The gas-phase interactions of TEMPO radicals with Fe^+ ions

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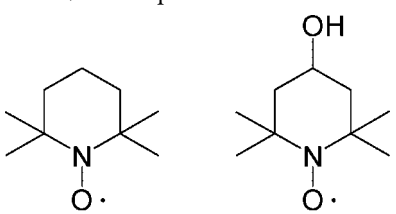
The gas-phase ligation of the 2,2,6,6-tetramethylpiperidyl-1-oxide (TEMPO) radical (1) and its 4-hydroxy derivative (2) with Fe^R ions in a 3 T Fourier transform ion cyclotron resonance (FTICR) mass spectrometer was investigated. Triple ligation may occur: the first ligation produces a transient species prone to either charge exchange or a stable second ligation; the third ligand adds slowly, with fragmentation. 1 and 2 differ in that 1 binds exclusively at the nitroxyl oxygen while 2 also binds at the OH site after the loss of a H-radical. Calculations combined with steric considerations support such a mechanism for 2. The site and the mechanism of the important side reaction of 1 that involvesOH addition from a water impurity to yield an FeR2R species remain unexplained.

Organic free radicals, compounds characterized by an unpaired valence electron, are usually highly reactive and short lived. Nonetheless, they have found use as spin traps, spin labels, antioxidants and polymerization initiators.[1] Such free radicals may be stabilized by appending bulky substituents in the vicinity of the unpaired electron, and the extent of stabilization is high enough to maintain the integrity of these radicals for several months. Nitroxide free radicals are prime examples of such persistent radicals.[2] Due to their inert and stable nature, they are widely used in electron spin resonance spectroscopy as probes for biological systems, as radical traps and as catalysts in oxidation of amines, phosphines, phenols, anilines and alcohols. Because they melt without decomposition, they can be transferred by heat into the gas phase in a mass spectrometer.

It is not surprising that these compounds have been intensively investigated both experimentally and theoretically. An approach that combined such efforts was a recent measurement of their ultraviolet (UV) photoelectron spectra[3–5] conjoined with sophisticated quantum chemical methods which enabled a determination of electronic structures. These results were surprising: they showed negligible substituent influence on the unpaired electron properties. The ionization behavior of such stable radicals was also studied under electrospray (ESI) and atmospheric pressure ionization (APCI) conditions. Depending on the conditions, R^+, $[R+1]^+$ or $[R+2]^+$ ions were observed as the main products in the mass spectra.[6,7] Electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (ESI-MS/MS) were found to be suitable tools for the detection and mass spectrometric characterization of radical intermediates[8] at the different stages of reactions that employed stable radicals (e.g. as radical traps[9] or catalysts in oxidation of alcohols[10]).

The coordination chemistry of stable free radicals is also an interesting field.[11] Studies of the kinetics of nitrosyl coordination to transition metals are limited because of the lability caused by the unpaired electron.[12] The interactions and reactions of NO with transition metal centers are important to the role of NO as a messenger molecule in diverse organisms.[13]

Our interest in the intrinsic activity of antioxidants (e.g. flavonoids,[14] for which we investigated gas-phase reactions with metal ions) drew our attention to nitroxide radicals. It was shown that, in the absence of redox-active metal ions in solution, a nitroxide radical removed a phenolic antioxidant (Trolox) in a simple bimolecular reaction that probably involves a hydrogen transfer from phenol to nitroxide.[15] We now investigate the 2,2,6,6-tetamethylpiperidyl-1-oxide (TEMPO) radical (**1**) and its 4-hydroxy derivative (**2**) as they react in the gas phase with Fe^+ ions within a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer.



