TEMPO reacts with oxygen-centered radicals under acidic conditions†

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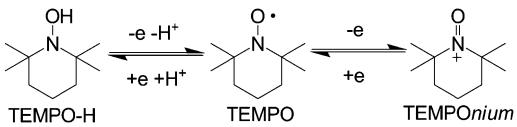
In the presence of organic acids in organic media, 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO) reacts with peroxyl radicals at nearly diffusion-controlled rates by proton-coupled electron transfer from the protonated nitroxide.

2,2,6,6-Tetramethylpiperidine-*N*-oxyl (TEMPO) is arguably the most famous odd-electron organic species. It was first isolated as a crystalline orange solid by Lebedev and Kazarnovskii in 1960,[1] and the characteristic three-line EPR spectrum was observed by Il'yasov[2] and Rassat's group[3] a short while later. Its persistence makes TEMPO and related nitroxides particularly versatile, *e.g.* as catalysts for controlled radical polymerization reactions,[4] spin labels,[5] radical probes,[6] or catalysts for the controlled oxidation of primary alcohols.[7] Being one electron away from hydroxylamines and oxoammonium ions (Scheme 1), nitroxides have a privileged redox position that is responsible for their role as oxidation catalysts. Paradoxically, the same chemistry is also the basis for the superoxide dismutase mimetic activity of TEMPO.[8]

Over the past two decades, considerable interest has emerged in the biological activities of nitroxides, which have been reported to range from neuroprotective to anticancer.[9] Many of these properties have been ascribed to the anti-oxidant activity of nitroxides, but the mechanistic rationale behind it is far from clear.[10] While it is quite clear that TEMPO and its analogs react readily with alkyl radicals (1×10^8–1×10^9 M^{-1} s^{-1}),[11] they have been found to be quite unreactive toward peroxyl radicals.[12] Since peroxyl radicals are the predominant chain-carrying species under atmospheric pressures of O_2, owing to the diffusion-controlled reactions of alkyl radicals with O_2 (2–5×10^9 M^{-1} s^{-1}),[13] nitroxides should be useless as antioxidants under most conditions.

Recent work by Ingold and co-workers,[14] as well as some of our own efforts,[15] has shown that reactions of phenolic antioxidants with peroxyl radicals, formal H-atom transfer processes, are highly medium-dependent. We wondered whether medium effects could account for the spurious antioxidant activity of nitroxides. Herein we report the results of our investigations, which demonstrate that nitroxides are among the most effective antioxidants, but only under acidic conditions.



Scheme 1 Redox behaviour of TEMPO.

We first set out to establish a baseline with which to compare medium effects, and found that when TEMPO (5–100 μM) was used as an inhibitor in the AIBN-initiated autoxidation of styrene (in MeCN or chlorobenzene),[15,16] only a modest retarding of the rate of autoxidation was observed, which was consistent with previous reports.[12] Interestingly, when we added trifluoroacetic acid (TFA) to the autoxidation mixture (in MeCN, see Fig. 1A), a very well-defined inhibition period was observed, and the inhibition rate constant (k_{inh}) derived from the slope of the inhibited rate of oxidation[16] was proportional to the concentration of the acid (Fig. 1B). In fact, the addition of TFA yielded values of k_{inh} as high as 2.2×10^7 M^{-1} s^{-1} (when 0.1 M TFA was included) thus outperforming the reference antioxidant, α-tocopherol, by more than 30-fold ($k_{inh} = 6.8 \times 10^5$ M^{-1} s^{-1} in MeCN[15]).

Qualitatively similar results were obtained by adding different acids (Table 1), including the weaker acetic acid or the stronger *p*-toluenesulfonic acid (*p*-TSA). In fact, the addition of *p*-TSA, at a concentration of 10 mM, resulted in the essentially diffusion-controlled quenching of peroxyl radicals, with $k_{inh} = 1.8 \times 10^8$ M^{-1} s^{-1}, equalling the performance of the most effective chain-breaking antioxidants known to date.[17] Extension of the investigation to a series of organic acids of different strengths showed that k_{inh} is clearly dependent on the pK_a of the acid (see Supporting Information†). Acids themselves (without TEMPO) did not affect the oxidizability of styrene, and we obtained similar results when cumene was employed as the oxidizable substrate.

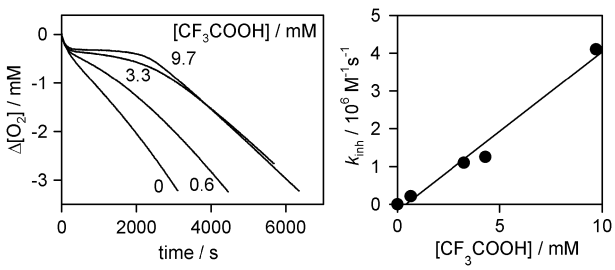


Fig. 1 (A) Oxygen uptake kinetics during the autoxidation of 4.3 M styrene in MeCN initiated by 0.05 M AIBN at 303 K inhibited by 12.5 μM TEMPO with variable amounts of trifluoroacetic acid, and (B) dependence of the measured inhibition rate constant (k_{inh}) on the concentration of acid.

