Bioinorganic Chemistry

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Bioinspired Dismutation of Chlorite to Dioxygen and Chloride Catalyzed by a Water-Soluble Iron Porphyrin**

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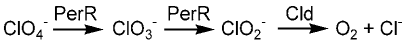
Oxychlorine species (ClO_x^-) are produced commercially for use as bleaching agents,[1] explosives,[2] herbicides,[3] and disinfectants.[4] The contamination of ecological and potable water supplies by these chemicals poses concerns for biology[5] and human health.[6] For these reasons, methods for environmental remediation of oxychlorine contamination are being sought.

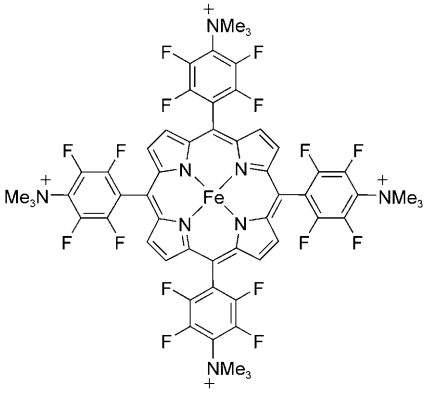
Perchlorate respiring bacteria (PRB) offer a potentially viable means for the removal of ClO_x^- from water supplies. These microorganisms contain two important enzymes: Perchlorate reductase (PerR), which reduces perchlorate to chlorate and chlorate to chlorite,[7] and chlorite dismutase (Cld), which catalyzes the dismutation of chlorite to Cl^- and O_2 (Scheme 1).[8]



Scheme 1. Chlorite respiration pathway for PRB respiring bacteria.

The chemistry of Cld, a tetrameric heme b enzyme, is the focus of this work. Besides some preliminary activity measurements,[8] little is known about the molecular mechanism of this enzyme. With the intent to better understand the enzyme chemistry and to develop potentially useful chemical catalysts for environmental chlorite remediation, we have explored water-soluble iron porphyrins as bioinspired catalysts for chlorite dismutation.

Synthetic systems that evolve dioxygen (O_2) from ClO_2^- are rare. Collman and co-workers have recently reported on a manganese porphyrin catalyst for alkane oxidation by ClO_2^- that evolves O_2 as a minor side reaction.[9] To our knowledge this is the only example of metal-catalyzed oxygen-evolving chlorite decomposition. Herein, we report on the water-soluble iron porphyrin system 5,10,15,20-tetrakis(tetrafluoro-*N*,*N*,*N*-trimethylanilinium)porphyrinatio iron(III) ([Fe(TF$_4$TMAP)], Scheme 2)[10] as a biomimetic chlorite dismutation catalyst.



[Fe^{III}(TF$_4$TMAP)]$^{5+}$[OTf$^-$]$_5$

Scheme 2. Structure of [Fe(TF$_4$TMAP)] catalyst. OTf = $CF_3SO_3^-$.

At pH 7.14, the addition of excess $NaClO_2$ (20 equivalents) to [Fe(TF$_4$TMAP)] results in a color change from brown to red and affords the appearance of bubbles. Mass spectrometric analysis using a residual gas analyzer (RGA) identifies the evolved gas as O_2 ($m/z = 32$). It is worth noting that simpler water-soluble porphyrin complexes such as [Fe(TMAP)] (TMAP = tetrakis(trimethylanillinium)porphyrin) and [Fe(TPPS)] (TPPS = tetrakis(*p*-sulfonatophenyl)porphyrin) exhibit different reactivity than [Fe(TF$_4$TMAP)], producing trace amounts of dioxygen, and are highly prone to bleaching by chlorite. Absorption spectroscopy (acquired on a stopped-flow analyzer) shows a red shift in the Soret band from 405 to 410 nm with a 12% increase in absorptivity, a spectrum consistent with the oxoferryl species [O=Fe^{IV}(TF$_4$TMAP)] (compound **II**).[11] This spectrum reverts back to the Fe^{III} form over the course of 30 min (Figure 1).