1 **2**

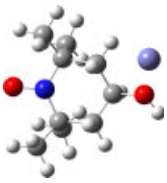
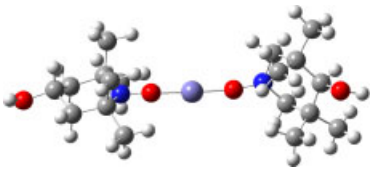
The products formed by reaction of laser-generated, mass-selected $^{56}Fe^+$ ions with the neutral radical, **1** and **2** in the gas phase were monitored as in previous work:[16,17] 'snapshot' mass spectra were recorded at various time delays after the moment of ion selection. Such studies of gas-phase reactions between bare metal ions and organic compounds are ideal for elucidation of the intrinsic properties and reactivity of chemical species.[18,19] The $^{56}Fe^+$ ions, according to previous gas-phase ligation experiments with organic ligands, are highly reactive and, in solution, play an important role in the Fenton reaction which yields hydroxyl radicals.[20–24] Thus, we tried to elucidate for **1** and **2**: (i) the site of metal ion attack, (ii) the ligation number, and (iii) the effect of the 4-substituent in **2** on product formation. Because the site of interaction of a metal ion with an organic molecule in the gas phase can be quite different from that in solution, we also performed calculations (*vide infra*) to determine these sites as well as the reaction enthalpies and the preferred structures of the Fe^+ chelation products with **1** and **2**.

EXPERIMENTAL

Experiments were carried out on a 3 T FTICR mass spectrometer (Extrel FTMS 2001 DD, Madison, WI, USA). Singly charged positive metal ions were generated by a single 1064 nm pulse of a Nd:YAG Quanta Ray DCR-11 laser (Spectra Physics Inc., Mountain View, CA, USA) on a stainless steel target. All but the desired $^{56}Fe^+$ ions were removed from the cell shortly after the laser pulse by a synthesized wave inverse FT SWIFT procedure. A steady-state concentration of 2,2,6,6-tetramethylpiperidyl-1-oxide (TEMPO) radical (**1**) and its 4-hydroxy derivative (**2**) was achieved in the FTICR mass spectrometer by sublimation from a small heated quartz tube mounted near the metal target. The mass spectra of products were recorded after various delay times (100 μs to 10 s).

The origin of some ions in the mass spectra was determined by MS/MS experiments: the presumed precursor ions were mass-selected in the ICR cell and their product ion spectra were recorded after various delay times. For some assignments, deuterium labeling produced by the introduction of D_2O into the sample tube and, thence, into the cell was found to be a useful tool.

Table 1. Energies for the optimized structures of Fe(**2**)$^+$, (**2**)$^+$ and **2**Fe(**2**)$_2^+$ ions in the gas phase

	$E_1 = -596965.70$ kJ/mol
	$E_2 = -596919.81$ kJ/mol
	$E_3 = -595236.91$ kJ/mol
	$E_4 = -272160.45$ kJ/mol
	$E_5 = -270460.95$ kJ/mol
	$E_6 = -869234.19$ kJ/mol
	$E_7 = -867814.98$ kJ/mol

CALCULATIONS

All calculations were density functional theory (DFT) in nature. They used Becke's three-parameter hybrid exchange functional (B3)[25] together with Perdew and Wang's 1991 (PW91) correlation functional.[26] The SDDALL basis sets were Gaussian 03 in nature.[27]

The calculations were carried out to determine why **2** reacts with Fe^+, not only additively at the nitroxyl oxygen site, but after release of a hydrogen atom, preferentially at the hydroxyl oxygen. Energies for the following optimized structures had to be determined (Table 1): (i) **2** bound through the nitroxyl oxygen atom, E_1; (ii) **2** bound through the hydroxyl oxygen without H-loss, E_2; (iii) **2** bound through the hydroxyl oxygen after loss of H, E_3; (iv) free **2**, E_4; and (v) free **2** without the OH hydrogen atom, E_5. The energy of the free hydrogen atom E(H) was assumed to be –1306.53 kJ/mol. Because of the release of H while forming a new bond to Fe^+, the OH bond is broken and its energy is given as:

$$E(OH) = E_4 - (E_5 + E(H)) = -392.97\ \text{kJ/mol}.$$

It follows that direct Fe^+ binding at the oxygen of ON vs. that of OH in **2** is preferred by $E_1 - E_2 = -45.89$ kJ/mol. However, the binding to the hydroxylic oxygen of **2** after loss of the H atom equals $E_3 + E(H) + E(OH)$ (−596936.40 kJ/mol), which is lower than E_2 but still higher than E_1. It is possible that steric factors also play an important role.

