

Am Chem Soc. Author manuscript; available in PMC 2013 January 18.

Published in final edited form as:

JAm Chem Soc. 2012 January 18; 134(2): 796–799. doi:10.1021/ja2095365.

# Characterization of an O<sub>2</sub> Adduct of an Active Cobalt-Substituted Extradiol-Cleaving Catechol Dioxygenase

Andrew J. Fielding<sup>†</sup>, John D. Lipscomb<sup>\*,‡</sup>, and Lawrence Que Jr.<sup>\*,†</sup>

<sup>†</sup>Department of Chemistry, University of Minnesota, Minneapolis, MN 55455

<sup>‡</sup>Department of Biochemistry, Molecular Biology and Biophysics, and Center for Metals in Biocatalysis, University of Minnesota, Minneapolis, MN 55455

#### **Abstract**

The first example for an  $O_2$  adduct of an active Co-substituted oxygenase is observed in the extradiol ring cleavage of the electron-poor substrate 4-nitrocatechol (4NC) by Co(II)-homoprotocatechuate 2,3-dioxygenase (Co-HPCD). Upon  $O_2$  binding to the high-spin Co(II) (S=3/2) enzyme-substrate complex, an S=1/2 EPR signal is observed exhibiting <sup>59</sup>Co hyperfine splitting (A=24 G) typical of a low-spin Co(III)-superoxide complex. Both formation and decay of the new intermediate are very slow in comparison to the analogous steps for turnover of 4NC by native high-spin Fe(II)-HPCD, which is likely to remain high-spin upon  $O_2$  binding. A similar, but effectively stable, S=1/2 intermediate is formed by the inactive [H200N-Co-HPCD(4NC)] variant. The observations presented shed light on the key roles played by the substrate, the second-sphere His200 residue, and the spin state of the metal center in facilitating  $O_2$  binding and activation.

Homoprotocatechuate 2,3-dioxygenase (HPCD) activates  $O_2$  to carry out the extradiol ring cleavage of homoprotocatechuate (HPCA) (Scheme 1) in the biodegradation of aromatic compounds by microorganisms.  $^{1-3}$  Recently metal substitution experiments showed that the native Fe(II) metal cofactor can be substituted with Co(II) to yield Co-HPCD with comparable activity,  $^4$  despite an apparent large difference in M(III/II) redox potential between Fe-HPCD and Co-HPCD as suggested by the observation that  $H_2O_2$  oxidatively inactivates Fe-HPCD, but not Co-HPCD and the large difference in the M(III/II) standard potentials of these metals.  $^5$  As the catalytic mechanism for Fe-HPCD involves  $O_2$  binding to the Fe(II) center,  $^6$ ,  $^7$ ,  $^8$  our observations raise the question as to how  $O_2$  activation and extradiol cleavage can be carried out by the higher potential Co(II) center of Co-HPCD.

Steady-state kinetics measurements show Co-HPCD to have a low apparent  $O_2$  affinity  $(K_M^{O2} = 1.2 \pm 0.1 \text{ mM} \text{ vs } 60 \,\mu\text{M}$  for Fe-HPCD at pH 7.8), perhaps reflecting the higher potential of the Co(II) center. However, under  $O_2$  saturating conditions, Co-HPCD shows a higher HPCA ring cleavage activity than Fe-HPCD  $(k_{\text{cat}} = 1120 \pm 70 \,\text{min}^{-1} \,\text{vs } 470 \pm 20 \,\text{min}^{-1}$  for Fe-HPCD at pH 7.8). Comparisons of transient kinetic experiments indicate different rate determining steps for Fe-HPCD- and Co-HPCD-catalyzed reactions. Whereas the rate limiting step for Fe-HPCD occurs in the product release phase of the catalytic cycle,  $^{6, \, 9, \, 10}$  that for Co-HPCD occurs in the  $O_2$  binding and activation phase of the reaction. Investigation of these steps has been facilitated in our past studies of extradiol dioxygenases by the use of the slow substrate analogue 4-nitrocatechol (4NC).  $^{6, \, 9, \, 10}$  This electron-poor

Corresponding Authors: lipsc001@umn.edu and larryque@umn.edu.

substrate is cleaved in the same position as HPCA by Co-HPCD, but the rate of the reaction is slowed 1000-fold (pH 6.0, 22  $^{\circ}$ C and 2 atm O<sub>2</sub>). This has allowed us to trap an O<sub>2</sub> adduct and characterize it as a low-spin Co(III)-superoxide complex, the first documented example of such a species for a functional cobalt oxygenase.

