Proton/Electron Transfer

How Single and Bifurcated Hydrogen Bonds Influence Proton-Migration Rate Constants, Redox, and Electronic Properties of Phenoxyl Radicals

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Tyrosine/tyrosyl radical redox systems are involved in many biological processes.[1] Among tyrosyl-radical enzymes, interest has been mainly focused on photosystem II,[2] prostaglandin H synthase,[3] galactose oxidase[4] and ribonucleotide reductase.[5] The phenolic (or phenolate) oxygen may be either bonded to a metal center (as in galactose oxydase) or hydrogen bonded to an appropriately orientated acidic residue of the protein. Despite the crucial role of hydrogen bonding on free radical properties,[6] only two pioneering works have appeared recently in the literature concerning simple biomimetic models of hydrogen bonded phenoxyl radicals.[7] The single intramolecular hydrogen-bonded phenoxyl radicals were evidenced through the reversibility of the phenol/phenoxyl redox couple, but unfortunately no rate constants for the proton transfer were reported. To better understand the way in which single and bifurcated hydrogen bonds influence the properties of phenoxyl-radical cations and phenoxyl radicals, we have synthesized and characterized several ortho-substituted 4,6-di(*tert*-butyl)-phenols in which the phenolic hydrogen atom is involved in hydrogen bonding with the nitrogen atom of a tertiary amine (Scheme 1). The strength of this hydrogen bond is modulated through the substituants of the amine. These compounds incorporate: 1) ester and pyridine groups that can establish weak or strong additional hydrogen bonds with the ammonium proton and 2) benzylic hydrogen atoms between the phenol group and the tertiary amine to obtain geometric information about the radical structure from EPR.

The electrochemical behavior of these substituted phenols has been studied in the nonpolar solvent CH_2Cl_2 + 0.1 M tetra *n*-butyl ammonium perchlorate (TBAP) by cyclic voltammetry (CV): On scanning towards the positive potentials, an electrochemical signal is observed at E_p^a, which corresponds to the oxidation of the phenol into a phenoxyl radical cation. On the reverse scan the cathodic peak at E_p^c is observed at a much lower potential ($\Delta E_p > 0.3$ V at 100 mV s^{-1}, 298 K), which suggests that a chemical reaction, attributed to a proton-migration process, is associated with the electron transfer (Scheme 2).[8] Similar potential values (referred to the Fc/Fc$^+$ system) were measured for HL^{Benz} ($E_p^a = +0.72$ V, $E_p^c = +0.32$ V, Figure 1 insert), HL^{Benzpy} ($E_p^a = +0.67$ V, $E_p^c = +0.30$ V) and HL^{py} ($E_p^a = +0.62$ V, $E_p^c = +0.32$ V). All these values are low compared to that obtained for the 2,4,6-tri(*tert*-butyl)phenol ($E_p^a = +1.20$ V): the hydrogen bonding increases the electron density on the phenolic oxygen making it easier to be oxidized. The rate constants of the proton-migration process were obtained from simulation and best fitting of the CV curves (see Supporting Information for details) recorded at several scan rates according to Scheme 2. The determined rate constants are: $k_1 = 10^{7.4\pm0.5}$ s^{-1}, $k_{-1} = 10^{4.1\pm0.7}$ s^{-1}, $k_2 = 10^{5.3\pm0.1}$ s^{-1}, and $k_{-2} = 10^{2.3\pm0.1}$ s^{-1} in the case of HL^{Benz}, with $E_1^0 = +0.65$ V, $E_2^0 = +0.28$ V. For HL^{Benzpy}, with $E_1^0 = +0.67$ V and $E_2^0 = +0.23$ V, these values are $k_1 = 10^{9.6\pm0.4}$ s^{-1}, $k_{-1} = 10^{5.1\pm0.4}$ s^{-1}, $k_2 = 10^{6.2\pm0.2}$ s^{-1}, $k_{-2} = 10^{3.4\pm0.1}$ s^{-1}.[10] The proton transfer from the phenoxyl cation to the tertiary amine is thus assisted, kinetically and thermodynamically, by the pyridine nitrogen atom as reflected by higher k_1 and K_1 (equilibrium constant) values obtained for HL^{Benzpy} relative to HL^{Benz}. Interestingly, in the presence of tetrabutylammonium hydroxide an irreversible signal corresponding to the phenolate/phenoxyl couple is observed for HL^{Benz} and HL^{Benzpy} at

