

# Superoxide Decay Kinetics in the Southern Ocean

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Kiel D-24105, GermanyReceived June 17, 2009. Revised manuscript received  
September 27, 2009. Accepted September 30, 2009.

Measurements of superoxide ( $O_2^-$ ) reaction kinetics were made during a transect with the research icebreaker Polarstern (ANT24-3) in the Antarctic through the Drake Passage in austral autumn 2008. Our sampling strategy was designed to investigate the sinks of superoxide in Polar waters; principally through reactions with dissolved organic matter (DOM) or metals (copper and iron). We modified an existing chemiluminescence flow injection system using methyl *Cypridina* luciferin analog (MCLA) for the detection of  $O_2^-$  and added  $O_2^-$  using  $KO_2$  as the source. Our results indicate that  $O_2^-$  in ambient seawater had a half-life ranging from 9.3 to 194 s. DTPA additions to seawater, to remove the effects of reactions with metals, revealed  $O_2^-$  decay rates consistent with a second order reaction, indicating that the dismutation reaction dominated and that reactions with DOM were not significant. Titrations of seawater by the addition of nanomolar amounts of iron or copper revealed the importance of organic chelation of Fe and/or Cu in controlling the reactivity with  $O_2^-$ . Throughout the water column reactions with Cu appeared to be the major sink for superoxide in the Southern Ocean. This new strategy suggests an alternative approach for speciation measurements of Fe and Cu in seawater.

## Introduction

The superoxide ( $O_2^-$ ) radical is potentially an important species involved in the redox cycling of metal ions in natural waters (1–4). In sunlit surface waters  $O_2^-$  is a major product of the photo-oxidation of colored dissolved organic matter (CDOM) (5) and can also be produced by metabolic processes in phytoplankton (6, 7). Inorganic complexes of Cu(II)/Cu(I) and Fe(II)/Fe(III) can react rapidly with superoxide (Table S1 Supporting Information) leading to a catalytic cycle for superoxide decay (2).

To assess the effect of superoxide on metal redox cycling it is necessary to understand both the rates of production and destruction. There are few direct measurements of superoxide production rates from the open ocean, with single studies published on photoproduction (5) and nonphotochemical production (8). However, studies of  $H_2O_2$  photoproduction are available and provide a reasonable estimate of the major  $O_2^-$  source in the ocean (9). Several reactions have been identified in seawater that could control superoxide decay rates and these principally include the following: (i) the second-order uncatalyzed dismutation reaction of superoxide with its conjugate acid (9); (ii) reactions with Cu species in seawater (2, 3); (iii) reactions with Fe species in

seawater (1, 4, 10); and (iv) reactions with CDOM or other organic matter (11). These pathways for superoxide decay are summarized in Figure S1.

Earlier work by Voelker and colleagues (1–3, 11) found that organically complexed Cu was a far more important sink for superoxide than the dismutation reaction and that reactions with Cu complexes could represent a significant source of Cu(I). Other workers have also suggested that superoxide could play a role in maintaining a significant concentration of Fe(II) in seawater (10, 12, 13) by reduction of inorganic or organically complexed iron.

The aim of the present work was to measure the decay kinetics of superoxide in Antarctic seawater from the Drake Passage to assess the sinks for superoxide and related effects on metal speciation in these waters. We examined here the role of natural Fe and Cu complexes in open ocean seawater to act as catalytic  $O_2^-$  sinks by relating the decay rate of  $O_2^-$  to Cu and Fe concentrations and speciation.  $O_2^-$  was added as  $KO_2$  to ambient, DTPA-complexed, and trace-metal-amended seawater samples from a range of depths throughout the water column and the decay of  $O_2^-$  was followed by a chemiluminescence technique.