Co-HPCD binds 4NC ( $K_D^{4NC} = 5 \pm 2 \mu M$ , Figure S1) in its purple dianionic form with an intense absorption band at 516 nm that slowly converts to the yellow extradiol ring-cleaved product with absorption bands at 330 and 390 nm at pH 6.0 (Figure 1).9 Time-dependent traces of the reaction at 516 and 390 nm were fit satisfactorily with single exponential equations to give nearly the same  $1/\tau$  value at these two wavelengths (Figure S2). The conversion exhibited a linear dependence on O<sub>2</sub> concentration (Figure 1 inset), suggesting that O<sub>2</sub> binding is the first and rate limiting step over the experimentally accessible [O<sub>2</sub>] range. The slope of the plot gives a second order rate constant for  $O_2$  binding of  $30 \pm 3 \text{ M}^{-1}$ min<sup>-1</sup>, which is at least 6 orders of magnitude smaller than that for O<sub>2</sub> binding to [Fe-HPCD(4NC)]. 9, 10 This is in accord with our previous proposal, based on steady-state kinetic analysis, that the O2 binding step in HPCA turnover by Co-HPCD is slow and rate limiting. Because of the slow turnover of 4NC by Co-HPCD, the observed activity could originate from contaminating Fe-HPCD. To rule out this possibility the enzyme was pretreated with H<sub>2</sub>O<sub>2</sub> to inactivate any contaminating Fe-HPCD. 11 Furthermore, in the single turnover of 4NC by Fe-HPCD none of the observed kinetic phases show an O2 concentration dependence, in contrast to our observations here for Co-HPCD (Figures 1 inset and S2).9

EPR freeze-quench experiments were performed by rapidly mixing the anaerobic [Co-HPCD(4NC)] complex (pH 6 at 22 °C) with  $O_2$ -saturated buffer and then placing the reaction under 2 atm of  $O_2$ . Aliquots from the reaction were then taken every few minutes over the course of 60 minutes and frozen in EPR tubes. Figure 2 shows representative EPR spectra from freeze-quench experiments. Co-HPCD exhibits an S = 3/2 EPR signal associated with a high-spin Co(II) center (Figure S3),<sup>4</sup> with the signal at g = 6.7 showing <sup>59</sup>Co (I = 7/2) hyperfine splitting (A = 80 G) (Table 1). The S = 3/2 signal changes upon anaerobic formation of the [Co-HPCD(4NC)] complex (Figure 2 and S3). Upon mixing with  $O_2$ , the amount of the S = 3/2 species decreases, as indicated by a decrease in the feature at g = 4.8, and a new S = 1/2 species forms with signals centered at g = 2. The new S = 1/2 species exhibits well-resolved eight-line hyperfine splitting from the <sup>59</sup>Co nucleus (A = 24 G). The intensity of the S = 1/2 species continues to increase over the first 2 minutes and then decreases as the S = 3/2 signal of Co-HPCD at g = 8.9 grows.

The reaction progress (Figure 3) was monitored by following changes in the intensities of EPR features unique to each species. A ~10% yield of the S=1/2 species was measured at 2 minutes by spin quantification. The ability to directly observe the intermediate allows accurate fitting of the time course to the appropriate equation for a 2-step reaction. The EPR and UV-Vis reaction progress curves (Figures 2 and S4) were fit simultaneously by global analysis to rate equations based on the reaction scheme shown in Figure 3 using the program DYNAFIT and the following rate constants were obtained:  $k_1 = 40 \pm 5 \,\mathrm{M}^{-1} \,\mathrm{min}^{-1}$ ,  $k_{-1} = 0.05 \pm 0.01 \,\mathrm{min}^{-1}$  and  $k_2 = 0.72 \pm 0.06 \,\mathrm{min}^{-1}$ . Under this model,  $k_1[\mathrm{O_2}]$  and  $k_2$  values are comparable in magnitude, with  $k_1[\mathrm{O_2}] < k_2$  at experimentally accessible  $\mathrm{O_2}$  pressures (< 5 atm). The ~10% yield of the S = 1/2 species observed in the freeze-quench EPR experiments at 2 atm  $\mathrm{O_2}$  is fully consistent with the rate constants obtained.

