoanion precatalyst are both present—after 90 min, only 2% of the added H_2O_2 remains in this latter case.

The implied conclusion from the reaction stoichiometry, which requires that 18% H_2O_2 is formed (concomitant with the 18% benzoquinone product, **6**); from the fact that, however, H_2O_2 is not detectable during the reaction ($\leq$0.4%) (vide supra); and from the above kinetic experiments in which added, authentic H_2O_2 is consumed fairly quickly is that H_2O_2 is the key product and the *kinetically competent intermediate*, B, that turns on the catalyst formation process *to yield the same main products*. Furthermore, confirming evidence that H_2O_2 is the key reagent, B, is provided by the fact that H_2O_2 stable[24] $PVW_{11}O_{40}^{5-}$ is the only V-containing polyoxoanion among many tested which failed to yield a dioxygenase catalyst. The novel part here is, again, that the usually undesirable, but often facile, autoxidation is what initiates the desired catechol dioxygenase reaction, an example of the *undesired autoxidation product initiating the desired dioxygenase catalysis!*

Pseudoelementary Step Treatment of the Autocatalytic Kinetics and Active Catalyst Formation Steps. The reader may well be wondering exactly how one measures the kinetics of $-d[O_2]/dt$, as we have done, yet uses this information to learn about the conversion of the polyoxometalate precatalyst to the catalyst. The answer is a connection that can be made through the employment of a concept very useful for studying more complicated reactions, the *pseudoelementary step* concept.[3,25] As discussed in greater detail elsewhere, the pseudoelementary concept was first used by Noyes as a kinetic tool for understanding oscillatory reactions. We have further developed this concept in our mechanistic work on a $\gg$300-step self-assembly reaction, which forms $Ir(0)_{\sim 300}$ nanoclusters, that, as it turns out, also displays an A → B and then A + B → 2B sequence of pseudoelementary reactions in, of course, a quite different way.[3]

As shown below, the pseudoelementary step concept applied to the present case allows us to use the overall reaction and precatalyst-to-catalyst conversion stoichiometry to connect the readily measurable $-d[O_2]/dt$ to the desired, but more difficult to obtain directly, values $-d[\text{precatalyst}]/dt$ and $+d[\text{catalyst}]/dt$. The pseudoelementary step treatment of the kinetics for the present catechol dioxygenase is summarized in the equations below in which A is the precatalyst (e.g., (n-$Bu_4N)_7SiW_9V_3O_{40}$) and B is H_2O_2; note that a key point is that reactions 1 and 2 also must form the catalyst (i.e., or catalyst resting state), Pierpont's dimer, $[VO(DBSQ)(DTBC)]_2$. The third step, (3) below, is a fast "reporter" reaction which catalytically amplifies how much catalyst has been made at a given moment: the more catalyst, the faster the O_2-uptake reaction, with, again, its overall sigmoidal shape due in large part to the A + B → 2B autocatalytic step. The sum reaction is a pseudoelementary reaction in that it is composed of other elementary (or even pseudoelementary) steps. Note also that DTBC and O_2 are involved in eqs 1 and 2 but have been deliberately omitted to simplify eqs 1-3 to their most essential features. In practice, if the DTBC and O_2 are in excess and if they remain relatively constant over the time course of the curve fitting (e.g., if one fits only the first half of the kinetics), then these [DTBC] and [O_2] concentrations can be treated as rough constants for the purposes of the semiquantitative work herein *designed to determine the basic reactions* rather than to measure accurate rate constants.

$$\underline{\mathbf{A \rightarrow B}}$$

$$x[\underset{\mathbf{A}}{\underline{SiW_9V_3O_{40}^{7-}}} \rightarrow \underset{\mathbf{B}}{\underline{H_2O_2}} + 1/2[VO(DBSQ)(DTBC)]_2] \qquad (1)$$

$$\underline{\mathbf{A + B \rightarrow 2B}}$$

$$(1-x)[\underset{\mathbf{A}}{\underline{SiW_9V_3O_{40}^{7-}}} + \underset{\mathbf{B}}{\underline{H_2O_2}} \rightarrow \underset{\mathbf{2B}}{\underline{2H_2O_2}} + 1/2[VO(DBSQ)(DTBC)]_2] \qquad (2)$$

$$\underline{\mathbf{O_2 - uptake\ Reporter\ Reaction}}$$

$$\underline{\sim 300[DTBC + O_2 \xrightarrow{\text{fast}} \text{Dioxygenase Products}]} \qquad (3)$$

$$\mathbf{Sum}: SiW_9V_3O_{40}^{7-} + \sim 300\ DTBC + \sim 300\ O_2 \rightarrow H_2O_2 + 1/2[VO(DBSQ)(DTBC)]_2 + \sim 300\ [\text{Dioxygenase Products}]$$