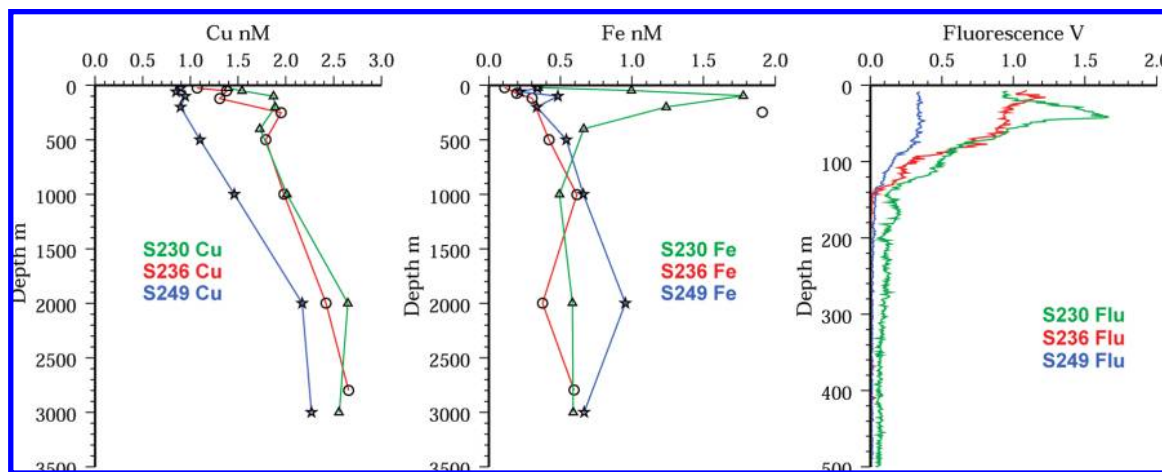
## Experimental Section

Complete descriptions of the experimental methods can be found in the Supporting Information (SI) accompanying this manuscript.

**pH Measurements.** In the present work we report seawater pH values using the total hydrogen scale ( $pH_{TOT}$ ) (14) while we use the NBS scale,  $pH_{NBS}$ , for pH measurements of buffers and other low ionic strength solutions. All pH measurements were made using a WTW pH meter 330i calibrated with Tris buffers (15).

**Reagents.** All reagents were prepared using 18M $\Omega$  cm resistivity water (hereafter MQ water) supplied by a combination of an ELIX-3 and Synergy 185 water systems (Millipore). High-purity HCl (6 M, hereafter abbreviated to Q-HCl) was made by redistillation of Merck trace-metal grade acids in a quartz sub-boiling still (16). MCLA ([2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3-one, HCl]) (Fluka) was used as received. A primary MCLA standard, 1 mM MCLA, was prepared by dissolving 10 mg of MCLA in 34.5 mL of MQ water; 1-mL aliquots of this solution were then pipetted into polyethylene vials and frozen at  $-80^\circ\text{C}$  until required for use. The working MCLA standard, 1  $\mu\text{M}$ , was prepared from a thawed vial of the primary stock by dilution into 1 L of MQ water. This solution was buffered in 0.05 M sodium acetate and adjusted to  $pH_{NBS}$  of 6 with Q-HCl. A 3.8 mM stock solution of diethylenetriaminepentaacetic acid (DTPA) was made up by dissolving 0.6 g in 400 mL of MQ water. A working Cu(II) standard (0.1 ppm) was prepared by serial dilution of the primary (1000 ppm) in MQ water with 1% 6 M Q-HCl. A 10 mM Fe(III) primary stock solution was prepared from  $FeCl_3 \cdot 6H_2O$  (Sigma) in MQ water with 1% 6 M Q-HCl. A working iron standard solution (1  $\mu\text{M}$ ) was prepared from the primary stock solution, diluted with MQ water, and acidified with 1% 6 M Q-HCl. For each experiment a fresh superoxide stock was prepared in a trace-metal-clean dark glass bottle by adding a specific amount (8–10 mg) of potassium superoxide,  $KO_2$ , to a 0.05 M NaOH solution (CAUTION:  $KO_2$  is explosive and must be handled with care). This pH ensured a relatively stable superoxide source concentration over the time frame of the experiment (half-life approximately 3 h). In the present manuscript we report seawater pH values using the total hydrogen scale ( $pH_{TOT}$ )

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**FIGURE 1.** Dissolved metal data from stations in the Drake Passage: (left) copper, (center) iron, and (right) arbitrary chlorophyll fluorescence (volts).