Given the facile appearance of compound **II** on the stopped-flow timescale (less than 1 s), we propose that oxoferryl species form by oxygen-atom transfer (OAT) to [Fe^{III}(TF$_4$TMAP)]. The resulting [O=Fe^{IV}(TF$_4$TMAP$^{\bullet+}$)] complex (compound **I**) has been shown to quickly comproportionate with iron(III) to give two equivalents of compound **II** [Eq (1)].[11a]

$$[Fe^{III}(TF_4TMAP)] + [O{=}Fe^{IV}(TF_4TMAP^{\bullet+})] + H_2O \rightarrow 2\,[O{=}Fe^{IV}(TF_4TMAP)] + 2\,H^+ \quad (1)$$

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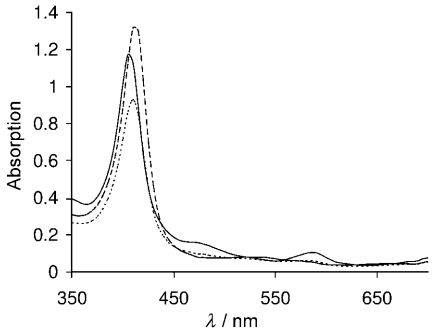
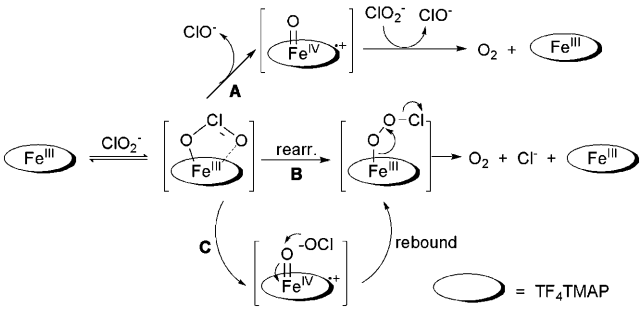


Figure 1. Absorption spectra showing [Fe^{III}(TF_4TMAP)] (——), [O=Fe^{IV}-(TF_4TMAP)] 1 s after addition of ClO_2^- (-----), and the return of catalyst to the Fe^{III} form ca. 30 min after reaction with some bleaching (•••••).



Scheme 3. Possible mechanisms for dismutation of chlorite: **A**, sequential OAT through [Fe(TF_4TMAP$^{+\cdot}$)] (compound **I**); **B**, concerted rearrangement of bound chlorite without formation of oxo iron; and **C**, OAT with subsequent rebound of compound **I** and ClO^- ion pair.

Monitoring of the absorption band of ClO_2^- at 260 nm indicated complete consumption of chlorite, which was present in at least twenty-fold excess relative to [Fe^{III}-(TF_4TMAP)]. Quantification of evolved dioxygen using an in-house-built time-dependent RGA mass spectrometer gave a yield of 18% O_2 based on ClO_2^-. Ion chromatography (IC) was employed to determine the fate of all chlorine-containing products. Ion chromatographic analysis afforded an approximate 2:3 ratio of chloride (Cl^-) to chlorate (ClO_3^-), with complete consumption of reactant chlorite. The 52% yield of chlorate coupled with 18% O_2 and 36% Cl^- yield accounts approximately for 80% of the oxygen and 88% of the chlorine atoms.

An ideal chlorite dismutation catalyst would transform ClO_2^- entirely to innocuous Cl^- and O_2 [Eq. (2)], as the enzyme Cld does. For this reason we sought to delineate the source of O_2 and the origin of chlorate formation, which could be either a by-product of O_2 formation (on pathway) or a result of a competitive (off) catalytic pathway. At pH 7.14, ClO_2^- does not exchange its oxygen atoms with solvent water. When ClO_2^- degradation catalyzed by [Fe(TF_4TMAP)] was carried out in $H_2{}^{18}O$ (95% enriched), exclusively $^{16}O_2$ (*m*/*z* 32) was detected. This result demonstrates that ClO_2^- is the sole source of dioxygen.

$$ClO_2^- \rightarrow Cl^- + O_2 \qquad (2)$$

Several metal systems have been documented for the disproportionation of hypochlorite to chloride and oxygen ($2\,ClO^- \rightarrow 2\,Cl^- + O_2$),[12] and our system is no exception.[13] However, at pH 7.14 and 25 °C ClO^- exchanges its oxygen atoms readily with water. Indeed, ClO^- disproportionation in the presence of 5 mol% [Fe(TF_4TMAP)] in 95% $H_2{}^{18}O$ gives isotopically enriched O_2 (*m*/*z* 32:34:36 in a 2:4:3 ratio). Therefore, the absence of ^{18}O-enriched O_2 from ClO_2^- decomposition demonstrates that hypochlorite cannot be the source of dioxygen. The exclusive formation of $^{16}O_2$ in $H_2{}^{18}O$ can be rationalized by reaction of chlorite with an oxoferryl species or by a concerted pathway (Scheme 3).

