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Proton-electron transfer pathways in the reactions of peroxyl and dpph[•] radicals with hydrogen-bonded phenols[†]

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The kinetics of the reaction of peroxyl and dpph[•] radicals with phenols H-bonded to N-bases have been studied for the first time. Electron-transfer processes are observed in MeCN but only with the dpph[•] radical.

The H-bond (HB) that tyrosine forms with several bases has a crucial role in some protein-catalysed redox reactions (*e.g.* photosynthetic system,¹ ribonucleotide reductase²). This is because the synchronous transfer of e^- to the oxidant and of H⁺ to the base is more efficient than the stepwise ET–PT or PT–ET processes.^{1,3} Several studies have shown that this concerted mechanism, named "multiple-site" electron–proton transfer (MS-EPT),^{1*a*} is dominant in the oxidation of phenols bearing pendant bases (*e.g.* compounds **1–3**), which can therefore be used as models of redox enzymes.⁴



However, attention has mainly been focused on the reactivity of these H-bonded phenols with one-electron oxidants and hence little is known about the kinetics involving oxidant

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Table 1Rate constants for the reactions of 4–7 with ROO* (at 303 K)and dpph* (at 298 K) radicals

| | $k_{\rm ROO}/10^3 \ ({\rm M}^{-1} \ {\rm s}^{-1})$ | | $k_{\rm dpph} (\mathrm{M}^{-1} \mathrm{s}^{-1})$ | | |
|---|--|-------------|--|--------------------|--|
| Phenols | PhCl | MeCN | Cyclohexane | MeCN | |
| 4 | 220 ± 20 | 22 ± 4 | 400 ± 30 | 280 ± 60 | |
| 5 | 1.5 ± 0.5 | 2.0 ± 0.5 | 2.5 ± 0.5 | 1200 ± 100^{a} | |
| 6 | 520 ± 40 | 43 ± 5 | 7800 ± 30 | 2340 ± 25 | |
| 7 | 260 ± 20 | 16 ± 2 | 238 ± 5^b | 5.0 ± 0.1^c | |
| ^a Rate constant measured before the equilibrium was established | | | | | |
| (first 300 ms), see text. ^b From ref. 6c. ^c From ref. 6d. | | | | | |

species able to accept *both* $e^- + H^+$ (H•), *i.e.* free radicals. Two noticeable examples of such oxidants are alkylperoxyl (ROO•) and 2,2-diphenyl-1-picrylhydrazyl (dpph•) radicals (eqn (1) and (2)). The former radicals are involved in the oxidative stress, in the synthesis of prostaglandins, in the degradation of food under air and in the oxidation of precursors of fine chemicals.⁵ The stable and coloured dpph• radical has widely been employed in the assessment of the antioxidant/antiradical ability of natural and synthetic products as this radical is supposed (in a few cases erroneously, *vide infra*) to behave similarly to ROO•.⁶

$$\mathsf{ROO} \cdot \xrightarrow{\mathbf{e}, \mathbf{H}^{+}} \mathsf{ROOH}$$
(1)



We therefore investigated the reaction of ROO[•] and dpph[•] radicals with phenols **4–6**, having structural motifs observed in natural phenols.⁷ The role of the pendant N-base and of the internal HB has been evaluated by comparing the relevant kinetic data with those of 4-methoxyphenol (7). The reactivity of alkylperoxyl radicals (k_{ROO}) was determined by measuring the oxygen consumption during the azobisisobutyronitrile-initiated autoxidation of cumene or styrene in the presence of **4–7**, ^{5c,d} and that of dpph[•] (k_{dpph}) by monitoring the bleaching of the 519 nm absorbance following the addition of phenols using a stopped-flow spectrophotometer (see Table 1).^{6b–f}

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Fig. 1 Correlation between O–H BDEs from Table 2 and log k_{ROO} in PhCl (\blacktriangle) and log k_{dpph} in cyclohexane (O). Data for 4-methylphenol (A) and phenol (B), taken from ref. 5*c* and 6*c*, were added to confirm the linear relationship. The best-fit equations are: log $k_{ROO} = -0.344BDE + 33.3$ ($R^2 = 0.92$) and log $k_{dpph} = -0.587BDE + 50.5$ ($R^2 = 0.97$).