The H200N-Co-HPCD mutant shows no extradiol ring-cleavage activity for 4NC at all pHs examined. Nevertheless, the [H200N-Co-HPCD(4NC)] complex can form an  $O_2$  adduct with an S=1/2 EPR spectrum similar to that of [Co-HPCD(4NC)O<sub>2</sub>] (Figure 4). In fact, because  $k_2=0$ , the intermediate is formed in higher yield. It reaches 50% under 2 atm  $O_2$ ,

facilitating investigation of its properties. Interestingly, no significant UV-Vis spectral changes are observed between [H200N-Co-HPCD(4NC)] and [H200N-Co-HPCD(4NC)O<sub>2</sub>] (Figure S5), suggesting that the 4NC chromophore is not affected by O<sub>2</sub> binding. Accordingly, O<sub>2</sub> binding to [H200N-Co-HPCD(4NC)] proved to be reversible, as purging the sample with argon caused the S=1/2 species to disappear, restoring the EPR signal of the enzyme-substrate complex (Figure S6). O<sub>2</sub> binding experiments monitored by EPR (Figure S7) show that [H200N-Co-HPCD(4NC)] has a low affinity for O<sub>2</sub> with a  $K_D^{O2}$  of  $2.8 \pm 0.2$  mM O<sub>2</sub> at pH 7.5, similar to the value of 1.3 mM O<sub>2</sub> deduced for Co-HPCD from the kinetic fits described above.

Both H200N- and wt-[Co-HPCD(4NC)]  $O_2$  adducts exhibit small  $^{59}$ Co hyperfine splittings of < 25 G (Table 1). These are comparable to other characterized low-spin Co(III)-superoxide species (Table S1), where the unpaired electron is localized on the superoxide moiety and the observed  $^{59}$ Co hyperfine splitting is attributed to spin polarization.  $^{14-16}$  Furthermore, the use of  $^{17}O_2$  (I = 5/2) results in significant broadening of the S = 1/2 EPR signals of both H200N- and wt-[Co-HPCD(4NC)O<sub>2</sub>] adducts (Figures 4C and S8), in strong support of the low-spin Co(III)-superoxo description for the adducts.

The binding of  $O_2$  to the ES complexes of Co-HPCD results in an apparent spin state change from high-spin Co(II) in the enzyme-substrate complex to low-spin Co(III) in the  $O_2$  adduct. However, as expected, such a spin transition is not observed for the corresponding  $O_2$  adducts of Fe- or Mn-HPCD because of the weak-field nature of the HPCD coordination environment.  $^{6, 17}$  Co(III) differs from the other two metal centers, because of its  $d^6$  electronic configuration and the consequent large ligand field stabilization energy that favors the low-spin state, as found for all Co(III)-superoxide complexes characterized thus far.  $^{15, 16}$  The formation of the low-spin adduct is likely to be an important factor that promotes  $O_2$  binding, despite the high Co(III/II) potential. However, the required reorganization that accompanies the spin transition as the  $O_2$  adduct forms and the high Co(III/II) potential work together to raise the barrier for  $O_2$  binding that shifts the rate determining step to the  $O_2$ -binding phase of the catalytic cycle for Co-HPCD.