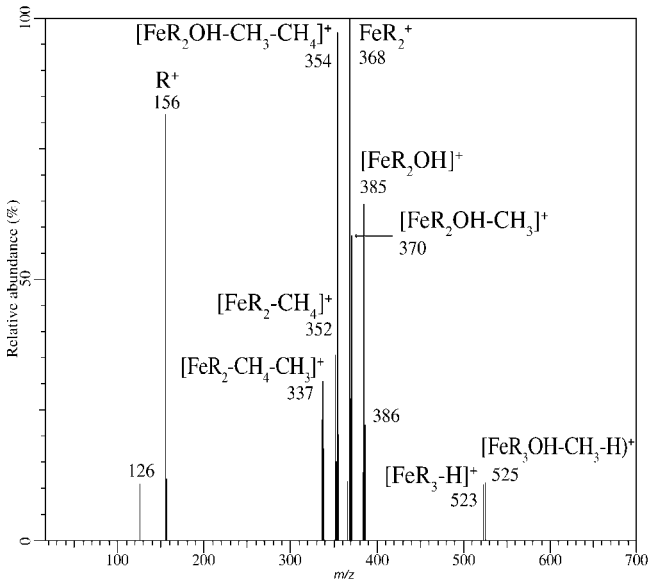


Figure 1. LDI FTMS of Fe^+ reaction products with TEMPO radical **1** after 10 s reaction time.

RESULTS AND DISCUSSION

The FT mass spectra of the reaction products of **1** and **2** with Fe^+ at a 10 s delay relative to the expulsion time of all but the $^{56}Fe^+$ ions are shown in Figs. 1 and 2, respectively. At this delay time, all the characteristic ligation and fragmentation products are seen, the sole exception being the singly ligated species which, even at the shortest delay of 100 μs, are minimally observable and evidently of a very transient nature. It is clear that **1** binds to Fe^+ additively while **2** binds preferably by losing one hydrogen atom and, to a lesser extent, additively in a 2:1 ratio. Even at 100 μs reaction time, substantial amounts of the positively charged ligands, R^+, and doubly ligated iron species, FeR_2^+, exist, indicating that

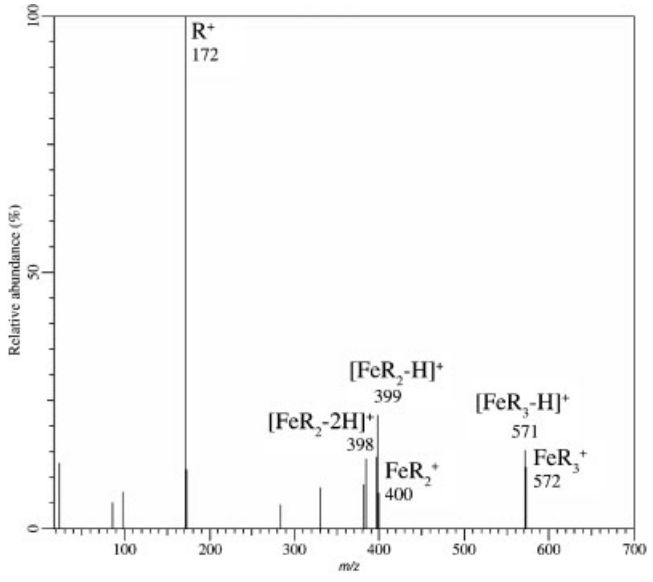


Figure 2. LDI FTMS of Fe^+ reaction products with TEMPO radical **2** after 10 s reaction time.

the reaction proceeds as:

$$Fe^+ + R \rightarrow [Fe + R]^+(\text{labile}) \rightarrow FeR_2^+(\text{ligation}) \text{ or}$$
$$\rightarrow Fe + R^+(\text{charge exchange})$$