(14) All plasticware used in this work was extensively acid cleaned before use.

**Superoxide Measurement Technique and Apparatus.** For this work, we adapted an existing chemiluminescence analysis method for superoxide which utilizes the reagent MCLA (17–20). The mechanism and specificity for the reaction of MCLA and  $O_2^-$  is well described (21). Our method is not substantially different from that recently published by Rose et al. (22) in which they also used a commercially available flow injection system (FeLume - developed by Whitney King at Colby College, Waterville, ME). All measurements were performed onboard the research icebreaker Polarstern in a Class 100 Clean Container at 23 °C. For full details of this method the reader is referred to the SI.

**Reaction Kinetics.** Our experimental design was based on previous research on superoxide reactions with Cu complexes (2) and organic matter (11) with the inclusion of reactions with Fe (23, 24). In this scheme the reactivity of  $O_2^-$  is determined by the following reactions:

$$\frac{\partial [O_2^-]}{\partial t} = 2k_2[O_2^-]^2 + \sum k_M[M]_X[O_2^-] + k_{org}[O_2^-] \quad (1)$$

where  $k_2$  is the second-order uncatalyzed dismutation rate constant and the rate constant for the metal reactions ( $k_M$ ) includes both the Cu(II)/Cu(I) and Fe(III)/Fe(II) redox pairs, the reaction with organic substances is described by the first order rate  $k_{org}$ .

$$\sum k_M[M]_X = (k_{Cu(I)}[Cu(I)] + k_{Cu(II)}[Cu(II)] + k_{Fe(II)}[Fe(II)] + k_{Fe(III)}[Fe(III)]) \quad (2)$$

The observed rate of superoxide decay can then be written as follows with only a single term each for the first-order and second-order rate components

$$\frac{\partial [O_2^-]}{\partial t} = -2k_2[O_2^-]^2 - k_{obs}[O_2^-] \quad (3)$$

where  $k_{obs}$  is described as the sum of the first order reaction rates.

$$k_{obs} = \sum k_M[M]_X + k_{org} \quad (4)$$

In the samples with added DTPA it is assumed that  $k_{obs} = k_{org}$  (11). For full details of the numerical methods used to solve  $k_2$  and  $k_{obs}$  simultaneously see the SI.

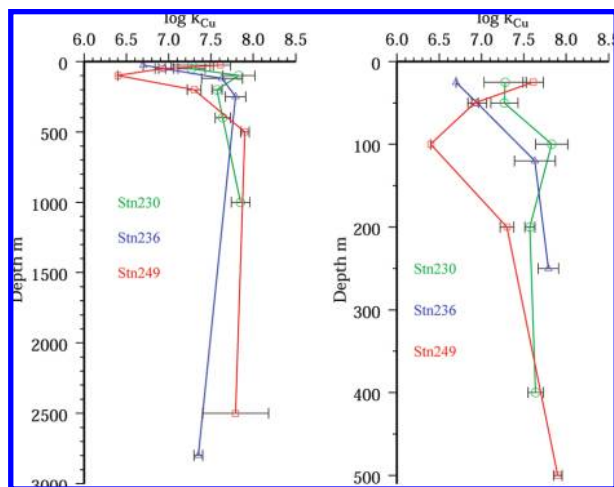
**Field Measurements of Superoxide Reaction Rates in Seawater.** Our approach is based on measuring the decay rate of known quantities of added  $O_2^-$  (as  $KO_2$ ) to seawater.  $O_2^-$  is detected via its chemiluminescence reaction with MCLA (see above). The experimental setup consists of a minimum of 6 experimental treatments: (i) control, reaction with unmodified seawater; (ii) metal-free reaction, achieved by complexation of trace metals in solution with DTPA, this reactivity measured here then only corresponds to reactions mechanism 1 and 2 shown above; (iii and iv) addition of Cu(II) to the seawater to form a titration series with the control, this approach was also used in a previous study (2); (v and vi) addition of Fe(III) to the seawater to form a titration series with the control (see Figure S3).