The decay rate of the observed low-spin Co(III)-superoxide species is at least 7 orders of magnitude slower than the rate of the analogous step using Fe-HPCD, 9, 10 even though one may expect Co(III) to be a stronger oxidant than Fe(III) in the subsequent 1-electron oxidation of 4NC. This slow rate of 4NC oxidation likely reflects the large kinetic barrier arising from an increase in the reorganization energy upon electron transfer as the low-spin Co(III)-superoxide converts to a high-spin Co(II)(semiquinone)-superoxide species (Scheme 2). 18

His200 has been shown previously by site-directed mutagenesis to be a catalytically important residue for Fe-HPCD.  $^{6, 10, 19, 20}$  The fact that H200N-Co-HPCD is not catalytically active but is nonetheless able to reversibly bind  $O_2$  when 4NC is bound to the cobalt center suggests that the H200 residue is not required for  $O_2$  binding, but must be essential for a subsequent step. In Fe-HPCD crystal structures of the substrate complex and intermediates following  $O_2$  binding, H200 is observed to be involved in weak hydrogen bonding interactions with the OH moiety of the monoanionically bound catecholate as well as stronger interactions with the various  $O_2$ -derived ligands.  $^{7, 21, 22, 23}$  His200 has been proposed to facilitate electron transfer from substrate to the M- $O_2$  unit by a proton coupled electron transfer mechanism, where it acts as a base to remove the proton from the substrate to assist in catechol oxidation. In turn, the protonated His200 residue can interact with the  $O_2$  adduct as it forms and stabilize the developing negative charge on the dioxygen moiety.  $^{7, 21}$  In the next step, the protonated His200 may also help to orient the superoxide moiety to optimize its attack on the substrate semiquinone radical to form the alkylperoxo

intermediate that leads to the extradiol cleavage of the substrate.<sup>6, 7</sup> These proposed roles are illustrated in Scheme 2.

Scheme 2 can also rationalize the dramatically different pH activity profiles of Co-HPCD with HPCA and 4NC as substrates. Optimal extradiol cleavage of HPCA occurs at pH 9.0 (Figure S9), but for 4NC the pH optimum is at pH 5.5 (Figure S10). This may arise from the drastically different p $K_a$ 's of HPCA and 4NC,<sup>24, 25</sup> resulting in HPCA binding to Co-HPCD as a monoanionic catecholate and 4NC binding as a dianion. <sup>9, 26, 27</sup> In the case of the latter, the intensity of its characteristic 516 nm band of the dianion decreases and the rate of 4NC turnover increases as the pH is lowered (Figure S10 and S11A). However the decrease in the 516 nm band is not accompanied by the appearance of a 425 nm chromophore associated with the 4NC monoanion (Figure S11A), 26, 27 so lowering the pH from 9.0 to 5.5 does not result in the protonation of the bound 4NC. Instead, we suggest that H200 becomes protonated and then hydrogen bonds to the bound 4NC to affect its chromophore. This notion is consistent with the observation that the 4NC chromophore of the [H200N-Co-HPCD(4NC)] complex is much less sensitive to changes in pH (Figure S11B). As Asn200 has a shorter side chain and cannot be protonated in this pH range, it is unable to interact productively with 4NC. The differences in the pH activity profiles for HPCA and 4NC as substrates can thus be rationalized by the key role proposed for H200 as an acid/base catalyst to promote electron transfer from the substrate to the M-O<sub>2</sub> unit to advance the reaction cycle beyond the O<sub>2</sub> binding step. In the case of HPCA, protonation of H200 occurs upon extraction of the proton from the bound substrate concomitant with its oxidation. The analogous proton transfer to H200 cannot occur for 4NC, as it is bound as a dianion. However, H200 can be protonated by solvent at lower pH, resulting in the observed increase in the rate of 4NC cleavage (Figure S10). The H200N mutation prevents the ring cleavage reaction by eliminating both protonation pathways as well as any effects on orientation of the oxymoiety.