The pseudoelementary step ("Sum" step after eq 3) allows the following desired differentials to be written in the usual

(24) (a) Nomiya, K.; Yanagibayashi, H.; Nozaki, C.; Kondoh, K.; Hirmatsu, E.; Simizu, Y. *J. Mol. Catal. A: Chem.* **1996**, *114*, 181−190. (b) Nomiya, K.; Yagishita, K.; Nemoto, Y.; Kamataki, T. *J. Mol. Catal. A: Chem.* **1997**, *126*, 43−53. (c) Note that the findings of the present work support, in a general way, Venturello's[23] as well as Nomiya's[24a] findings that oxygenations with H_2O_2 and V-based polyoxoanions involve V-fragments of the polyoxoanions as the true catalysts, rather than intact polyoxoanion catalysis.

(25) Original work using the pseudoelementary step concept: (a) Field, R. J.; Noyes, R. M. *Acc. Chem. Res.* **1977**, *10*, 214−221. (b) Noyes, R. M.; Field, R. J. *Acc. Chem. Res.* **1977**, *10*, 273−280. (c) Noyes, R. M.; Furrow, S. D. *J. Am. Chem. Soc.* **1982**, *104*, 45−48.

Scheme 2. Plausible, More Detailed Reactions for H_2O_2 Autoxidation-Product-Mediated Conversion of a Vanadium−Polyoxometalate to Pierpont's Dioxygenase Catalyst, $[V^V(O)(DBSQ)(DTBC)]_2$

$$\underline{\mathbf{A} \rightarrow \mathbf{B}} \qquad (1)$$

$$\text{DTBC} + O_2 \rightarrow H_2O_2 + \text{DBQuinone} \qquad (1a)$$

$$SiW_9V_3O_{40}^{7-} + H_2O_2 \rightarrow V(O)O_2^{+} + SiW_9V_2O_{39}^{10-} + 2H^+ \qquad (1b)$$

$$V(O)O_2^{+} + 2\text{DTBC} + 0.25\,O_2 \rightarrow 1/2[VO(DBSQ)(DTBC)]_2 + H_2O_2 + H^+ + 0.5H_2O \qquad (1c)$$

Sum

$$\underbrace{\underline{SiW_9V_3O_{40}^{7-}}}_{\mathbf{A}} + 3\text{DTBC} + 1.25O_2 \rightarrow \underbrace{\underline{H_2O_2}}_{\mathbf{B}} + 1/2[VO(DBSQ)(DTBC)]_2 + 0.5H_2O + 3H^+ + SiW_9V_2O_{39}^{10-} + \text{DBQuinone} \qquad (1')$$

$$\underline{\mathbf{A} + \mathbf{B} \rightarrow 2\mathbf{B}} \qquad (2)$$

$$\underbrace{\underline{SiW_9V_3O_{40}^{7-}}}_{\mathbf{A}} + \underbrace{\underline{H_2O_2}}_{\mathbf{B}} + 1.25O_2 + 3\text{DTBC} \rightarrow \underbrace{\underline{2H_2O_2}}_{2\mathbf{B}} + SiW_9V_2O_{39}^{10-} + 1/2[VO(DBSQ)(DTBC)]_2 + 3H^+ + 0.5H_2O + \text{DBQuinone} \qquad (2')$$

fashion: $-d[O_2]/300 \cdot dt = -d[A = \text{precatalyst}]/dt = +d\{[VO(DBSQ)(DTBC)]_2\}/0.5 \cdot dt$, where the value of 300 results from our (initial, picked for convenience) stoichiometry of 1.8 mmol of DTBC (eq 3 above) vs ca. 6 μmol of precatalyst, A. Hence, we can follow the kinetics of the dioxygenation of DTBC but learn about the desired formation of the catalyst, $-d[A = \text{precatalyst}]/dt = +d[B = H_2O_2]/dt = +d[\text{catalyst}]/0.5 \cdot dt$, *as long as* eq 3 is faster than (1) and (2). The earlier steps, (1) and (2), are co-rate-determining in this treatment.

