Complexes of a Pendant Phenoxyl Radical Ligand, Including a New Model for the Ribonucleotide Reductase R2 Protein

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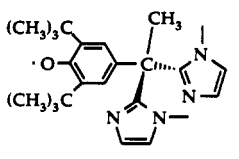
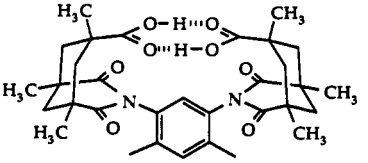
Phenoxyl radicals have recently been identified as an essential component of several metalloenzyme active sites.[1] They have been implicated as cofactors required for the functional activity of ribonucleotide reductase R2 protein,[2-4] galactose oxidase,[5] prostaglandin H synthase,[6-8] and the oxygen-evolving center of photosystem II.[9,10] Defining the steps in the iron-mediated formation of the tyrosyl radical in R2, as well as understanding the inherent stability of this redox-active organic radical in the native protein, are topics of considerable current interest.[3,4,11] In order to provide fundamental information about the properties and stability of metal complexes containing pendant phenoxyl radical ligands and, in particular, to prepare a small-molecule mimic for the R2 protein active site of *Escherichia coli* ribonucleotide reductase, we have designed and synthesized a ligand containing biomimetic imidazole donors and a dangling phenoxyl radical arm. We report the synthesis, quantitation, and physical properties of the ligand 1,1-bis[2-(1-methylimidazolyl)]-1-(3,5-di-*tert*-butyl-4-oxylphenyl)ethane (BIDPhE). Complexes of BIDPhE with divalent zinc and an oxo/carboxylato-bridged diiron(III) core are described.



BIDPhE



$XDKH_2$

The di-*tert*-butyl phenol precursor to BIDPhE, BIDPhE-H, was obtained by the route shown in Scheme I.[12] This ligand was designed to resemble BIPhMe, a bis-imidazole donor previously employed by us in the synthesis of models for diiron oxo proteins.[13] Anaerobic oxidation of a solution of BIDPhE-H in C_6H_6 with an aqueous solution of $K_3Fe(CN)_6/NaOH$[14] afforded BIDPhE on a 3-g scale. The amount of radical was established iodometrically[15] to be 94%. Its electronic spectrum in CH_3CN displays bands at λ_{max} (ϵ, M^{-1} cm^{-1}) = 378 (1500), 394 (1700), and 638 nm (430), closely resembling those of other 2,4,6-trisubstituted phenoxyl radicals.[16] The resonance Raman spectrum of the radical in CH_2Cl_2 solution (Figure 1) has a characteristic, intense, sharp C–O stretching mode at 1503 cm^{-1}, which falls within 2 cm^{-1} of the value reported for phenoxyl radical.[17]

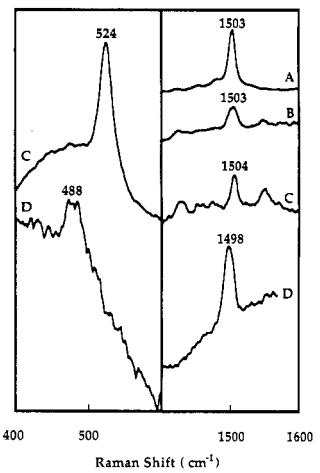
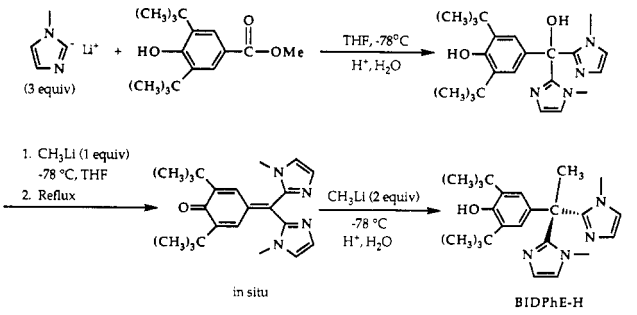


Figure 1. Resonance Raman spectra of (A) BIDPhE, (B) [Zn(BIDPhE)-Cl_2], (C) [Fe_2O(XDK)(BIDPhE)$_2$(NO_3)$_2$] dissolved in CH_2Cl_2, and (D) wild type ribonucleotide reductase R2 protein from *E. coli*, strain N6405/PSPS2, dissolved in Tris buffer, pH 7.6, 5% glycerol. All spectra were recorded at room temperature with Kr ion laser excitation at 406.7 nm.