 Table 2
 Calculated and experimental (in round brackets) standard redox potentials (in MeCN, reference electrode NHE) and bond dissociation enthalpies (in benzene)

| | $E^{\mathrm{o}}/\mathrm{V}^{a}$ | $BDE^{b}/kcal mol^{-1}$ |
|--------------------------------|--|---|
| Phenols | ArOH ^{+•} /ArOH | $ArOH/ArO^{\bullet} + H^{\bullet}$ |
| 1 2 3 4 | $ \begin{array}{c} 1.06 (1.01)^c \\ 1.12 (1.07)^d \\ 1.27 (1.21)^e \\ 1.42 \end{array} $ | |
| 5 6 7 | 1.42 1.04 0.96 1.51 | $\begin{array}{c} 81.3 \\ 86.2 \\ 79.5 \ [86.0]^{f} \\ 81.4 \ (81.7)^{g} \end{array}$ |
| Radicals | X^{ullet}/X^- | $XH/X^{\bullet} + H^{\bullet}$ |
| PhCH ₂ OO• dpph• | 0.17 $0.76 (0.69)^{i}$ | 84.1 $(84.3)^h$ 79.5 $(78.9)^j$ |

^{*a*} These values were obtained from the experimental E° of α -tocopherol (α -TOH), 1.13 V,¹² and the isodesmic reaction: X^{red} + α -TOH⁺• \rightarrow X^{ox} + α -TOH; calculated at the B3LYP/6-31+g(d,p) level using the implicit solvation model (PCM) as implemented in Gaussian 03.¹³ For those OH groups exposed to the solvent and not involved in intramolecular HB, an explicit HCN molecule was included as a reduced analogue of MeCN (see ESI for further details). ^{*b*} BDE values were obtained from the experimental BDE of phenol (86.7 kcal mol⁻¹)¹⁴ and the isodesmic reaction (computed at the B3LYP/6-31+g(d,p) level in the gas phase): XH + PhO• \rightarrow X• + PhOH. ^{*c*} From ref. 4*a*. ^{*d*} From ref. 4*c*. ^{*e*} From ref. 4*b*. ^{*f*} H-bonded OH group. ^{*g*} From ref. 9*b*. ^{*h*} Value for *t*BuOOH, from ref. 3. ^{*i*} From ref. 15*a*. ^{*j*} From ref. 15*b*.

The rate constants of the reactions of 4–7 with both ROO[•] and dpph[•] radicals, in chlorobenzene and cyclohexane, respectively, were dependent on the bond dissociation enthalpy (BDE) of the phenolic O–H bond (Table 1 and Fig. 1). In particular, the low reactivity of phenol 5 (~100-fold lower in comparison with 4 and 7) arises from the fact that its O–H is engaged in a relatively strong intramolecular HB (as assessed by FT-IR spectroscopy, see ESI†),^{5c} which increases the O–H BDE by *ca*. 5 kcal mol⁻¹ (see Table 2) relative to 4 and 7. Conversely, the high reactivity of 6 implies that the H-donor in this phenol is the HB-free OH.^{6c,8a} The higher reactivity of 6 relative to 4 (despite the apparent similarity of the chemical milieu of their free OH groups) is a consequence of its "remote" intramolecular HB which weakens the HB-free O–H by about 2 kcal mol^{-1.8} When changing the solvent to MeCN the two radicals behaved quite differently. We found that k_{ROO} for **4**, **6** and **7** decreased significantly by 10–16 fold relative to PhCl (Table 1). These kinetic solvent effects (KSEs), largely documented in H-atom abstractions from polar X–H bonds, are due to formation of intermolecular hydrogen bonds between MeCN and the reactive phenolic groups.^{9,10} In the case of **5**, no KSE was detected because its intramolecularly H-bonded OH can be only weakly solvated by MeCN. We conclude, therefore, that the ROO[•] radicals react with **4–7** in both PhCl and MeCN *via* a "formal H-atom abstraction".¹¹ In the case of dpph[•], the rates of reaction in MeCN with **4–6** were large and did not comply with KSE (only 7 displayed the expected KSE, see Table 1). These large rates therefore demand mechanisms quite dissimilar to the one proposed for ROO[•] radicals, most likely ET processes, as thermodynamic data suggest (Table 2).