The ionization energies of **1** and **2** are both 7.3 eV[3] whereas that of Fe is 7.87 eV. These values suggest that electron transfer from nitric oxide to atomic metal cations[26] is feasible, particularly via an exchange path. However, there are striking differences in the behaviors of **1** and **2** that must be addressed. While **1** binds additively both as a first and second ligand, **2**, because of its preference to bind by losing a hydrogen atom (*vide supra*), forms three different doubly ligated species: FeR_2^+, $[FeR_2{-}H]^+$ and $[FeR_2{-}2H]^+$, for which the labeling experiments show that the H originates in the hydroxyl group. The other striking difference is that the doubly ligated species of **1** is prone to the addition of OH from the water that is ubiquitously present in the instrument, namely as:

$$FeR_2^+ + OH \rightarrow FeR_2OH^+$$

While FeR_2^+ (*m*/*z* 368) is fairly stable, losing methane and methyl in two steps or in a single step (yielding *m*/*z* 352 or 337, respectively), the FeR_2OH^+ (*m*/*z* 385) species fragments readily losing methyl and methane (yielding *m*/*z* 370 or 354, respectively). The third difference between **1** and **2** refers to the attachment of a third ligand: thus, **1**, while very slowly and simultaneously losing a hydrogen atom, either attaches to FeR_2^+ (*m*/*z* 523) or to $[FeR_2OH{-}CH_3]^+$ (*m*/*z* 370) yielding *m*/*z* 525 for an OH group or *m*/*z* 526 for an OD group (when D_2O is added). Every triple ligation of **2** yields *m*/*z* 571 (i.e. has the $[FeR_3{-}H]^+$ composition).

All these processes were confirmed by MS/MS and deuterium-labeling measurements. On the basis of these results, it follows that both the metal ion attack and the fast subsequent second ligation yielding FeR_2^+ take place at the oxygen atom of the nitroxide because both steps involve negligible fragmentation or loss; these processes seem to start with attachment of the third ligand (i.e. with the OH and R addition). Actually, the presence of water is responsible for most of the product ions observed in the ligation of **1**. The FeR_2^+ ion of *m*/*z* 368, when mass selected under minimal water presence, slowly yields *m*/*z* 156, 352, 385 and 523 with the ion of *m*/*z* 385 being the most abundant (Fig. 3). Its main product ion, *m*/*z* 371^+, that arises from loss of non-labeled methyl from *m*/*z* 386 (D_2O added), if mass selected, yields (in descending order) ions of *m*/*z* 156, 352, 368, 355 and 526 where the presence of the latter two indicates that the label remains intact in the corresponding loss processes of methyl and hydrogen atom, respectively (Fig. 4). All these suggest that the attack of the third radical ligand on the already crowded iron ion, while it probably results in addition, also causes substantial rearrangement and provokes charge-exchange and fragmentation reactions instead. We were not able to select the FeR_3^+ species for an MS/MS experiment. Therefore, we consider that they, like the monocoordinated species, may also be of transient nature and prone to fragmentation. Because we observe that the third ligand addition requires loss of a hydrogen atom from the complex, its binding seems to proceed by a mechanism that involves

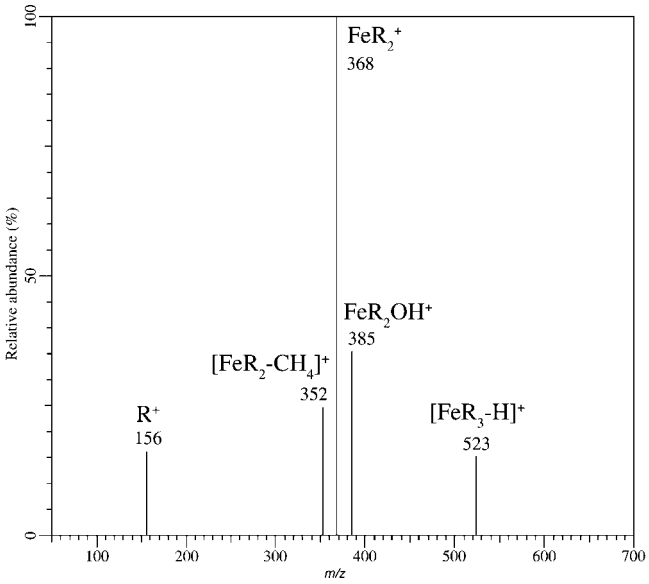


Figure 3. LDI FTMS of mass-selected *m/z* 368, FeR_2^+, R = TEMPO radical **1** after 15 s reaction time.

insertion of the metal into one of its bonds. When **2** is the ligand, the loss of hydrogen, methyl and methane from both the FeR_2^+ and FeR_3^+ species involves some of the label, thus pointing to possible rearrangements within the complexes; however, no addition of an OH group from water is ever observed (Fig. 2) despite its presence in the gas phase.