Scheme I



The complex [Zn(BIDPhE)Cl_2]·MeOH was prepared in order to determine the properties of the radical ligand coordinated to a spectroscopically silent and redox-inactive metal ion. Reaction of anhydrous $ZnCl_2$ with BIDPhE-H in MeOH led to the precipitation of a white solid, which was recrystallized from CH_3CN/Et_2O to yield [Zn(BIDPhE-H)Cl_2]·CH_3CN.[18] As shown in Figure 2, the zinc coordination geometry is approximately tetrahedral, with two chloride ions and two imidazole nitrogen donors from BIDPhE-H comprising the coordination sphere. The phenol oxygen atom does not coordinate to the metal center. The analogous phenoxyl radical metal complex was similarly prepared from $ZnCl_2$ and BIDPhE in MeOH and isolated as a deep green microcrystalline solid, [Zn(BIDPhE)Cl_2]·MeOH.[19] An X-ray powder pattern of this radical species matched that of the phenol

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(12) Details of the preparation and characterization of all compounds shown in Scheme I are provided in the supplementary material.
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(18) Satisfactory elemental analysis was obtained for [Zn(BIDPhE-H)-Cl_2], a white microcrystalline solid isolated directly from MeOH. Anal. Calcd for $C_{24}H_{34}Cl_2N_4OZn$: C, 54.30; H, 6.46; N, 10.55. Found: C, 54.25; H, 6.46; N, 10.49. Crystal data for [Zn(BIDPhE-H)Cl_2]·CH_3CN ($C_{25}H_{37}Cl_2N_5OZn$, M_r = 571.90) at 194 K: size ca. 0.2 × 0.2 × 0.15 mm, triclinic, space group $P\bar{1}$, a = 11.236(2) Å, b = 13.293(3) Å, c = 11.150(1) Å, α = 98.52(2)°, β = 97.91(2)°; γ = 114.26(2)°, V = 1465(1) Å^3, Z = 2. For 3545 unique, observed reflections with $F^2 > 3\sigma(F^2)$ and 319 variable parameters, the current discrepancy indices are R = 0.040 and R_w = 0.048.

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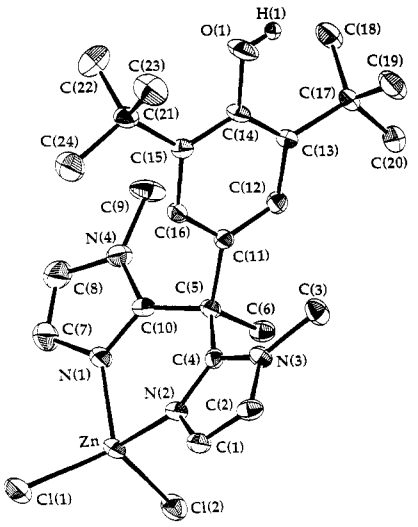

Figure 2. ORTEP diagram of $[Zn(BIDPhE\text{-}H)Cl_2]\cdot CH_3CN$ showing the 50% probability thermal ellipsoids. Selected interatomic distances (Å) and angles (deg) are as follows: Zn–Cl1, 2.224(1); Zn–Cl2, 2.237(1); Zn–N1, 2.003(3); Zn–N2, 2.012(3); Cl1–Zn–Cl2, 120.19(4); Cl1–Zn–N1, 113.4(1); Cl1–Zn–N2, 112.57(9); N1–Zn–N2, 89.4(1).

complex isolated directly from MeOH, proving the structures to be isomorphous. The radical content of $[Zn(BIDPhE)Cl_2]$ was determined to be 87% by iodometric titration.[20]

The UV–visible spectrum of $[Zn(BIDPhE)Cl_2]$ in CH_3CN exhibits three peaks at λ_{max} = 404 (sh), 422, and 638 nm. The resonance Raman spectrum in CH_2Cl_2 solution (Figure 1) displays an intense band at 1503 cm^{-1} due to the C–O stretch of the radical ligand, the same frequency as found for the radical alone. Since the UV–visible and resonance Raman spectra are essentially unperturbed compared to those of free BIDPhE, we conclude that the radical has no direct interaction with the zinc ion.