To distinguish the mechanisms outlined in Scheme 3 for O_2 formation, we performed a double-crossover experiment with a 1:1 ratio of standard $Cl^{16}O_2^-$ and isotopically labeled $Cl^{18}O_2^-$ (77% enrichment). Concerted dismutation of this mixture by [Fe(TF_4TMAP)] via mechanisms **B** or **C** would be expected to result in predominantly $^{16}O_2$ and $^{18}O_2$ with no additional $^{16}O^{18}O$ resulting from crossover. If oxygen scrambling occurs (pathway **A** in Scheme 3), the expectation is a 1:2:1 binomial distribution of $^{16}O_2$, $^{16}O^{18}O$, and $^{18}O_2$.

Analysis of the gas mixture by RGA demonstrates *m*/*z* peak distribution of 54:17:29 (Figure 2) for the three isotopomers. The larger $^{16}O_2$ and $^{18}O_2$ peaks suggest concerted dismutation, while the smaller $^{16}O^{18}O$ peak is consistent with

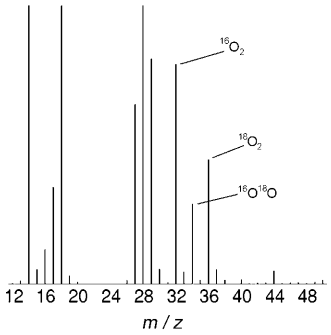


Figure 2. Mass spectrum of gaseous product from double crossover experiment. Mass peaks at *m*/*z* 32, 34, and 36 correspond to $^{16}O_2$, ^{16}O–^{18}O, and $^{18}O_2$, respectively. Other peaks are those of carrier gas N_2 (*m*/*z* 28 and 14) and water (*m*/*z* 18).

the incomplete (77%) enrichment of the starting $NaCl^{18}O_2$ sample. This result strongly suggests that each oxygen atom in the molecular O_2 product originated from the same chlorite ion [Eq. (2)]. Dismutation of chlorite therefore most likely occurs by either a concerted rearrangement mechanism, pathway **B**, or a rebound mechanism, pathway **C** in Scheme 3.

The data suggest that chlorate formation results from a competing catalytic pathway and is not a byproduct of dioxygen formation. Indeed, we have found that chlorate (ClO_3^-) forms in a competing catalytic cycle that involves [O=Fe^{IV}(TF_4TMAP)] (compound **II**). The use of compound **II** as catalyst produces ClO_3^- and Cl^- with diminished O_2 yield. Hence, chlorate is formed by OAT from [O=Fe^{IV}-

(TF_4TMAP)] to ClO_2^-. The details of the chlorate pathway and its kinetics will be the subject of another report.

Irrespective of the pathway (**B** or **C** in Scheme 3), we propose that O=O bond formation proceeds via a fleeting peroxyhypochlorite intermediate, which is poised to evolve O_2 and Cl^-. This intermediate could form either from rearrangement of a bound chlorite ion as in mechanism **B** or by a nucleophilic attack of ClO^- on the oxoferryl oxygen atom (mechanism **C**), reminiscent of the "radical rebound" recognized for heme and nonheme oxygenases.[14] The observation of oxoferryl species compound **II** lends support to mechanism **C**. However, the absence of an enzyme pocket in this model system would lead to some diffusion of hypochlorite into the solvent, which would compete with the rebound of the ion pair. This observation may explain the low yield (18%) of O_2. The absence of isotopically labeled oxygen when the reaction is carried out in $H_2{}^{18}O$ suggests that diffused hypochlorite does not accumulate in solution but instead partakes in the chlorate formation pathway by fast reaction with [Fe(TF_4TMAP)] catalyst (see above).

We have shown that [Fe(TF_4TMAP)] catalyzes the dismutation of chlorite to O_2 and Cl^-. Isotope labeling studies established that both oxygen atoms in O_2 come from chlorite. The catalyst produces chlorate in addition to O_2 and Cl^- in a competing pathway. The mechanistic insights gained from this study should aid in the development of chemical catalysts that depress chlorate formation and favor ClO_2^- conversion to innocuous chloride and dioxygen.