The H200N-Fe-HPCD, mutant is similarly unable to catalyze extradiol cleavage of 4NC, but instead catalyzes the 2-e $^-$  oxidation of 4NC to yield quinone and  $\rm H_2O_2$ .  $^{6,\,9,\,10}$  Initial  $\rm O_2$  binding to [H200N-Fe-HPCD(4NC)] is very rapid and reversible ( $k_1=9\pm1.2\times10^6\,\rm M^{-1}$  min $^{-1}$ , and  $k_{-1}$  3120 min $^{-1}$  at 4 °C and pH 7.5) and leads to the formation of a long-lived Fe(III)-superoxo species analogous to the Co(III)-superoxo species observed in this study, This shows that the initial electron transfer step from M(II) to  $\rm O_2$  proceeds with either metal in the absence of H200. However, the subsequent electron transfer step from substrate to the M–O<sub>2</sub> unit is clearly blocked for the unreactive low-spin [H200N-Co-HPCD(4NC)O<sub>2</sub>] species, but can still occur for the high-spin [H200N-Fe-HPCD(4NC)O<sub>2</sub>] intermediate, ( $k=1.32\,\rm min^{-1}$  at 4 °C and pH 7.5) to form a high-spin Fe(III)(4-nitrobenzosemiquinone)peroxo intermediate.  $^{6,\,10}$ 

In summary, we have characterized the  $O_2$  adduct of a cobalt-substituted iron dioxygenase that exhibits comparable activity to its iron analog. The  $O_2$  adduct is best described as a low-spin Co(III)-superoxo complex,  $^{14-16}$  the first example of such a species for a functional cobalt oxygenase. We also show that H200 plays a crucial role in promoting the subsequent oxidation steps that lead to extradiol cleavage of the substrate by acting as an acid/base catalyst and properly orienting the superoxide to attack the substrate. The substitution of Co(II) for the native Fe(II) center in HPCD shifts the rate determining step from the product release phase of the catalytic cycle in the case of the Fe(II) enzyme to the  $O_2$  binding and catechol oxidation phase for the Co(II) enzyme.  $^{9, 10}$  This shift probably results from the higher kinetic barrier associated with  $O_2$  binding to Co-HPCD due to the higher M(III/II) redox potential of the cobalt center and the spin transition from high-spin Co(II) to low-spin Co(III) upon  $O_2$  binding to Co-HPCD. It may also involve the reverse spin transition in the subsequent electron transfer from the electron poor 4NC substrate to the low-spin Co-

superoxo unit. A similar change in oxidation and spin state presumably occurs as [Co-HPCD(HPCA)] binds  $O_2$ , but this remains to be demonstrated. However, the fact that the HPCA reaction is at least 1000-fold faster than that for 4NC suggests that the lifetime of the putative low-spin Co(III)-superoxo intermediate significantly decreases as the electron-poor 4NC is replaced with the more easily oxidized substrate. Further efforts are aimed at trapping the analogous superoxo intermediate with substrates other than 4NC.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## **Acknowledgments**

This work was supported by NIH grants GM 24689 to J.D.L. and GM 33162 to L.Q. A.J.F. thanks the University of Minnesota Chemical Biology Initiative for financial support in conjunction with the NIH-supported Chemistry Biology Interface Training Grant (GM 08700).