The first oxo/carboxylato-bridged diiron(III) phenoxyl radical model, $[Fe_2O(XDK)(BIDPhE)_2(NO_3)_2]\cdot CH_2Cl_2$, was synthesized by the addition of 2 equiv of BIDPhe to the unusually stable solvento complex $[Fe_2O(XDK)(MeOH)_5(H_2O)](NO_3)_2$[21] in methylene chloride (XDK = xylenediamine bis(Kemp's triacid imide), a chelating dicarboxylate obtained by condensing two Kemp triacid moieties with a xylyl spacer).[22,23] The BIDPhE complex was isolated as a crystalline solid and characterized by elemental analysis, UV–vis, Raman, and infrared spectroscopy.[24]

The presence of the radical was substantiated by the occurrence of a broad peak at 644 nm in the CH_2Cl_2 solution absorption spectrum, which closely matches the shape and position of the band for the free radical ligand. The optical transition between 650 and 750 nm expected for the $\{Fe_2O\}^{4+}$ unit is masked by the radical absorbance in this region.[25] Convincing evidence for the structure of $[Fe_2O(XDK)(BIDPhE)_2(NO_3)_2]$ is its methylene chloride solution resonance Raman spectrum (Figure 1). The 1504-cm^{-1} peak confirms the presence of the radical, and the intense band at 524 cm^{-1} is a characteristic signature of an oxobis(carboxylato)diiron(III) core.[26] The terminal sites are most likely occupied by coordinated BIDPhE and NO_3^- ligands, as in the $[Fe_2O(XDK)(bpy)_2(NO_3)_2]$ analogue.[21]

Although systems in which a metal complex containing a pendant phenol group have been oxidized to give a phenoxyl radical,[27,28] in all cases there was a pathway for delocalization of the radical spin onto the metal center. This property distinguishes these species from the isolated tyrosyl radical found in R2 and the present iron and zinc model complexes. Moreover, the earlier systems were neither characterized structurally, analyzed quantitatively for radical content, nor comprised of biomimetic donor groups. A recent report describes the use of several dinuclear Fe(III) compounds in the peroxide-induced oxidation of uncoordinated phenols, but no discrete phenoxyl radical containing complex was characterized.[29]

The placement of quarternary carbon atoms in the ortho and para positions of BIDPhE prevents the radical coupling decomposition pathways that occur for unsubstituted analogues.[16] Because of these substituents, however, BIDPhE lacks the *o*-H and *p*-CH_2 nuclei that have been used to assess the extent of spin delocalization at these sites by EPR spectroscopy in the tyrosyl radical.[30,31] Although characteristic proton hyperfine features are absent in the EPR spectra of BIDPhE and its complexes, it should be possible to use spin saturation–recovery experiments[32,33] to evaluate the interaction between the dinuclear iron center and dangling phenoxyl radicals in $[Fe_2O(XDK)(BIDPhE)_2(NO_3)_2]$.

The compound $[Fe_2O(XDK)(BIDPhE)_2(NO_3)_2]$ represents an important first step in the development of functional models for the ribonucleotide reductase R2 protein because it demonstrates that both redox-active partners, the (μ-oxo)diiron(III) core and a proximate phenoxyl radical, can be accommodated in a single molecule. The juxtaposition of these two critical components of the natural system in $[Fe_2O(XDK)(BIDPhE)_2(NO_3)_2]$ is illustrated in Figure 1 where, for comparison, the spectrum of the protein is also displayed. From these spectra it is apparent that the model compound accurately mimics the protein active site.

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Supplementary Material Available: Synthetic details and tables of atomic positional and thermal parameters for $[Zn(BIDPhE\text{-}H)Cl_2]\cdot MeOH$ (9 pages). Ordering information is given on any current masthead page.

(19) Anal. Calcd for $C_{25}H_{37}N_4Cl_2O_2Zn$: C, 53.44; H, 6.64; N, 9.97. Found: C, 53.91; H, 6.40; N, 9.66.

(20) Based on an average of six titrations performed on 34–56 mg of $[Zn(BIDPhE)Cl_2]\cdot MeOH$ per trial.

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(24) Anal. Calcd for $C_{81}H_{106}N_{12}Cl_2O_{17}Fe_2$: C, 57.15; H, 6.28; N, 9.87. Found: C, 57.52; H, 6.20; N, 9.83. The occurrence of CH_2Cl_2 in the lattice was confirmed by the ^1H NMR spectrum in $CDCl_3$. Selected IR data (KBr) ν, cm^{-1}: 1737 and 1695 (XDK, CO_2^-); 1091, 887, 812, 760, and 712 ("fingerprint", BIDPhE-H).

(25) The radical content of $[Fe_2O(XDK)(BIDPhE)_2(NO_3)_2]\cdot CH_2Cl_2$ was estimated to be >95% by assuming ϵ_{644} to be the same as ϵ_{638} for free BIDPhE (see text). The small contribution from the $\{Fe_2O\}^{4+}$ moiety was neglected.

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