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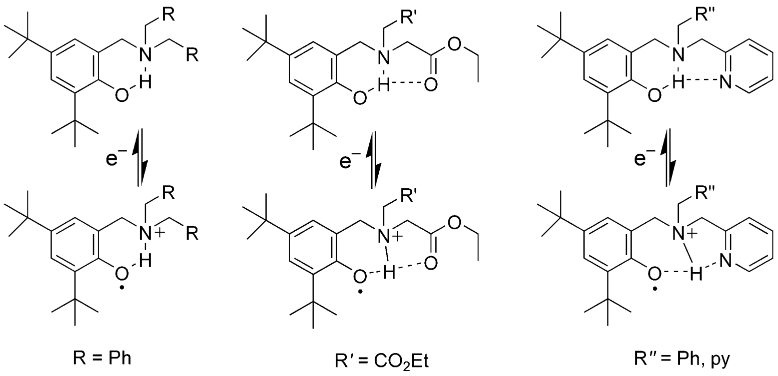
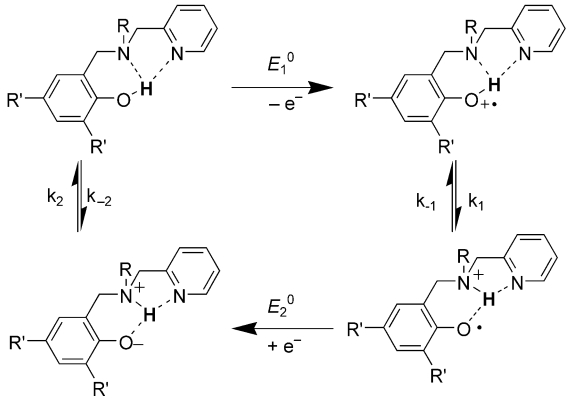


Scheme 1. Phenol derivatives HL^{Benz} (R = Ph), HL^{est} (R′ = CO$_2$Et), HL^{Benzpy} (R″ = Ph) and HL^{py} (R″ = py) and their corresponding hydrogen-bonded phenoxyl radicals.



Scheme 2. Minimum proton-migration process during electron transfer; $K_1 = k_1/k_{-1}$ and $K_2 = k_2/k_{-2}$.

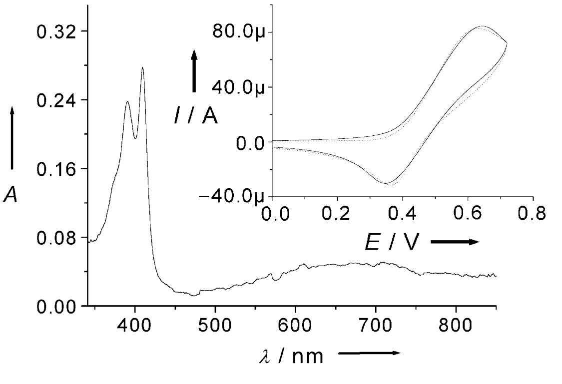
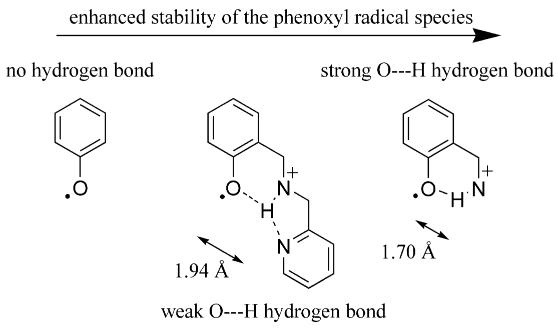


Figure 1. Electronic spectrum of the electrogenerated $HL^{Benz\bullet+}$ 0.32 mM recorded in CH$_2$Cl$_2$ 0.01 M TBAP at 233 K (path length l = 1 cm); Inset: CV curve of HL^{Benz}, 2.4 mM in CH$_2$Cl$_2$ 0.1 M TBAP at 298 K; Scan rate: 0.1 Vs^{-1}, solid line: experimental, dotted line: simulation by using the parameters given in the text. (A = absorption, arbitrary units; I = electrical current).