Experimental Section

General: All reactions were carried out in deionized water obtained from a Millipore Milli-Q Academic TC water purification system. Reagents were used as obtained from Fisher, Baker, Acros, Sigma–Aldrich, GFS, Mid-Centruy, Frontier, Cambridge Isotope Labs, and Strem. Phosphate buffers were prepared by dissolution of mono- and dibasic sodium phosphate. UV/Vis spectra were recorded on a Shimadzu UV-2501PC scanning spectrophotometer. Gas evolution was analyzed using an in-house-built RGA mass spectrometer. Typically, the reaction solution (1–2 mL) was stirred in a custom-made glass RGA cell with a minimum (1–2 mL) head space. An inert carrier gas (Ar or N_2) was drawn over the reaction head space at 2 mL min^{-1} by a Varian model SH 100 vacuum pump and analyzed by a Stanford Research Systems RGA 100 mass spectrometer equipped with an Alcatel ATH31 Series turbopump. Ion chromatography was performed on a Dionex DX-500 Liquid Chromatography System equipped with a Dionex LC25 Chromatography Oven, a Dionex ED40 Electrochemical Detector, and a Dionex Ion-Pac AS9-HC ion exchange column. 9 mM Na_2CO_3 was used as eluant. Chromatography calibration standards were prepared in the 0.5–60 mM concentration range. Peaks were identified by comparison to standard samples and quantified by comparison of the integrals of the signals to standard curves for the corresponding ion. ESI mass spectra were obtained using a Finnigan LTQ linear ion trap mass spectrometer in negative ion mode. Sample was introduced by direct infusion from a syringe pump.

[Fe(TF_4TMAP)][OTf]$_5$ was prepared from 5,10,15,20-tetrakis-(pentafluorophenyl)porphine (Frontier) according to the procedure of Miskelly and co-workers.[10a]

RGA calibration: Yields of dioxygen were determined by integration of the RGA signal and comparison to a calibration curve prepared by the injection of known volumes of oxygen gas. The calibration plots were obtained in the following way: For a 4 mL RGA glass cell, 2 mL water was added into the cell and stirred to simulate a typical reaction. 50, 125, 250, 375, and 500 μL air was injected into the RGA cell through a rubber septum. Partial pressure (torr) of oxygen was monitored against time (seconds) for each calibration point. Integration of the partial pressure versus time graph is plotted against mole oxygen injected (assuming 20.95% of air is oxygen).

Ion chromatography on reaction products: A 20 mM solution of $NaClO_2$ in 50 mM pH 7.14 phosphate buffer (0.5 mL) was added to a 1.125 μM solution of [Fe(TF_4TMAP)] in 50 mM pH 7.14 phosphate buffer (20 mL) and stirred for 30 min. This solution was analyzed and quantified by IC.

Preparation of isotopically labeled $NaCl^{18}O_2$: Isotopically labeled chlorite was prepared and used in situ based on a modified protocol for the production of sodium chlorite from chlorate.[15] In a thick-walled Schlenk tube, $NaClO_3$ (2.1 g, 20 mmol) was stirred in 95% enriched $H_2{}^{18}O$ (5 mL). Concentrated sulfuric acid (0.83 mL) was added to this mixture, and the mixture was capped and stirred for 1 h at 70 °C. After this period, the tube was removed from heat, cooled to room temperature, and frozen in liquid N_2. The tube was opened to air, and solid Na_2SO_3 (1.1 g, 9 mmol) was added to the frozen mixture. The tube was sealed and warmed to melt the solution, and the mixture was placed back in the heating bath and stirred for 1.5 h in the dark to afford the appearance of yellow ClO_2 gas. This gas was bubbled through an ice-cold solution of NaOH (9 mmol), 30% H_2O_2 (1.1 mL, 9.7 mmol), and H_2O (2 mL). The resulting solution was stirred with MnO_2 (0.5 g) for 2 h to disproportionate unreacted H_2O_2. The solution was filtered, and an aliquot was titrated to neutrality with 3 M H_3PO_4 (typically 1–1.5 mL) for use in labeling experiments. The resulting solution is 77% ^{18}O-enriched ClO_2^- in phosphate buffer (0.6 M). For ESI analysis, the basic solution was instead titrated to neutrality using formic acid. Methanol was added to improve ionization. Further details are available in the Supporting Information.

Double crossover chlorite dismutation experiment: A mixture of unlabeled chlorite and ^{18}O chlorite (1 mL, ca. 75 mM in each) in 0.3 M phosphate buffer, pH 7.2, was disproportionated by the addition of [Fe(TF_4TMAP)] (1 mM, 0.1 mL). The resulting O_2 was analyzed by an RGA mass spectrometer.

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[13] A 2.6 mmol solution of [Fe(TF_4TMAP)] disproportionates a 20-fold excess of hypochlorite to oxygen and chloride in 100% yield based on RGA and IC.

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