Plausible Stoichiometries for the Conversion of $SiW_9V_3O_{40}^{7-}$ to the Active Catalyst, $[V^V(O)(DBSQ)(DTBC)]_2$, Plus Initial Experimental Tests of Those Stoichiometries. It is possible to write more specific stoichiometries for the conversion of A, $SiW_9V_3O_{40}^{7-}$ (the prototype polyoxometalate studied herein), to the catalyst, $[V^V(O)(DBSQ)(DTBC)]_2$, via the involvement of the autocatalytic product, B, H_2O_2 generated by the autoxidation of 3,5-DTBC to the benzoquinone, **6**. Those stoichiometries, in turn, provide predicted reactions that can be subjected to experimental verification or refutation. In what follows in Scheme 2, we use the precedent of $V(O)O_2^+$ leaching from polyoxometalates by H_2O_2 as a key part of our scheme of hypothesized reactions (e.g., the precedent of decavanadate plus hydrogen peroxide:[20,21] $V_{10}O_{28}^{6-} + H_2O_2 \rightarrow V(O)O_2^+$).

We performed an initial test of the predicted stoichiometry in eq 1′ above via two experiments in which $SiW_9V_3O_{40}^{7-}$ was treated with 4-6 equiv of DTBC and the O_2 uptake stoichiometry and reaction products were determined. Key differences in the experimental conditions of the *stoichiometry* experiments vs the *catalytic* experiments examined so far, which will prove important (vide infra), are (1) a different stoichiometric ratio of the precatalyst to DTBC had to be used in the stoichiometry experiments (ca. 0.05 mmol of $SiW_9V_3O_{40}^{7-}$ vs 0.2-0.3 mmol of DTBC) in order to produce sufficient material for analysis (a ratio of ca. 0.006 mmol of precatalyst vs 1.8 mmol of DTBC in the catalytic runs) and (2) DTBC was added to the precatalyst in the stoichiometry experiments (whereas the reverse order of addition was employed in the catalytic runs).

In one experiment, $SiW_9V_3O_{40}^{7-}$ was treated with 4 equiv of DTBC and 1 equiv of H_2O_2. The measured O_2-uptake was 29 ± 3% relative to DTBC, and GC showed 56 ± 6% conversion of DTBC to DBQuinone as the primary product. Significantly, ≤3% of the dioxygenase products **2-5** were formed, *indicating that the conditions of the* stoichiometry experiments are producing different chemistry than the catalytic conditions. The observed O_2/DBQuinone ratio is 0.52(±0.08)/1 vs a predicted ratio of 1.25/1 from Scheme 1. In a second experiment employing 6 equiv of DTBC, the O_2-uptake was 37 ± 2% relative to DTBC for a ratio of O_2/DBQuinone of 0.63(±0.05)/1 (vs the predicted 1/1 ratio) and GC revealed a 59 ± 4% conversion of DTBC to DBQuinone with a small percentage (≤2%) of dioxygenase products **2-5** being formed.

The product solutions from the two experiments above were examined by negative ion ESI-MS to determine which V species were present. Two major peaks assignable, as before,[6] to $[VO(DTBC)_2]^-$ and $[V(DTBC)_3]^-$ were observed (Figure S6). Interestingly, Pierpont's catalyst resting state, $[VO(DBSQ)(DTBC)]_2$, was *not* detectable in the product solution by either ESI-MS or EPR, despite our previous ready detection of this catalyst resting state by both of these methods.[6] This is a key finding: we have already shown in our earlier work that $[V(DTBC)_3]^-$ yields diagnostic dioxygenase products **2−5** with short induction periods (∼10 min) when treated with excess DTBC under catalytic conditions (see Figure 4 of ref 6). It appears, then, that the catalytic conditions involving a large excess of DTBC to vanadium (i.e., which are not the conditions for the stoichiometry studies employed above) are required for the formation of $[VO(DBSQ)(DTBC)]_2$. It is actually reassuring that Pierpont's catalyst resting state is *not* seen under conditions where the dioxygenase products **2−5**, that are the hallmark of its presence,[6] are also *not* seen. Moreover, the difference in the observed value of ca. 0.6/1 vs the predicted value of 1.25/1 for the O_2/DBQuinone stoichiometry makes it clear that a competing, parallel reaction of DTBC + $H_2O_2 \rightarrow$ DBQuinone + $2H_2O$ is occurring under the conditions of the stoichiometry experiment. If one corrects for this by subtracting out the amount of DBQuinone formed from the DTBC consumed, then one finds that ca. 2 equiv of DTBC reacts with 1 equiv of $SiW_9V_3O_{40}^{7-}$ and 1 equiv of H_2O_2 to produce detectable amounts of $V(DTBC)_3^-$ and $VO(DTBC)_2^-$.