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† Electronic supplementary information (ESI) available: Cartesian coordinates, electronic energies and thermochemical corrections for all relevant structures. See DOI: 10.1039/c0cc00547a

Table 1 Rate constants for the reaction of peroxyl radicals with TEMPO obtained from inhibited autoxidations of styrene at 303 K in the presence of various organic acids

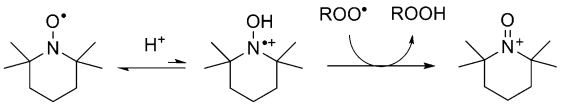
Acid[a]	pK_a[b]	k_{inh}^{MeCN}(M^{-1} s^{-1}) [Ac] = 4.3 mM	k_{inh}^{MeCN}(M^{-1} s^{-1})/ [Acid (M)]	n[c]	$k_{inh}^{PhCl}/k_{inh}^{MeCN}$ [d]
AA	23.5	2.6×10^5	3.1×10^7	>1	1.2
BA	21.5	3.8×10^5	—	>1	—
DCA	13.2	5.6×10^5	—	1	—
TFA	12.7	1.0×10^6	7.0×10^8	1	2.9
TCA	10.8	9.6×10^5	—	1	—
p-TSA	8.0	7.0×10^6	1.4×10^{10}	1	—

[a] AA = acetic acid; BA = benzoic acid; DCA = dichloroacetic acid; TFA = trifluoroacetic acid; TCA = trichloroacetic acid; *p*-TSA = *p*-toluenesulfonic acid. [b] Measured in MeCN from ref. 19. [c] Stoichiometric coefficient = moles of peroxyl radicals trapped by one mole of TEMPO. [d] Kinetic solvent effect.

On changing the solvent from the relatively polar, H-bond accepting (HBA) solvent MeCN to the relatively non-polar, non-H-bond accepting chlorobenzene, the effect was maintained and, interestingly, the inhibition rate constants became even larger. This type of kinetic solvent effect (KSE) is typical of phenolic antioxidants, since in HBA solvents, the phenolic H-atom is H-bonded to the solvent, making it unavailable for abstraction by the peroxyl radical.[14] This finding was particularly intriguing since TEMPO itself does not possess an acidic hydrogen, or for that matter, any abstractable hydrogen. A similarly surprising result arose when we measured the isotope effect on the kinetics of the reaction. When parallel sets of autoxidations inhibited by 12.5 μM TEMPO were run in MeCN to which either 4.4 mM CD_3COOD/CH_3COOH or 3.3 mM CF_3COOD/CF_3COOH was added,[18] deuterium kinetic isotope effects (DKIEs), k_H/k_D, of 2.4 and 2.2, respectively, were obtained. These values happen to be quite close to the values reported for the reaction of peroxyl radicals with structurally related hydroxylamines (k_H/k_D = 2–3).[12]

Since TEMPO is known to disproportionate in acidic media to yield TEMPO-H and the corresponding oxoammonium ion (TEMPO*nium*),[7,20] we figured that the DKIE values and solvent effects could be explained by the intervention of TEMPO-H as the antioxidant. In fact, the structurally related 4-oxo-TEMPO-H has been reported to react rapidly with peroxyl radicals (k_{inh} = 5.0×10^5 M^{-1} s^{-1}).[12] In our hands, rate constants of k_{inh} = 2.4×10^6 M^{-1} s^{-1} and 3.0×10^6 M^{-1} s^{-1} were measured at 303 K for TEMPO-H in MeCN and chlorobenzene, respectively ($k_{inh}^{PhCl}/k_{inh}^{MeCN}$ = 1.3), with a stoichiometric coefficient (n) of 1. No acid catalysis was observed for the reaction of TEMPO-H with peroxyl radicals. While this result confirms that TEMPO-H is an effective antioxidant, it cannot account for the much greater reactivity of TEMPO under acidic conditions. When we monitored TEMPO disproportionation by EPR under the conditions of our inhibited autoxidations, we found that the kinetics were much too slow to be relevant (see Supporting Information for further details†). In fact, with the stronger acids, less than 1% of TEMPO would be converted to TEMPO-H during the inhibited period of the autoxidations (~2000 s).

We next considered the possibility that protonation of the nitroxide under the reaction conditions, although unfavourable



Scheme 2 Proposed mechanism for the reaction of TEMPO with peroxyl radicals under acidic conditions.[23]

(pK_a of TEMPOH$^{+\bullet}$ is −5.8 in water),[20] may yield an effective H-atom donor (Scheme 2). In fact, in a preliminary set of calculations carried out using the CBS-QB3 approach,[21] we found that the O–H bond dissociation enthalpies (BDEs) of protonated nitroxides are very low—even lower than those in equivalently substituted hydroxylamines, which are commonly thought of as having the weakest O–H bonds of any closed shell molecule. For example, while *N*,*N*-di-*tert*-butylhydroxylamine has a calculated O–H BDE of 69.0 kcal mol^{-1},[22] the O–H BDE calculated for the corresponding protonated *N*,*N*-di-*tert*-butyl nitroxide is 58.2 kcal mol^{-1}.