−0.48 V: the non-hydrogen bonded phenoxyl-radical species thus degrade at the time scale of the experiment.

Upon one-electron electrochemical oxidation at 233 K, the colorless solutions of the phenolic compounds turn deep-green. Their UV/Vis spectra exhibit π–π* transitions typical of a phenoxyl radical at around 400 nm and a weaker band at 650 nm (Figure 1).[9] Under similar electrolysis conditions, in CH$_2$Cl$_2$, the 2,4,6-tri(*tert*-butyl)phenoxyl-radical species was too unstable to be characterized, thus attesting that hydrogen bonds are of prime importance to stabilize a phenoxyl radical electrochemically generated from a phenol. The electrochemically generated-radical species are moderately stable at 298 K. $HL^{py\bullet+}$ and $HL^{Benzpy\bullet+}$ are less stable ($k_{dec} > 5$ min^{-1}) than $HL^{est\bullet+}$ and $HL^{Benz\bullet+}$ ($k_{dec} = 1.20$ min^{-1} and 0.18 min^{-1} respectively). An additional hydrogen bond in $HL^{py\bullet+}$, $HL^{Benzpy\bullet+}$ and $HL^{est\bullet+}$ decreases the strength of the O(phenoxyl)–H(ammonium) hydrogen bond, thus destabilizing the phenoxyl radical (Scheme 3).[10] The effect is more pronounced in $HL^{py\bullet+}$and $HL^{Benzpy\bullet+}$ than in $HL^{est\bullet+}$ according to the strength of the O(phenoxyl)–H(ammonium) hydrogen bond.



Scheme 3. Stability of phenoxyl radical species (O–H distances were obtained from DFT calculations, see text) under our experimental conditions.

The X-Band EPR spectra of the electrochemically oxidized species recorded at 213 K in CH$_2$Cl$_2$ (0.1M TBAP) show an isotropic signal typical of an organic radical (Figure 2a). It is dominated by a large hyperfine coupling constant (hfc) of the spin with one benzylic hydrogen (A_H ranging from 0.51 to 0.80 mT). This is a signature of a rigid conformation of the phenoxyl radical arising from a six-membered ring involving hydrogen bonding. Assuming that the distance d (O(phenoxyl)–H(ammonium)) depends only on θ and the validity of a Mc Connell-type angle-dependent relationship between the hfc and the benzylic hydrogens, the magnitude of A_H is correlated to $\cos^2\theta$ and consequently to d (Figure 3a). The strongest hfc (lowest θ value) obtained for $HL^{Benz\bullet+}$ suggests a shorter d distance. The lowest hfc values were obtained for the compounds bearing one or two pyridines, in which the additional hydrogen bond established between the nitrogen atom of the pyridine and the ammonium proton displaces the latter farther from the oxygen.

Density functional theory (DFT) calculations were performed on two model systems, namely the *N*,*N*-dimethyl-2-

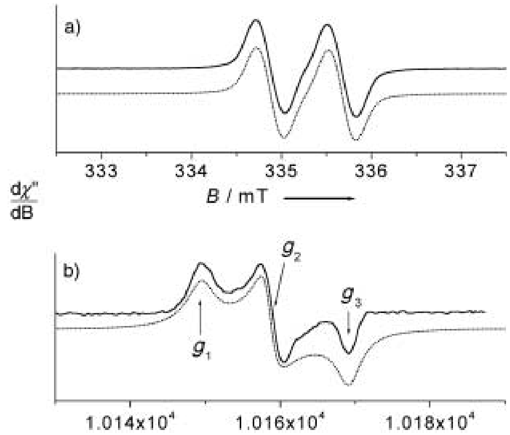


Figure 2. EPR spectra of electrochemically oxidized $HL^{Benz\bullet+}$ radical species recorded in CH_2Cl_2 solution (0.1 M TBAP): a) isotropic X-band spectrum at 213 K (modulation: 100 KHz, 0.0393 mT, Frequency: 9.4103 GHz, 10 mW, $[HL^{Benz\bullet+}] = 1.6$ mM); b) 285 GHz spectrum at 5 K ($[HL^{Benz\bullet+}] = 5$ mM); solid lines: experimental spectrum, dotted lines: simulation by using the parameters given in the Supporting Information. (B = magnetic-flux density).