Overall, the more detailed reactions hypothesized in Scheme 2 and these initial tests (i) provide independent evidence that H_2O_2-assisted V-leaching from polyoxoanions to form species such as $V(DTBC)_3^-$ and $VO(DTBC)_2^-$ can and does occur (as detected by ESI-MS and their respective *m*/*z* values of 711 and 507) and (ii) detect $VO(DTBC)_2^-$ by ESI-MS, implying that it is at least a possible intermediate en route to $[VO(DBSQ)(DTBC)]_2$ but (iii) show that *neither* the formation of diagnostic dioxygenase products **2−5** nor the previously demonstrated[6] conversion of $V(DTBC)_3^-$ into the active catalyst capable of forming **2−5** occurs *under noncatalytic conditions*.

Confirmed Formation of $[VO(DBSQ)(DTBC)]_2$ from $V(DTBC)_3^-$ under Catalytic Conditions Using EPR. The experimental procedures are detailed in a previous paper.[6] The reaction conditions are as follows: 1.8 mmol of DTBC and 0.003 μmol of $[Na(CH_3OH)_2]_2[V^V(DTBC)_3]_2\cdot 4CH_3OH$ were allowed to react with O_2 at 40 °C in 8 mL of toluene. The reaction solution was sampled for EPR experiments. The postreaction solution gives the 9-line EPR spectrum (g = 2.006, A_{51V} = 3.08 G) that is the signature of Pierpont's catalyst resting state, $[VO(DBSQ)(DTBC)]_2$.[6] The organic products established by GC (and in toluene) are as follows: **2**, in 25 ± 1% yield; **3**, in 5 ± 1% yield; **4**, in 8 ± 1% yield; and **6**, in 20 ± 1% yield. These yields in toluene are somewhat different from the product yields in our standard solvent (1,2-$C_2H_4Cl_2$), as shown in Scheme 1. Nevertheless, the EPR results confirm the finding that the specific condition of an excess of DTBC to vanadium is key to the formation of $[VO(DBSQ)(DTBC)]_2$.

A Return to the Question of the Broader Generality of Autoxidation-Initiated Dioxygenases or Other Reactions. An important question is are there other examples of autoxidation-product-initiated chemistry? Is this a more general concept? As noted in the Introduction, this concept is very likely much more widespread even if rarely detected, *given that autoxidation and the formation of the resultant ROOH products is the primary way that organic compounds degrade in air*.

One highly relevant case that may be autoxidation-initiated chemistry is that of the oxidation of saturated hydrocarbons with 2,6-dichloropyridine-*N*-oxide as the oxygen donor and $[Ru^{II}(TPFPP)(CO)]$ as the precatalyst (TPFPP is the 5,10,15,20-tetrapentafluorophenylporphyrinato ligand). This system shows induction periods similar to those seen here, and evidence exists that a Ru(V)-oxo species or a Ru(IV)-oxo porphyrin radical cation is the reactive species.[26] In addition, the CH_2Cl_2 solvent and O_2 have been described as "noninnocent"[26b] and would seem to be prime candidates for at least a plausible autoxidation-product-induced reaction and catalyst formation step.

We found at least one clear example of autoxidation-product-enhanced *reduction* catalysis as well; notably, the ethylbenzene autoxidation product $C_6H_5CH(OOH)CH_3$ enhances the rate of ethylbenzene reduction by the triruthenium cluster cation precatalyst[27,28] $[Ru_3(\mu_2\text{-}H)_3(\eta^6\text{-}C_6H_6)(\eta^6\text{-}C_6Me_6)_2(\mu_3\text{-}O)]^+$. The catalysis-triggering process is known in some detail in that case: this substitutionally inert complex gets oxidized by the $C_6H_5CH(OOH)CH_3$ hydroperoxide autoxidation product, and the resultant complex is more readily turned into the true $Ru(0)_n$ nanocluster ethylbenzene reduction catalyst[29] under H_2—an example of autoxidation-product-enhanced *reduction* catalysis.