#### References

- 1. Smith MR. Biodegradation. 1990; 1:191. [PubMed: 1368147]
- 2. Orville AM, Lipscomb JD. Met Ions Biol Syst. 1992; 28:243.
- 3. Miller MA, Lipscomb JD. J Biol Chem. 1996; 271:5524. [PubMed: 8621411]
- Fielding AJF, Kovaleva EG, Farquhar ER, Lipscomb JD, Que L Jr. J Biol Inorg Chem. 2010; 16:341. [PubMed: 21153851]
- 5. Bratsch SG. J Phys Chem Ref Data. 1989; 18:1.
- Mbughuni MM, Chakrabarti M, Hayden JA, Bominaar EL, Hendrich MP, Münck E, Lipscomb JD. Proc Natl Acad Sci USA. 2010; 107:16788. [PubMed: 20837547]
- 7. Kovaleva EG, Lipscomb JD. Science. 2007; 316:453. [PubMed: 17446402]
- Mbughuni MM, Chakrabarti M, Hayden JA, Meier KK, Dalluge JJ, Hendrich MP, Münck E, Lipscomb JD. Biochemistry. 2011; 50:10262. [PubMed: 22011290]
- 9. Groce SL, Miller-Rodeberg MA, Lipscomb JD. Biochemistry. 2004; 43:15141. [PubMed: 15568806]
- 10. Groce SL, Lipscomb JD. Biochemistry. 2005; 44:7175. [PubMed: 15882056]
- Emerson JP, Kovaleva EG, Farquhar ER, Lipscomb JD, Que L Jr. Proc Natl Acad Sci USA. 2008; 105:7347. [PubMed: 18492808]
- 12. Aasa R, Vanngard T. Mag Reson. 1975; 19:308.
- 13. Kuzumic P. Anal Biochem. 1996; 237:260. [PubMed: 8660575]
- 14. (a) Niswander RH, Taylor LT. J Mag Reson. 1977; 26:491.(b) Hoffman BM, Damon LD, Basolo F. J Am Chem Soc. 1970; 92:61.(c) Tovrog BS, Kitko DJ, Drago RS. J Am Chem Soc. 1976; 98:5144.
- 15. Smith TD, Pilbrow JR. Coord Chem Rev. 1981; 39:295.
- 16. Jones RD, Summerville DA, Basolo F. Chem Rev. 1979; 79:139.
- Gunderson WA, Zatsman AI, Emerson JP, Farquhar ER, Que L Jr, Lipscomb JD, Hendrich MP. J Am Chem Soc. 2008; 130:14465. [PubMed: 18839948]
- 18. Basolo, F.; Pearson, RG. Mechanisms of Inorganic Reactions: A Study of Metal Complexes in Solution. 2. Vol. Chapter 6. John Wiley and Sons, Inc; New York: 1967.
- 19. Emerson JP, Wagner ML, Reynolds MF, Que L Jr, Sadowsky MJ, Wackett LP. J Biol Inorg Chem. 2005; 10:751. [PubMed: 16217642]
- 20. Groce SL, Lipscomb JD. J Am Chem Soc. 2003; 125:11780. [PubMed: 14505375]
- Vetting MW, Wackett LP, Que L Jr, Lipscomb JD, Ohlendorf DH. J Bacteriol. 2004; 186:1945.
   [PubMed: 15028678]
- 22. Davis MI, Wasinger EC, Decker A, Pau MYM, Vaillancourt HF, Bolin JT, Eltis LD, Hedman B, Hodgson KO, Solomon EI. J Am Chem Soc. 2003; 125:11214. [PubMed: 16220940]

23. Vaillancourt FH, Barbosa CJ, Spiro TG, Bolin JT, Blades MW, Turner RFB, Eltis LD. J Am Chem Soc. 2002; 124:2485. [PubMed: 11890797]

- 24. Cornard J-P, Rasmiwetti, Merlin J-C. Chem Phys. 2005; 309:239.
- 25. Perrin, DD.; Dempsey, B.; Serjeant, EP. pKa Prediction for Organic Acids and Bases. Chapman and Hall; London: 1981.
- 26. Tyson C. J Biol Chem. 1975; 5:1765. [PubMed: 163257]
- 27. Reynolds MF, Costas M, Ito M, Jo D-H, Tipton AA, Whiting AK, Que L Jr. J Biol Inorg Chem. 2003; 8:263. [PubMed: 12589562]

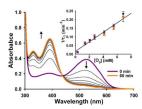


Figure 1. UV-Vis absorption spectra observed for the single-turnover reaction of [Co-HPCD(4NC)] (40  $\mu M$  4NC and 150  $\mu M$  Co-HPCD) (purple) with  $O_2$  to form the extradiol ring-cleaved product (orange). Reaction conditions: 50 mM MES, pH 6.0, 2 atm  $O_2$ , and 22 °C. Intermediate lines were obtained at 5 min intervals. Inset:  $O_2$  dependence of average  $1/\tau$  values from single exponential fits of data (Figure S2) at either 516 (purple points) or 390 nm (orange points). The thin black line represents a linear fit of the  $1/\tau$  data points.

