Stereoselective Oxidation of a Coordinated Phenoxazinylate Radical with Molecular Oxygen

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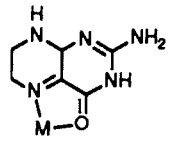
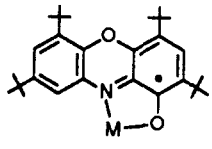
Phenylalanine hydroxylase (PAH), a pterin monooxygenase enzyme, catalyzes the oxidation of phenylalanine to tyrosine.[1] One non-heme iron per subunit is required for activity in mammalian forms of the enzyme, and a bacterial form has been found to have the requirement of one copper atom per subunit. EPR studies on the oxidized Cu(II) enzyme have indicated that the metal is bonded directly to the 5a nitrogen atom of the reduced dihydropterin ring.[2] It is likely that the ring is chelated to the copper through the nitrogen and oxygen atoms of the redox-active region to give a structure that is similar to that found in simple synthetic pterin complexes (I).[3] Recently, it was shown that dioxygen



I



II

coordination to the copper atom of the reduced enzyme occurs at the initial step in the catalytic cycle.[4] Dioxygen addition to a single enzymatic copper center is unusual[5] and may, in this case, involve a Cu(II)–superoxo–pterin semiquinone or a Cu(II)–peroxo–pterin quinone species in the dioxygen coordination/reduction step. An intermediate with a closely related structure has been proposed for the iron intradiol dioxygenase enzymes.[6]

The *tert*-butyl-substituted phenoxazinol semiquinone (II, HPhenox) can be easily prepared by the reaction of 3,5-di-*tert*-butylcatechol with ammonium hydroxide.[7] In its deprotonated form, the molecule readily coordinates with transition metals through the imineolate region of the ring to give complexes which may resemble chelated semiquinone forms of either the flavin isoalloxazine or pterin rings.[8] Ruthenium(II) shows a Ru(II)/Ru(III) oxidation potential that is remarkably dependent upon the composition of the coordination sphere.[9] The metal atom of $Ru(PPh_3)_3Cl_2$ becomes strongly reductive when nitrogen and oxygen donor ligands replace chloro and phosphine ligands. The reaction between HPhenox and $Ru(PPh_3)_3Cl_2$ carried out under argon in 95% ethanol has been found to give the Ru(III) complex *cis*-$Ru(PPh_3)(Cl)(Phenox)_2$.[10] Hydrogen ion released upon co-

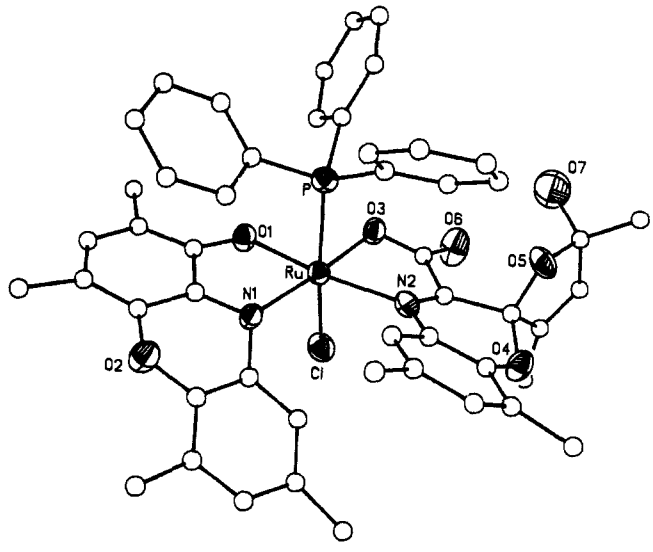
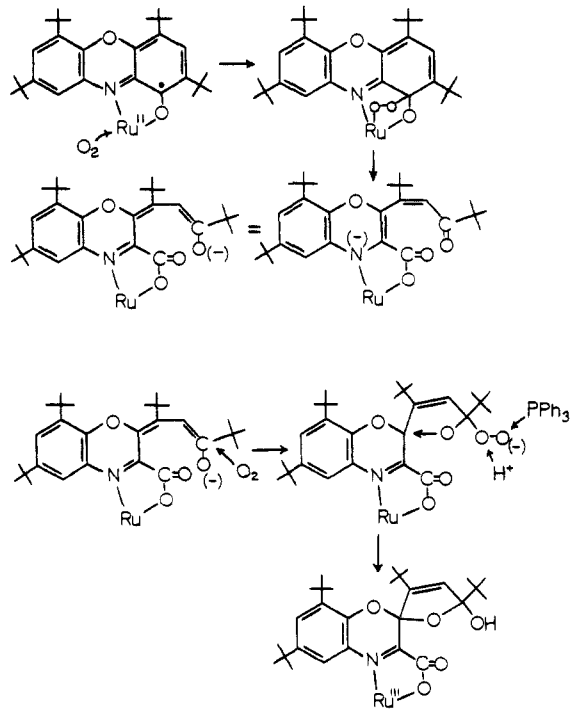


Figure 1.