Although it is gratifying that the thermodynamics are quite reasonable (the reaction enthalpy would be ~30 kcal mol^{-1} exothermic, since the O–H BDE in hydroperoxides are *ca.* 88 kcal mol^{-1}),[24] it is not obvious that the kinetics would be. Therefore, we next computed the transition state (TS) structure corresponding to the transfer of the H-atom from a protonated nitroxide to a peroxyl radical.[25] The TS structure (Fig. 2A and B) is characterized by a co-planar arrangement of all of the heteroatoms and the H-atom being transferred. This geometry is consistent with a proton-coupled electron transfer (PCET)[26] between these two species: with proton transfer taking place between the lone pairs of the terminal oxygen atoms of the nitroxide and peroxyl, which are separated by only 2.46 Å (the highest doubly-occupied MO is shown in Fig. 2C), and electron transfer across the 2p orbitals of the nitroxide and peroxyl SOMOs which are orthogonal to the molecular framework (one of the two singly occupied MOs is shown in

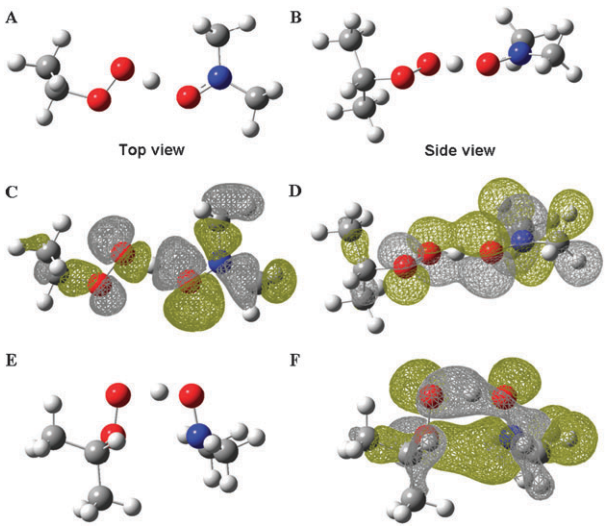


Fig. 2 (A,B) Transition state structure for the formal H-atom transfer from a protonated nitroxide to a peroxyl radical, and (C,D) its corresponding highest occupied molecular orbitals. (E) Transition state structure for the formal H-atom transfer from the equivalently-substituted hydroxylamine to a peroxyl radical, and its corresponding (F) highest doubly-occupied molecular orbital, for comparison.

Fig. 2D).[27] This structure lies 23.3 kcal mol^{-1} lower in enthalpy than the separated reactants, and is connected to them *via* a H-bonded pre-reaction complex which lies 27.9 kcal mol^{-1} beneath them. In contrast, the lowest energy TS structure for H-atom transfer between the equivalently-substituted hydroxylamine and peroxyl radical (Fig. 2E) shows a cyclic PCET process,[28] due to the exchange of the electron between the nominally doubly-occupied lone pair of the hydroxylamine N-atom and the peroxyl SOMO as the proton is passed between the terminal oxygen atoms. This TS structure lies 4.2 kcal mol^{-1} above the separated reactants, and is connected to them by a H-bonded pre-reaction complex that lies 3.5 kcal mol^{-1} beneath them. Therefore, while theory predicts a modest barrier for the reaction of the hydroxylamine with the peroxyl, it predicts a diffusion-controlled reaction between the protonated nitroxide and the peroxyl, consistent with the foregoing experimental findings.

An important point that remains to be clarified is the unusually large stoichiometric factor observed with the weaker acids we have examined. Indeed, when either acetic or benzoic acids were used as a proton source in the TEMPO-inhibited autoxidations, an apparently infinite inhibited period was observed. Furthermore, when the concentration of TEMPO was monitored in these reactions, there was nearly no decay of the EPR spectrum of the nitroxide (see Supporting Information†). This result, which stands in stark contrast with the consumption of TEMPO by the end of the *ca.* 2000 s inhibited period of autoxidations in the presence of the stronger acids, implies that TEMPO is regenerated from TEMPO*nium* when the weaker acids are used as proton source. We are currently investigating this exciting and unexpected feature further.

We believe the foregoing observations to have very important implications in both health and industry. In biological systems, various environments within the cell have acidic functionalities that can provide a proton to unleash the antioxidant activity of TEMPO. For example, lipid-derived peroxyl radicals can be expected to be trapped by TEMPO when in proximity to acidic sidechains of transmembrane proteins of the lipid bilayer or apoproteins of circulating lipoproteins. These reactions may very well underlie the biological activities of nitroxides, and may help in the design of experiments aimed at deconvoluting the purported neuro-protective and anticancer roles of nitroxides.

Hindered amine light stabilizers (HALS) are derivatives of 2,2,6,6-tetramethylpiperidine and are extremely efficient stabilizers against light-induced degradation of most polymers. Nitroxides are observed in these reactions, and have a key role in trapping alkyl radicals.[29] Since organic acids are often generated during the photo-induced polymer degradation, our results suggest they may catalyze additional reactions of the nitroxides with peroxyl radicals, and also work to regenerate them.

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