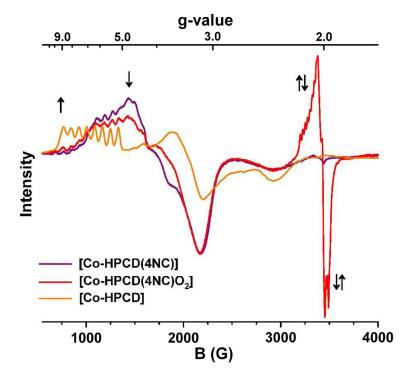


Figure 2. EPR spectra of freeze-quench samples of anaerobic enzyme-substrate complex [Co-HPCD(4NC)] (purple) rapidly mixed with  $O_2$ -saturated buffer under 2 atm  $O_2$  at 22 °C in 50 mM MES buffer pH 6.0, showing formation of the [Co-HPCD(4NC) $O_2$ ] intermediate (red) at 2 min and subsequent decay to Co-HPCD (orange) and the extradiol ring-cleaved product after 60 min. Initial concentration of reactants were 0.5 mM [Co-HPCD(4NC)] and 2.75 mM  $O_2$ .

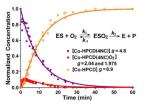
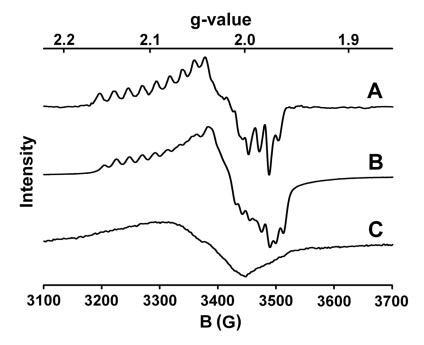


Figure 3.

Time dependence of the various EPR-active species observed in EPR freeze-quench experiments. The change in concentration of each species was measured by monitoring the change in the intensity of EPR features unique to each species. The initial and final spectra were used to normalize the concentrations of [Co-HPCD(4NC)] and Co-HPCD, respectively. The maximum yield of [Co-HPCD(4NC)O<sub>2</sub>] was measured by spin quantification and used to normalize its concentration. <sup>12</sup> Solid lines represent fits of data by global analysis to rate equations based on the reaction scheme shown in Figure 3.



**Figure 4.** EPR spectra of (A) [Co-HPCD(4NC)<sup>16</sup>O<sub>2</sub>] at pH 6.0, (B) [H200N-Co-HPCD(4NC)<sup>16</sup>O<sub>2</sub>] at pH 7.5, and (C) [H200N-Co-HPCD(4NC)<sup>17</sup>O<sub>2</sub>] at pH 7.5 (prepared with 70%  $^{17}$ O<sub>2</sub>). Spectra were obtained at 9.64 GHz, 20 dB microwave power at 20 K.

HO R Fe/Co-HPCD HO R

$$R = CH_2CO_2H \text{ (HPCA) or } NO_2 \text{ (4NC)}$$

#### Scheme 1.

Extradiol ring cleavage of catechol substrates by Fe- or Co-substituted HPCD.

Scheme 2.
Proposed O<sub>2</sub> activation mechanism by Co-HPCD with HPCA at pH 9.0 and 4NC at pH 5.5.<sup>4</sup>

Table 1

# EPR data for Co(II)-HPCD complexes

Sample	s	g values	A <sub>x</sub> ( <sup>59</sup> Co)
Co-HPCD <sup>a</sup>	3/2	6.7, 3.4, 2.4	80 G
[Co-HPCD(HPCA)] <sup>a</sup>	3/2	7.6, 2.5, 1.9	98 G
[Co-HPCD(4NC)] <sup>b</sup>	3/2	5.6, 3.5, 2.1	75 G
[Co-HPCD(4NC)O <sub>2</sub> ]	1/2	2.10, 2.02, 1.99	24 G
[H200N-Co-HPCD] <sup>b</sup>	3/2	6.9, 3.7, 2.6	90 G
[H200N-Co-HPCD-(4NC)] <sup>b</sup>	3/2	6.3, 3.2, 1.9	53 G
[H200N-Co-HPCD-(4NC)O <sub>2</sub> ]	1/2	2.10, 2.02, 1.99	22 G

 $<sup>^</sup>a$ Reference 4.

 $b_{\hbox{Figure S3}}$