For each experiment 6 depths from throughout the water column were sampled. From each depth six 60-mL Teflon bottles were filled with 40 mL of the fresh collected seawater. One was spiked with DTPA (3.8  $\mu$ M), two were spiked with Cu (0.85 nM, 1.7 nM), two were spiked with Fe (1 nM, 2 nM), and one was left with seawater only. Metal additions were left to equilibrate for 3 h, while DTPA samples were left overnight (12 h). After equilibration, 20  $\mu$ L of a freshly prepared  $KO_2$  solution ( $\sim 100 \mu$ M  $O_2^-$  as checked by UV spectrophotometry and corrected for  $H_2O_2$  absorbance) was added to the samples and immediately injected in the flow injection system where the chemiluminescence signal was detected and followed for up to 5 min. Full details of the data analysis methods used can be found in the SI.

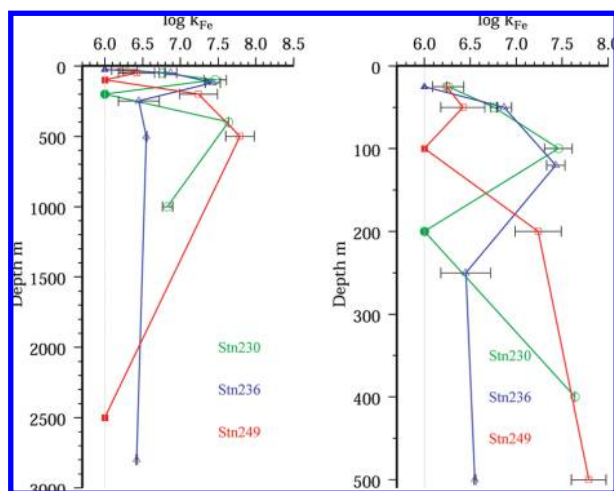
## Results

Measurements of total dissolved Cu and Fe and the chlorophyll fluorescence at the stations occupied in the Drake Passage are shown in Figure 1. Dissolved Cu increased monotonically with depth as has been seen previously in this region (25). Profiles of dissolved Fe also increased with depth with the exception of station 230 where an iron-rich plume originating from the Antarctic Peninsula could be clearly observed from the surface to 500 m. This iron rich plume was coincident with enhanced chlorophyll with the highest levels found closest to the Antarctic Peninsula (Figure 1) at station 230.

In all experiments the decay of  $O_2^-$  in natural seawater was predominantly first order with  $k_{obs}$  ranging from 0.004 to 0.074  $s^{-1}$  (Tables S3–S4). In the vertical profiles there was a clear minimum in  $k_{obs}$  in the depth range 50–75 m which was the lower part of the mixed layer but still within the euphotic zone (Figure 1). For samples with added DTPA, the observed decay was strongly second-order and did not fit a



**FIGURE 2.** Calculated Cu reaction rates ( $k_{Cu}$ ,  $M^{-1} s^{-1}$ ) with superoxide in the Drake Passage, Station 230 (green, circles), Station 236 (blue, triangles), and Station 249 (red, squares): (left) full depth range; (right) top 500 m of the profile. Error bars represent the 95% confidence intervals.



**FIGURE 3.** Calculated Fe reaction rates ( $k_{Fe}$ ,  $M^{-1} s^{-1}$ ) with superoxide in the Drake Passage, Station 230 (green, circles), Station 236 (blue, triangles), and Station 249 (red, squares): (left) full depth range; (right) top 500 m of the profile. Closed symbols indicate the value was below the detection limit of  $\log k_{Fe} = 6$ . Error bars represent the 95% confidence intervals.

first-order decay curve. Second-order rate constants were fitted by simultaneous estimation of the MCLA sensitivity and rate data (see SI) for each experimental run (Tables S3–S4). In only a few DTPA-amended samples, from intermediate depths (200–500 m) and from the near surface close to the Antarctic Peninsula (Station 230), were significant first-order components detectable.