The large E° of phenol 4 (Table 2) leads to the conclusion that the reactive species must be its anion 4' which is produced from the self-ionization (eqn (3)) and is characterized by a low E° (calculated as 0.07 V vs. NHE).¹⁶ Furthermore, formation of the dpph• anion (eqn (4)) was spectroscopically confirmed (see ESI†). The low concentration of 4' ($K_3 \sim 6 \times 10^{-9}$, 4' ~ 0.008% of 4)¹⁷ and its low E° require that ET be fast, $k_4 \approx 3.5 \times 10^6$ M⁻¹ s⁻¹.



On the other hand, autoionization of 5 and 6 with formation of a zwitterion seems to be improbable,^{4b} and hence the reactivity of 5 and 6 in MeCN must be due to ET from the phenols themselves which are characterized by low E° . Surprisingly, the kinetics of 5 + dpph[•] showed biphasic behaviour with an initial fast process (300 ms, k_{obs}) and a second slower dpph[•] loss for some tens of seconds, as shown in Fig. 2. We attribute the first stage to a fast approaching to equilibrium. The linearity of the k_{obs} vs. [ArOH] plot suggests an equilibrium of the type, $\mathbf{A} + \mathbf{B} \Leftrightarrow \mathbf{C}$, where \mathbf{C} might be the contact ion-pair {ArOH $^{\bullet+}$ /dpph⁻} formed after MS-EPT. Once the equilibrium is established, a PT from the ammonium ion of 5°⁺ to dpph⁻ drives the reaction to completion (second kinetic phase), see Scheme 1. Simulations performed with the program SPECFIT confirmed this reaction scheme and provided the value for the rate constant $k_{\rm PT}$. The mechanism shown in Scheme 1 is also compatible with the kinetics of 6 +dpph[•], although we observed in this case only a rapid and 'clean' first-order process. The lack of pre-equilibrium is most likely due to the fact that $k_{\text{PT}} \gg k_{\text{-MS-EPT}}$ since the radical 6°⁺ is about 3 orders of magnitude more acidic than $5^{\bullet+}$ because the H⁺ can be released from the HB-free OH (see ESI⁺ and Scheme 1). The strong intramolecular HB in the radical 5^{•+} slows down the proton release to dpph⁻ and determines a surprisingly long survival of the 5^{•+} + dpph⁻ contact ion-pair ($\tau_{1/2} \approx 3$ s).



Fig. 2 Decay of the 519 nm absorbance of dpph• (0.1 mM) following the addition of **5** (2.7 mM) in MeCN. The inset shows the observed rate constants measured during the fast dpph• decay.



Scheme 1 Reaction of 5 and 6 with dpph[•] in MeCN at 298 K.

In conclusion, our work shows that the dpph• radical behaves very differently from ROO• in MeCN. The relatively high E° of dpph• activates MS-EPT/PT reactions with phenols **5** and **6** or PT–ET with **4** which are not observed with ROO• because of its low E° (0.17 V, see Table 2).^{19,20} In fact, we calculate from the data in Table 2 that the ET process from **6** to ROO• is endergonic by about 17 kcal mol⁻¹ while in the case of dpph• only by 4.6 kcal mol⁻¹. Therefore, caution must be exercised when the antioxidant ability of phenols is evaluated through their reactivity with dpph• in polar solvents.

Our results also show that peroxyl radicals are ineffective in the H-atom abstraction from phenols H-bonded to N-bases. This implies that the H-bond that tyrosines may form with basic residues in proteins protect them from the attack of peroxyl radicals formed during oxidative stress.

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