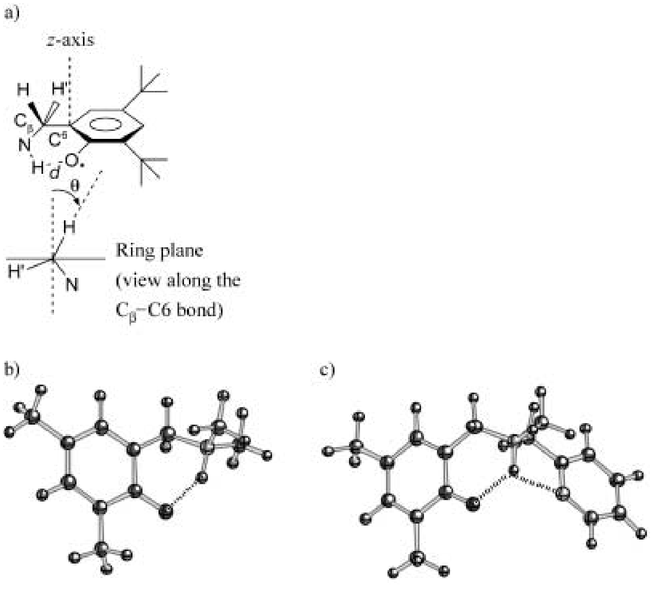
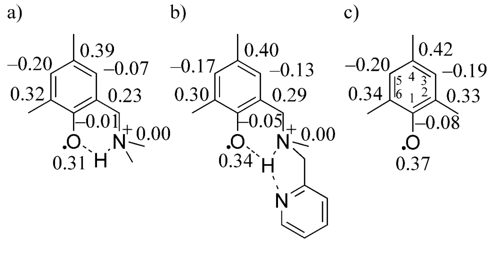


Figure 3. a) Model for the hydrogen bonded phenoxyl radicals; b) (U)B3LYP6-31g* optimized structure of a single hydrogen bonded phenoxyl-radical model system; c) (U)B3LYP6-31g* optimized structure of a bifurcated hydrogen bonded phenoxyl-radical model system.



Scheme 4. Spin density in a a) single hydrogen bonded phenoxyl-radical model system b) bifurcated hydrogen bonded phenoxyl-radical model system and c) "free" 2,4,6 tri(methyl)phenoxyl radical.

methylammonium-4,6-di(methyl)phenoxyl radical ($L^{Me2\bullet+}$, Scheme 4a) and the *N*-(pyrid-2-ylmethyl)-*N*-methyl-2-methylammonium-4,6-di(methyl)phenoxyl radical ($L^{Mepy\bullet+}$, Scheme 4b).[11] All calculations were performed by using the B3LYP hybrid density functional. The efficiency of this method to describe geometries of radical complexes has been recently illustrated.[12] The proton-transferred (phenoxyl–ammonium) and non-proton-transferred (phenoxyl cation–amine) forms were first considered. No non-proton-transferred form could be finally found on the potential energy surface, in agreement with the electrochemical and high field EPR (HFEPR) (see below) data. The optimized structures (Figure 3b,c) of the proton-transferred forms confirmed that the benzylic hydrogen atoms are not equivalent. In $L^{Mepy\bullet+}$ (Figure 3c) the pyridine nitrogen interacts with the ammonium proton, as reflected by a N(pyridine)–H(ammonium) distance of 2.25 Å. Consequently the ammonium proton is displaced farther from the phenoxyl oxygen than in $L^{Me2\bullet+}$: the O(phenoxyl)–H(ammonium) distance is 1.70 Å in $L^{Me2\bullet+}$ (single hydrogen bonded), while it is 1.94 Å in $L^{Mepy\bullet+}$ (bifurcated hydrogen bonded). The spin-density distributions were compared with the values of the "free" 2,4,6 tri(methyl)phenoxyl radical (Scheme 4c). A redistribution of spin density from the oxygen atom to the carbon atom that bears the O atom arises from the presence of the hydrogen bond. Spin density is lost at the C2 atom position and increases at the C3 position. The effect is more pronounced in $L^{Me2\bullet+}$ in agreement with a stronger O–H hydrogen bond. Similar redistribution has been previously reported for a phenol–imidazole hydrogen bonded complex.[12b]