The addition of Cu and Fe to seawater increased the rate of superoxide decay (Figure S7); in general this effect was more pronounced below 500 m and with added Cu. For many samples from the euphotic zone (0–120 m) the addition of Cu or Fe did not appreciably change the rate of superoxide decay, most likely indicating an excess of strong superoxide inert organic chelators in the sample. Estimates of  $\log k_{Cu}$  are shown in Figure 2 and varied from 6.4 to 7.9 with a clear minimum at station 249 at 100 m. Values of  $\log k_{Cu}$  were relatively constant below 200 m at all stations. Estimates of  $\log k_{Fe}$  are displayed in Figure 3 and varied from below detection (<6) to 7.9 and tended toward maximal rates in mid-depth waters (100–500 m) with lowest values in the upper 200 m.

## Discussion

**Superoxide Decay Rates in Seawater.** Measurements of superoxide decay in open ocean waters are limited, with earlier results being undetectable ( $<1.5 s^{-1}$ ) using spectrophotometry (9), while recent results in the Tropical Pacific using a chemiluminescence approach similar to ours (8) found values of  $9.7 \times 10^{-3} s^{-1} > k > 1 \times 10^{-4} s^{-1}$ . Higher values have been found in coastal waters (3, 11, 26). The kinetics of the dismutation reaction has been extensively examined in pure water (27, 28) and Zafriou (9) made the first direct measurements of  $K_{HO_2}$  in seawater ( $pK_a^* = 4.60 \pm 0.15$ ) and measured the dismutation rate constant  $k_2$  ( $5 \pm 1 \times 10^{12} [H^+] M^{-1} s^{-1}$ ) on the  $pH_{NBS}$  scale. In the present work from the experiments performed with DTPA and seawater we measured the dismutation rate using  $pH_{TOT}$  as  $4.4 \pm 1.6 \times 10^{12} [H^+] M^{-1} s^{-1}$  ( $n = 14$ , 95% CI), in reasonably good agreement with the earlier data of Zafriou (9) given the differences in the pH scales used and that Zafriou did not state at what temperature his work was conducted.

A recent study in the Tropical Pacific by Rose et al. (8) observed first-order rates with DTPA-amended seawater and these authors suggested it was from reactions with organic species or strong metal organic species that did not react with DTPA rapidly. There is a significant difference between our study and theirs with regard to the experimental setup as in their work they added DTPA immediately before measuring the superoxide decay while in the present study we equilibrated the DTPA with the seawater for a minimum of 12 h before the addition of superoxide. The longer equilibration time used in our work was based on the findings of Hering and Morel (29) and experiments in our own lab which indicated a minimum of 1 h was required. We suggest here that the addition of DTPA immediately before measurement does not prevent trace metal reactions with superoxide and thus work performed in this manner needs to be interpreted carefully.

**Importance of Nonmetal (Organic) Reactions with Superoxide.** Previous work by Goldstone and Voelker (11) found a significant reaction between superoxide and DOM. In the present work we found this reaction pathway to be minor as we observed in DTPA-amended samples reaction rates that were distinctly second order and similar to the rate of the dismutation reaction in seawater (9). However closer examination at stations close to the Antarctic Peninsula (Station 230) suggests that there were small contributions from DOM, most noticeably in the productive surface waters (25 m:  $k_{obs} = 0.006 \pm 0.001$ ) and in the region of the nutricline (200 m:  $k_{obs} = 0.004 \pm 0.001$ ). The observed pattern in our work is partially consistent with the production of new organic matter in the surface waters by phytoplankton and the remineralization of organic matter at depth via zooplankton grazing and bacterial activity.

Goldstone and Voelker (11) found a correlation between CDOM absorption at 300 nm and  $k_{org}$  for DTPA-amended coastal seawater samples. Unfortunately during our cruise no measurements of CDOM absorption were made, however a previous photochemical study in the same region (30) found values of  $\sim 0.25 m^{-1}$  for surface water CDOM absorption at 300 nm which would suggest a value of  $k_{org} = 0.1 s^{-1}$  based on the work of Goldstone and Voelker. The much lower values for  $k_{org}$  found here suggest that open ocean CDOM is significantly less reactive with superoxide than coastal waters and this may be related to the lack of riverine humic materials.