Scheme I



ordination of HPhenox appears to be the oxidant in this reaction. Electrochemical characterization on *cis*-$Ru(PPh_3)(Cl)(Phenox)_2$ has shown that the Ru(II)/Ru(III) redox couple occurs reversibly at −1.02 V (Fc/Fc$^+$), with two reversible Phenox$^-$ oxidations observed at −0.25 and +0.87 V. The oxidation potential of the Ru(II) form of the complex, *cis*-$Ru^{II}(PPh_3)(Cl)(Phenox)_2^-$, which may form initially in the synthesis, would be sufficient to reduce hydrogen ion and dioxygen. A further point of interest regarding the neutral Ru(III) complex is that it contains a single unpaired electron arising from strong coupling between the two radical ligands and the low-spin d^5 metal ion. The complex shows an EPR

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(10) Synthesis of *cis*-$Ru(PPh_3)(Cl)(Phenxo)_2$: $Ru(PPh_3)_3Cl_2$ (500 mg, 0.52 mmol) and HPhenox (490 mg, 1.16 mmol) were combined in a flask, and degassed ethanol (50 mL) was added to the solids under Ar. The solution was refluxed under Ar for 2.5 h with stirring and then reduced in volume. Ru(PPh$_3$)Cl(Phenox)$_2$ precipitated from solution as a dark brown solid accompanied by a small quantity of unreacted $Ru(PPh_3)_3Cl_2$. The crude product was purified and recrystallized from a dichloromethane/hexane solution. Yield: 500 mg (77%). Details of the crystal structure and spectroscopic characterization on this complex will appear in a separate publication.

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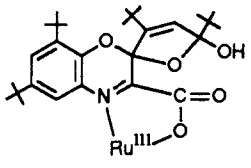
resonance at room temperature centered about a g value of 1.996 with no resolvable hyperfine coupling and a peak to peak width of 28 G. Similar spectra have been reported for Ru(bpy)-$(DBSQ)_2^+$ and $Ru(py)_2(DBSQ)_2^+$.[11] The related Ru(III) complex containing a single Phenox$^-$ radical ligand, $Ru(PPh_3)_2$-$(Cl)_2$(Phenox), is diamagnetic and shows a sharp NMR spectrum.

When the reaction between HPhenox and $Ru(PPh_3)_3Cl_2$ was carried out in air, the product obtained was the neutral Ru-(PPh_3)Cl(Phenox)(oxPhenox) molecule shown in Figure 1.[12] The oxidized Phenox ligand (III, oxPhenox) contains two chiral carbon



III

centers, and the metal center of the complex is also chiral. Strong magnetic coupling between the remaining Phenox radical and the Ru(III) center results in overall diamagnetism for the complex. NMR spectra indicate that Ru(PPh$_3$)Cl(Phenox)(oxPhenox) is obtained exclusively as the diastereomer shown in Figure 1.

The mechanism of oxidation of the coordinated Phenox$^-$ ligand is pertinent to mechanistic models for both oxygenase and dioxygenase metalloenzymes. We belive that the initial step in the process is coordination and one-electron reduction of molecular oxygen at a vacant site of the Ru(II) form of the complex (Scheme I, part A). A vacancy in the Ru(II) coordination sphere may be created by dissociation of the chloro ligand; indirect evidence for this is seen in the change in stereochemistry of the complex, from cis to trans. The coordinated superoxide radical may then add to the Phenox ring at the radical carbon atom. The succeeding steps in oxygen atom rearrangement remain unclear, but appear to follow a mechanism that is similar to that of the extradiol dioxygenases.[13] The result is addition of two oxygen atoms across the C1–C2 bond of the ring to give a coordinated carboxylic acid and a conjugated ketone. Protocatechuate-4,5-dioxygenase and catechol-2,3-dioxygenase both catalyze the addition of two oxygen atoms of dioxygen across an outer carbon–carbon bond of catechol to give an aldehyde–carboxylic acid in a reaction that appears to occur at an iron center of the enzyme.[13] Addition of a second molecule of dioxygen at the ketonic carbon center is thought to occur next,[14] with cyclization of the ketonic oxygen to form the dihydrofuran ring (Scheme I, part B). In a subsequent step, one oxygen atom of the bound peroxo group is abstracted by triphenylphosphine in a reaction that parallels iron abstraction of oxygen from the 4a peroxo group of the oxygenated dihydropterin of PAH.[1] Protonation of the remaining oxo anion gives the coordinated oxPhenox ligand. From the structure of *trans*-Ru-(PPh$_3$)Cl(Phenox)(oxPhenox) it appears that dioxygen addition to the ketonic carbon occurs selectively at the side of the complex away from the bulky phosphine. Cyclization is directed by the steric interactions between *tert*-butyl groups of the dihydrofuran ring and the phosphine phenyl rings.