Thus, the experimental results show that the ligation of $^{56}Fe^+$ ions by TEMPO radicals **1** and **2** yields highly unstable single-addition complexes that are prone to charge exchange; the formation of products with two ligands is preferred and is influenced by the 4-hydroxy substituent of **2**. With **1**, both

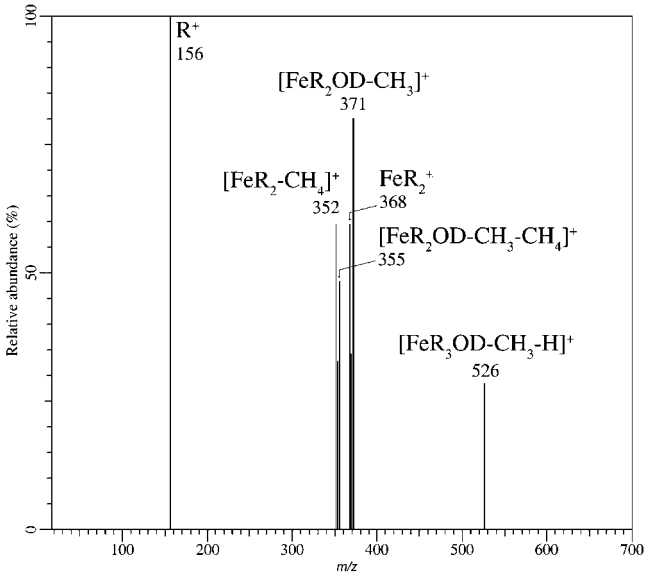


Figure 4. LDI FTMS of mass selected *m/z* 371, ($[FeR_2OD - CH_3]^+$, R = TEMPO radical **1** after 5 s reaction time (D_2O was introduced directly into the ICR cell before measurement).

ligands bind, without loss, at their nitroxide group oxygens whereas, with **2**, binding at the 4-hydroxy group with expulsion of the OH hydrogen atom also occurs. Thus, three different bisligated products (i.e. with loss of two, one or zero hydrogen atoms) are formed. Addition of a third ligand destabilizes the complexes and extensive fragmentation ensues.

Calculations show that the attack by Fe^+ on the OH group in **2** is not energetically preferred over an attack at the NO site. However, the energy differential is small and the former attack is certainly preferred sterically. Once attack occurs at the OH site, calculations show that the H-radical loss precludes the choice of direct addition, thus partially explaining our finding. It is possible, however, that purely electronic energy considerations are inadequate and that vibrations may also play a role.

CONCLUSIONS

It is shown that the TEMPO radicals **1** and **2** react with Fe^+ ions in the gas phase to produce rather unstable transient 1:1 adducts which either decompose by charge exchange (electron transfer) or stabilize by addition of a second TEMPO radical. While binding to **1** occurs exclusively at the nitroxyl oxygen, binding to **2** induces a competition with alternative binding at the hydroxyl oxygen after loss of an H-radical. Binding of a third ligand in both cases is slow and takes place along with fragmentation of that ligand. The addition of OH from traces of water was observed in **1** only and it led to ligand addition and ligand fragmentation. Calculations indicate a preference of an H-loss mechanism over simple addition at the OH site; however, the higher yield of this reaction over addition at the nitroxyl oxygen seems to be a result of steric hindrance in the latter. A structure for such FeR_2^+ ions is proposed.

Acknowledgements

Funded by the Ministry of Science, Education and Sports of Republic of Croatia (Grant number: 098-0982915-2945).

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