**Half Life and Steady State Concentration for  $O_2^-$ .** In the present study we observed half-lives for superoxide in ambient seawater ranging from 9.3 to 193.6 s (mean 43.5 s,  $n = 27$ ). In general the slowest rates were found around 50–75 m in the euphotic zone, though there was no statistically significant trend with depth. Earlier estimates for seawater were significantly longer; 5–20 min (26) based solely on the

dismutation reaction indicating the importance of including reactions with metals in estimating superoxide lifetimes.

Previous workers (2, 8, 11) have utilized the concept of a pseudo-steady-state concentration of  $O_2^-$  in seawater based on equal production and decay terms:

$$[O_2^-]_{ss}(2k_2[O_2^-]_{ss} + k_{obs}) = \text{production} \quad (5)$$

Estimates of the steady-state concentration can be obtained by solving eq 5 as a quadratic equation. For Antarctic waters we can find no published values for superoxide formation but we can estimate an upper limit of  $9 \text{ nM h}^{-1}$  by using  $H_2O_2$  production rates (31) of  $4.5 \text{ nM h}^{-1}$  for sunlit surface waters at midday in the vicinity of the Antarctic Peninsula. Using this approach we estimate  $[O_2^-]_{ss} \approx 116 \pm 28 \text{ pM}$  ( $n = 3$ , 95% CI) for samples in the Drake Passage at 25 m depth. Our estimate is similar to that suggested by Voelker and colleagues based on midday photoproduction in coastal and open ocean waters of  $[O_2^-]_{ss} \sim 10\text{--}200 \text{ pM}$  (2, 11).

**Implications for Steady-State Deep Water  $H_2O_2$  Concentrations.** Measurements of  $H_2O_2$  in deep waters in the Drake Passage ranged from 2 to 6 nM (Croot et al. in preparation) similar to other deep waters (32). Yuan and Shiller (32) suggested a 12-day residence time in the deep North Pacific for  $H_2O_2$  based on the assumption of a  $0.01 \text{ nM h}^{-1}$  biological production rate and an extrapolation of measured biological decay rates ( $0.009 \text{ nM h}^{-1}$ ) for  $H_2O_2$ . If we apply the same deep water production rate here to the decay constants we determined it suggests a  $O_2^-$  production rate of  $20 \text{ pM h}^{-1}$  resulting in a  $[O_2^-]_{ss}$  for deep water (>500 m) of  $0.26 \pm 0.24 \text{ pM}$  (95% CI: range  $0.15\text{--}0.46 \text{ pM}$ ,  $n = 7$ ). This contrasts sharply with recent data from intermediate waters in the Tropical Pacific (8) which indicated  $[O_2^-]_{ss}$  of  $20\text{--}150 \text{ pM}$  with superoxide first order decay rates of  $0.001\text{--}0.01 \text{ s}^{-1}$ . Such high  $[O_2^-]_{ss}$  would indicate elevated deepwater  $H_2O_2$  production ( $0.08\text{--}0.6 \text{ nM h}^{-1}$ ) and concentrations ( $8\text{--}61 \text{ nM}$ , assuming a 4 day residence time for  $H_2O_2$  (33)) which have currently not been observed in the field. Alternative pathways for  $H_2O_2$  production that do not involve superoxide (34) would further suggest that the  $[O_2^-]_{ss}$  are pM or less in deep waters as our study suggests.

**Effect of  $O_2^-$  on Metal Speciation in Seawater.** The significant decreases observed in our study for the decay rate of superoxide in DTPA-amended seawater indicate that for ambient seawater, reactions with metals must be the dominant superoxide decay process. For a  $[O_2^-]_{ss} = 100 \text{ pM}$  our study predicts that the dismutation reaction would represent less than 1% of the loss of superoxide in all samples without DTPA. Under the higher concentrations of superoxide used in the decay experiments we performed the uncatalyzed-dismutation pathway represents  $78 \pm 24\%$  ( $n = 27$ , 95% CI, range 42–94%) of the initial loss rate (Figure S6). Thus it is necessary to include the uncatalyzed dismutation pathway for our experiments but it is clearly a minor pathway in the open ocean.