There are surely other examples of autoxidation-product-enhanced catalysis as well, although they are either buried in the literature and, hence, hard to find, simply unrecognized, or, in some cases, are related but conceptually distinct chemistry.[30] Cyclopentadienyl ligand removal under O_2 and activation of hydrogenation catalysts most likely qualifies as one example;[31] another is probably the better-known phosphine oxidation to $O{=}PR_3$ in the presence of trace O_2, a process which opens a site of coordinative unsaturation, thereby turning on catalysis.[32] However, unless an ROOH autoxidation product is the actual oxidant for the PR_3, this latter case would not fall under the definition provided.[9]

Of course, there is the present, apparently best-studied example of an autoxidation-product-initiated reaction, that of a record catalytic lifetime vanadium catechol dioxygenase initiated autocatalytically via the autoxidation product H_2O_2 to form, ultimately, Pierpont's catalyst resting state $[VO(DBSQ)(DTBC)]_2$. That is, we have discovered an example of the seemingly improbable situation where *undesired* autoxidation turns on the *desired dioxygenase chemistry*, a novel *autoxidation-initiated dioxygenase*. Since the series of radical-chain reactions known as autoxidation is the common, central pathway by which organic compounds such as fats, plastics, gasoline, lubricating oils, rubbers, etc. degrade to hydroperoxides with ROOH as the primary initial products (or H_2O_2 and benzoquinones, in the case of catechols),[33] it seems highly likely that autoxidation-product-initiated reactions are much more common than heretofore appreciated.

Summary and Conclusions

The main findings from this study can be summarized as follows:

(i) An example of the seemingly unlikely case of the undesired reaction of autoxidation producing a product that

(26) (a) Groves, J. T.; Bonchio, M.; Carofiglio, T.; Shalyaev, K. *J. Am. Chem. Soc.* **1996**, *118*, 8961−8962. (b) Grover, J. T. Mechanisms of Metalloporphyrin-Catalyzed Oxidations. Presented at the ADHOC 2002 Conference, Activation of Dioxygen and Homogeneous Catalytic Oxidation, Emory University, Atlanta, GA, June 2−7, 2002. (c) Professor Grove's aerobic olefin epoxidation dioxygenase: Groves, J. T.; Quinn, R. *J. Am. Chem. Soc.* **1985**, *107*, 5790−5792. This classic system has not been commercialized due, it is our understanding, to its low catalytic turnover frequencies (ca. 1 turnover/h), irreversible inactivation to Ru(III), product inhibition, and eventual oxidative inactivation of the porphyrin-based catalyst system. It is a remarkable system nevertheless. (d) A pertinent review is the following: Groves, J. T. *Models and Mechanisms of Cyctochrome P_{450}: Structure, Mechanism and Biochemistry*, 3rd ed.; Ortiz de Montellano, P. R., Ed.; Kluwer Academics/Plenum Publishers: New York, 2004; pp 1−44.

(27) Schissler, D. O.; Stevenson, D. P. *J. Chem. Phys.* **1954**, *22*, 151−152. The easily autoxidized benzylic C−H bond has a bond-dissociation energy of 77 ± 3 kcal mol^{-1}.

(28) Süss-Fink, G., Therrien, B., Vieille-Petit, L., Tschan, M., Romakh, V. B., Ward, T. R., Ed. In *Abstracts of Papers, 226th ACS National Meeting, New York*; American Chemical Society: Washington, DC, 2003; p INOR-369.

(29) Hagen, C. M.; Vieille-Petit, L.; Laurenczy, G.; Süss-Fink, G.; Finke, R. G. *Organometallics* **2005**, *24* (B), 1819−1831.

(30) For example, RH autoxidation to ROOH and, then, decomposition of that metal-catalyzed ROOH to products such as the ketone and water are well-known processes but distinct chemistry from that herein where H_2O_2 is formed *and then is crucial in helping to create a new (dioxygenase) catalyst*. See: Chen, J. D.; Sheldon, R. A. *J. Catal.* **1995**, *153*, 1−8.

(31) Hamlin, J. E.; Hirai, K.; Gibson, V. C.; Maitlis, P. M. *J. Mol. Catal.* **1982**, *15*, 337−347.