This particular spin distribution, suggested to be responsible for the persistency of phenoxyl radicals in solution,[7a] is reflected by the g_1-tensor value determined by HFEPR experiments[2d,13] (within the molecular frame chosen by van Dam et al.).[5c] The 285 GHz EPR spectra recorded at 5 K exhibit a $S = 1/2$ signal (Figure 2b). The g_2 and g_3 values are known to be independent of the surrounding of the tyrosyl radical. The relatively low g_1 values, 2.0061, 2.0064 and 2.0066 for $HL^{Benz\bullet+}$, $HL^{Benzpy\bullet+}$, $HL^{est\bullet+}$ respectively,[14] are far from that of a "free" tyrosyl radical (usually 2.009). This further confirms that an intramolecular hydrogen bond is established between the phenoxyl-radical oxygen atom and the ammonium proton in our model compounds. These are within the range of that reported (2.0067) for the strongly hydrogen bonded γ-irradiated tyrosine HCl crystals,[15] HSV1 ribonucleotide reductase, prostaglandin synthase tyrosyl radicals (2.0075)[3] or the hydrogen bonded Photosystem II $TyrD^{\bullet}$ (2.0076).[2a]

The stability of phenoxyl radicals is thus correlated to the presence (preventing formation of a phenoxyl radical cation) and strength of an intramolecular hydrogen bond established between the phenoxyl oxygen and an adjacent ammonium proton. The proton-coupled electron transfer from the phenoxyl cation to the tertiary amine is assisted kinetically and thermodynamically by a neighboring hydrogen acceptor: the induced multiple hydrogen-bond network makes the proton move farther and faster. Such processes could play a crucial role in biological systems.[16]

Experimental Section

Starting materials were obtained commercially. *N*-benzyl-*N*-(2-methylpyridine)[17] and HL^{py}[8] were prepared according to the reported procedures.

2,4-di-*tert*-butyl phenol (5 mmol) and the corresponding amine (5 mmol) were dissolved in methanol (40 mL). Aqueous formaldehyde solution (2 mL, 37%) were then added and the mixture was refluxed for two days. The resulting white suspension was filtered, washed with cold methanol and dried under vacuum.

HL^{Benz}: Yield: 65%. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 10.71$ (s, 1H), 7.36–7.22 (m, 10H), 7.19 (d, 1H), 6.84 (d, 1H), 3.71 (s, 2H), 3.60 (s, 4H), 1.43 (s, 9H), 1.26 ppm (s, 9H); MS (DCI, NH_3/isobutane): *m*/*z* (%) 416 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{29}H_{37}NO$: C 83.81, H 8.97, N 3.37; found: C 83.42, H 8.95, N 3.12.

HL^{est}: Yield: 40%. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 9.22$ (s, 1H), 7.24 (d, 1H), 6.82 (d, 1H), 4.20 (q, 4H), 3.97 (s, 2H), 3.53 (s, 4H), 1.42 (s, 9H), 1.24–1.22 ppm (m, 15H); MS (DCI, NH_3/isobutane) : *m*/*z* (%) 408 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{23}H_{37}NO_5$: C 67.78, H 9.15, N 3.44; found: C 67.21, H 9.29, N 3.39.

HL^{Benzpy}: Yield: 51%. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 10.66$ (s, 1H), 8.56 (d, 1H), 7.70–7.61 (m, 1H), 7.37–7.13 (m, 9H), 6.86 (d, 1H), 3.78 (s, 4H), 3.69 (s, 2H), 1.45 (s, 9H), 1.26 ppm (s, 9H); MS (DCI, NH_3/isobutane): *m*/*z* (%) 417 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{28}H_{36}N_2O$: C 80.73, H 8.71, N 6.72; found: C 80.60, H 8.59, N 6.60.

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