Superoxide reacts rapidly with inorganic Cu species in seawater (3) and also with organic Cu complexes (2) despite their apparent strong complexation. In the present study we also saw evidence for a fast reaction between organic Cu species and superoxide, as voltammetric speciation measurements for Cu (Heller and Croot, manuscript in preparation) indicated free metal concentrations for Cu below  $10^{-13} \text{ M}$  throughout the water column. Such low values of free copper would not be sufficient to generate the values of  $\log k_{Cu}$  we observed and thus the reaction must involve organically complexed Cu also. This is consistent with the work of Voelker et al. (2) who found that the strong Cu organic chelators produced by *Synechococcus* reacted rapidly with superoxide and suggested that this may be related to an exchangeable water coordinated to the chelated copper.

Fast reactions with Cu(I) resulting from the reduction of Cu(II) would help to catalyze the decay of superoxide. The impact of superoxide reactions on the Cu redox cycle can be estimated by using the observed value of  $\log k_{Cu}$  and its relationship to  $k_{Cu(I)}$  and  $k_{Cu(II)}$  (2).

$$k_M = \frac{2k_{M^{n+}}k_{M^{n+1}}}{k_{M^{n+}} + k_{M^{n+1}}} \quad (6)$$

Assuming that  $k_{Cu(I)} = 1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ , which is simply the reaction with inorganic Cu(I) as there is insufficient time to form organic complexes, and that the reactions with superoxide are much faster than the oxidation of Cu(I) by  $O_2$  (3), then eq 6 can be solved for  $k_{Cu(II)}$  and the steady state ratio of Cu(I) can be calculated from the following:

$$\frac{[M^{n+}]}{[M^{n+1}]} = \frac{k_{M^{n+1}}}{k_{M^{n+}}} \quad (7)$$

Using this approach we can estimate for our study that Cu(I) under steady state conditions made up between 0.1 and 4% of the total copper pool. For samples from the sunlit upper 100 m in our study this we estimate  $1\text{--}63 \text{ pM}$  Cu(I) which is on the limit of current analytical detection schemes (35). Actual measurements of Cu(I) in the subtropical Atlantic indicate that Cu(I) is 5–10% of the total copper (35) in the euphotic zone, suggesting an additional contribution from direct photoreduction of Cu complexes.

Additions of Fe to seawater in this study saw only small increases in superoxide decay rates when compared to copper (Figure S7). For many water samples from the upper water column the addition of iron saw almost no change in the decay kinetics (Figures 2 and 3) suggesting strong organic complexation of iron by chelators in excess of the ambient iron concentration in agreement with previous observations in the Southern Ocean (36, 37). Estimation of the reaction rates of Fe(II) ( $k_{Fe(II)}$ ) and Fe(III) ( $k_{Fe(III)}$ ) with superoxide using  $k_{Fe}$  via the same approach as for Cu as above is problematic, if we assume that the reaction is only due to free Fe(II), then the usual value used in the literature,  $k_{Fe(II)} = 1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  (Table S1), is inconsistent with the observed values of  $k_{Fe}$  (eq 6) in many cases. This suggests that  $k_{Fe(II)}$  under seawater conditions may represent faster reactions with superoxide than with free Fe(II). There is precedence for this as studies on Fe(II)-EDTA suggest faster reaction rates with superoxide ( $k_{Fe(II)} = 1.2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ ) (38) and model findings where reactions with Fe(II) species that predominate in ambient seawater ( $Fe(OH)^+$ ,  $Fe(OH)_2$ ,  $Fe(CO_3)_2^{2-}$ ) were suggested to react faster than free Fe(II), though these model results are currently in dispute (39). Using a higher estimate for  $k_{Fe(II)} = 5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  (1) we then estimate that under steady-state conditions Fe(II) made up only 0.05–4% of the total iron in our experiment resulting in <1 pM Fe(II) from superoxide in the euphotic zone in the Drake Passage. Thus the elevated levels of Fe(II) in Southern Ocean waters seen during a diel cycle must be produced via a direct photoreduction pathway (40).