(32) (a) van Bekkum, E.; van Rantwijk, F.; van de Putte, T. *Tetrahedron Lett.* **1969**, 1. (b) Augustine, R. L.; van Peppen, J. F. *J. Chem. Soc., Chem. Commun.* **1970**, 571. (c) Strohmeier, W.; Hitzel, E. *J. Organomet. Chem.* **1975**, *91*, 373. (d) Strohmeier, W.; Hitzel, E. *J. Organomet. Chem.* **1975**, *102*, C37. (e) Strohmeier, W.; Lukacs, M. *J. Organomet. Chem.* **1977**, *129*, 331. (f) Strohmeier, W.; Lukacs, M. *J. Organomet. Chem.* **1977**, *133*, C49. (g) Strohmeier, W.; Weigelt, L. *J. Organomet. Chem.* **1977**, *133*, C43.

(33) Weiner, H.; Trovarelli, A.; Finke, R. G. *J. Mol. Catal. A: Chem.* **2001**, *191*, 217−252 and references therein to the extensive prior literature of autoxidation.

initiates the desired dioxygenase reaction has been discovered. Autoxidation of the DTBC substrate to the corresponding benzoquinone, with coproduction of H_2O_2, was shown to be key to the catalyst evolution process, with the H_2O_2 being a key to the V-leaching, autocatalytic catalyst production and the resultant, observed sigmoidal kinetic curves. Of interest here is that this result is very non-biomimetic; H_2O_2 *inhibits* at least some dioxygenase enzymes.[34]

(ii) Our studies confirm the conclusion in our earlier paper[6] that attempts to support and prepare nonleachable V-based dioxygenase catalysts will require new strategies, especially if H_2O_2 and powerful σ- and π-donor ligands, such as $[DTBC]^{2-}$, will be present or can be formed.

(iii) The novel concept of *autoxidation-product-induced* catalysis is hypothesized to be more general but little recognized. This hypothesis is supported by independent examples of both oxidative and reductive catalysis extracted from the literature and presented herein.

(iv) Our previous finding[6] that Pierpont's important, structurally characterized complex $[VO(DBSQ)(DTBC)]_2$ is directly connected to the catalytic cycle remains consistent with all our findings. In a separate paper, we provide compelling kinetic evidence that this complex is in fact the catalyst resting state and that it fragments to 2 equiv of its active monomer, VO(DBSQ)(DTBC), prior to reaction with O_2 in a rate-determining step.[35]

(34) Que, L., Jr. *Coord. Chem. Rev.* **1983**, *50*, 73−108.
(35) Yin, C.-X.; Finke, R. G. Kinetic and Mechanistic Studies of Vanadium-Based, Extended Catalytic Lifetime Catechol Dioxygenases. *J. Am. Chem. Soc*., published online Sept 17, http://dx.doi.org/10.1021/ja052998+.

These results, along with our other work, go far in simplifying and unifying the previously disparate literature about V-based DTBC dioxygenase catalysts. It is hoped that these and our additional kinetic and mechanistic studies will help expedite progress in the area of highly active, long-lived, man-made dioxygenase catalysts.

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Supporting Information Available: O_2-uptake of DTBC and the precatalyst $(n\text{-}Bu_4N)_7SiW_9V_3O_{40}$ with the addition of H_2O_2 and 3,5-DBQuinone; O_2-uptake of DTBC and the precatalyst $(n\text{-}Bu_4N)_7SiW_9V_3O_{40}$ with the addition of AIBN; O_2-uptake of DTBC and the precatalyst $(n\text{-}Bu_4N)_7SiW_9V_3O_{40}$ with the addition of NaDBSQ; O_2-uptake of DTBC and the precatalyst $(n\text{-}Bu_4N)_7SiW_9V_3O_{40}$ in water-saturated 1,2-$C_2H_4Cl_2$; O_2-uptake of DTBC and the precatalyst $(n\text{-}Bu_4N)_7SiW_9V_3O_{40}$ in *o*-dichlorobenzene; negative ion ESI-MS spectrum of the product solution from an experiment probing the catalyst evolution stoichiometry; control experiments testing the effectiveness of transferring H_2O_2 with a stainless steel needle; and stirring rate controls—the establishment of O_2 mass-transfer limitations. This material is available free of charge via the Internet at http://pubs.acs.org.

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