Oxygen addition to the Ru(II)–Phenox$^-$ unit may parallel oxygen binding to the Cu(I)-reduced pterin center of the bacterial PAH. Both reactions involve one-electron oxidation of the metal, with additional charge required for dioxygen activation provided by a reduced ligand. However, the 2-aminopyrimidone ring of the pterin cofactor provides protection against oxidation, and oxygen transfer occurs to substrate. The phenoxazenylate radical and also the catecholate ring, in the case of the catechol dioxygenase, lack this protection and are themselves oxidized.

Acknowledgment. We gratefully acknowledge the help of Dr. Anthony Spek with solution of the Ru(PPh$_3$)(Cl)(Phenox)(oxPhenox) crystal structure and Professor David Walba and Dr. Anthony Arduango for providing helpful comments on the oxidation mechanism. This research was supported by the National Science Foundation (Grant No. CHE-88-09923); ruthenium trichloride was provided by Johnson Matthey, Inc., through their Metal Loan Program.

Supplementary Material Available: Tables giving crystal data and details of the structure determination, anisotropic thermal parameters, hydrogen atom locations, and bond lengths and angles for Ru(PPh$_3$)Cl(Phenox)(oxPhenox) (15 pages); observed and calculated structure factors for Ru(PPh$_3$)Cl(Phenox)(oxPhenox) (23 pages). Ordering information is given on any current masthead page.

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(12) Synthesis of Ru(PPh$_3$)Cl(Phenox)(oxPhenox): An ethanol solution (50 mL) containing 260 mg (0.26 mmol) of $Ru(PPh_3)_3Cl_2$ and 280 mg (0.66 mmol) of HPhenox was gently refluxed in air for 4 h. The resulting blue-green solution was filtered to remove unreacted starting material and reduced in volume to give a dark green precipitate. The crude product was separated from solution by filtration and recrystallized from a dichloromethane/hexane solution. Yield: 220 mg (63%). *tert*-Butyl resonances appear in the NMR spectrum for the complex at 0.78, 0.81, 1.04, 1.23, 1.39, 1.42, 1.47, and 1.63 ppm. X-ray analysis of Ru(PPh$_3$)(Cl)(Phenox)(oxPhenox): monoclinic, space group $P2_1/c$, a = 19.092 (6) Å, b = 16.018 (7) Å, c = 24.889 (10) Å, β = 111.74 (3)°, V = 7070 (5) Å^3, Z = 4, R = 0.060 for 3679 unique observed reflections. Details of the structure determination are given with the supplementary material.

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(14) Since the ketonic carbon atom is less nucleophilic than other sites on the opened ring, initial oxygen attack may occur elsewhere with subsequent transfer to this atom. To give the observed stereochemistry, attack and migration must occur at the face of the partially oxidized Phenox ligand that is opposite from the triphenylphosphine.

Formation and Properties of a $NiFe_3S_4$ Cluster in *Pyrococcus furiosus* Ferredoxin

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The NiFe-enzyme CO dehydrogenase (or acetyl-CoA synthase) catalyzes CO oxidation in vitro and the formation of a carbon–carbon bond in vivo. EPR studies of the enzyme from the acetogen *Clostridium thermoaceticum*[1] and from the methanogen *Methanosarcina thermophila*[2] have provided evidence for a CO-binding, NiFe-containing catalytic site, in addition to multiple Fe–S clusters. Although the stoichiometry of the novel mixed-metal center is unknown, EXAFS studies indicate substantial S ligation at the Ni.[3] A second type of CO dehydrogenase, which catalyzes only CO oxidation, is found in the photosynthetic bacterium *Rhodospirillum rubrum*. Recent EPR studies of this enzyme also indicate a mixed Ni- and Fe-containing center as the site of CO activation, although the spectroscopic properties of this center are quite distinct from those of other CO dehydrogenases.[4] Since these studies suggest that CO activation by these enzymes occurs at a novel Ni–Fe–S center and synthetic model compounds are not available, we have initiated a program to investigate the properties of cubane-type [$NiFe_3S_4$] clusters. Here we report

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