If iron in our samples is organic complexed then the reduction of the naturally occurring Fe(III) complexes by superoxide is significantly faster than for model siderophores such as ferrioxamine B (Table S1), and is more consistent with reactions with inorganic or weakly complexed iron (1, 24). It may also indicate the importance of rapid superoxide reactions with the reduced form Fe(II) where organic complexation may occur on a slower time scale (41). Recent ultrafiltration studies (42) found that much of the Fe that passed through an  $0.2\text{-}\mu\text{m}$  filter did not pass through a 10- or 200-kDa ultrafilter indicating that much of the dissolved Fe is colloidal in nature though still possibly bound to an organic matrix. Thus in the present work we suggest that

where we observed elevated  $k_{Fe}$  values it is suggestive of iron weakly bound to soluble complexes or labile sites on organic colloids. Observations of  $\log k_{Fe} < 6.5$  are indicative of strong soluble complexes such as siderophores which are relatively unreactive to superoxide.

An important consideration in the present work is whether the presence of up to 500 nM of  $H_2O_2$  derived from the  $KO_2$  source may influence the results for Cu and Fe. We believe this was not the case for Cu based on two lines of reasoning: (1) the reaction of Cu(I) with  $H_2O_2$  is very slow  $7 \times 10^3 M^{-1} s^{-1}$  (43) and  $O_2$  would remain the major pathway for Cu(I) oxidation (44); and (2) similarly the reaction of Cu(II) with  $H_2O_2$  is also a minor pathway under seawater conditions (44). As the oxidation of Fe(II) is sensitive to  $H_2O_2$  concentrations (45) in our experiments Fe(II) oxidation would be influenced by  $H_2O_2$ . However the rate-determining step for the superoxide loss was still the reduction of Fe(III) and thus faster oxidation of Fe(II) would not give rise to an acceleration of the overall loss rate of superoxide.

**Environmental Significance: Superoxide decay and Biogeochemical Cycles.** The concept of "Oceanographic Consistency" was first applied to dissolved trace metal data by Boyle and Edmond (46) and should also be applicable to redox data such as we present here. However the criteria must be extended to include both the effects of fast kinetics and physical mixing processes which can strongly determine the shape of a vertical profile in sunlit waters (41) and to include consistency between production and decay terms. Thus superoxide data should be consistent with both  $H_2O_2$  data and trace metal redox speciation data. In the current study it appears that superoxide decay kinetics in ambient seawater are related to other biogeochemical processes through the reactions with the Cu and Fe species present. Changes in Cu and Fe speciation within the water column caused by release/exudation of chelators by phytoplankton/bacteria in the photic zone and remineralization of metals by zooplankton grazing at intermediate depths are all plausible interpretations of the changes in superoxide decay we observed here. It is apparent here that Cu, based on both the higher concentrations and faster reaction rates than Fe, is the major pathway for superoxide decay in Southern Ocean waters. These reactions occur despite the Cu being strongly organically complexed and has clear implications for both Cu redox cycling and superoxide lifetimes.

### Acknowledgments

We gratefully acknowledge the officers and crew of the *P.S. Polarstern* whose help and cooperation made this work possible. Special thanks also to the chief scientist Prof. Eberhard Fahrbach (AWI) for making our participation possible. Shipboard work was only possible with the help of the onboard NIOZ team led by Hein de Baar, and our IFM-GEOMAR colleagues Oliver Baars and Katrin Bluhm. Thanks also to Peter Streu (IFM-GEOMAR) for the laboratory analysis in Kiel. The comments of Dr. Andrew Rose and two anonymous reviewers are gratefully appreciated in helping to improve this manuscript. This work is a contribution to IPY GEOTRACES and was financed by the DFG Antarctic Program (SPP 1158) via a grant to P.C. (CR145/10-1).

### Supporting Information Available

Full details of station locations and the experimental setup and related analysis methods; the cruise track and stations locations (Figure S2); experimental set up (Figure S3) and design (Figure S1); and an example of the signal data from the FeLume (Figure S4). This information is available free of charge via the Internet at <http://pubs.acs.org>.

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ES901766R