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The effects of intramolecular hydrogen bonding on the reactivity of phenoxyl radicals in model systems

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ABSTRACT

The effects of hydrogen bonding and spin density at the oxygen atom on the gas-phase reactivity of phenoxyl radicals were investigated experimentally and theoretically in model systems and the dipeptide LysTyr. Gas-phase ion-molecule reactions were carried out between radical cations of several aromatic nitrogen bases with the neutrals nitric oxide and *n*-propyl thiol. Reactivity of radical cations **4–6** correlated with the spin density. The possibility of hydrogen bonding was explored in compounds which allowed four-, five-, and six-membered ring to be formed between the protonated nitrogen and the phenoxyl oxygen, while possessing similar spin density at the oxygen atom. The N^+-H···O$^\bullet$ bond length was calculated to decrease in the series (**1–3**), consistent with the theoretical calculations finding weak hydrogen bonding in **2** and strong hydrogen bonding in **3**. This coincided with the decrease in reaction rates of **1–3** with both nitric oxide and *n*-propyl thiol. DFT calculations found that the lowest energy structure of the distonic radical cation of the dipeptide $[LysTyr(O^\bullet)]^+$ has a short hydrogen bond between the protonated Lys side chain and the phenoxyl oxygen, 1.70 Å, which is consistent with its low reactivity.

1. Introduction

Q3 Free radicals play key roles in protein chemistry due in part to their ability to transform substrates within the active sites of enzymes [1,2]. Radical sites in proteins are normally found on reactive amino acid side chains, of which, tyrosine phenoxyl radicals are among the most prominent (Scheme 1, **A**). These tyrosyl radicals are thought to be important intermediates in the action of the enzyme Class I Ribonucleotide Reductase (RNR) of *Escherichia coli* [3–5], production of oxygen in photosystem II [6–8], and the oxidation of peroxides in cytochrome C oxidases [9]. They have also been implicated in radical-induced protein damage, and are the precursors of various post-translational modifications (3,3′-dityrosine, 3-nitrotyrosine, and tyrosine-cytosine cross-linking) [10–13].

With the abundance of recent experimental data obtained via X-ray crystallography, high-frequency EPR, and ENDOR spectroscopy, which revealed structural information about local protein environments, it is widely accepted that tyrosyl radicals are often stabilized by hydrogen bonding [14–23]. Hydrogen bonding between a phenolic hydrogen and a properly oriented basic group such as histidine residue (Scheme 1, **B** [19]) modulates the formation and chemical behavior of the ensuing tyrosyl radical by changing redox potential of the tyrosine/tyrosyl radical pair [19,24–29].

There has been considerable interest in developing model systems to better understand the effects of hydrogen bonding on the properties of tyrosyl radicals [17,30–33]. Most of these systems incorporate both a phenol and a basic nitrogen atom in a close proximity to facilitate the formation of hydrogen-bonded phenoxyl radical, as shown for **C** in Scheme 1 [31]. Varying the substituents in the phenyl ring and the chemical surroundings of the nitrogen was shown to affect both redox potentials and the EPR signals [17,27,28,34,35].

Despite the success of solution-based approaches, there are several advantages of using mass spectrometry-based approaches to examine the fundamental gas-phase chemistry of relevant distonic ion model systems [36]. Apart from significantly reducing the time and sample quantity required for these studies, they shut down the possibility of radical self-termination reactions due to the coulombic repulsion of the charge sites. In addition, they reduce the overall

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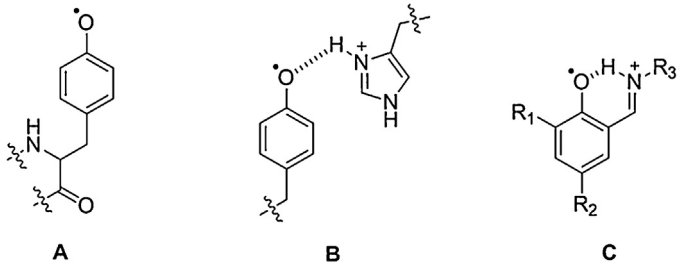
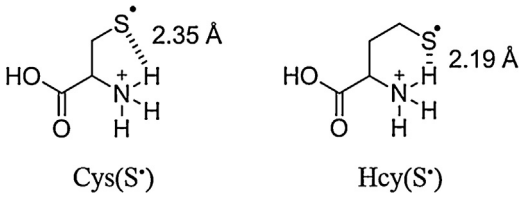


Scheme 1.



Scheme 2.

complexity of the system by limiting the chemistry of intramolecular interactions and avoiding complications from intermolecular hydrogen bonding with solvent molecules. Notwithstanding the recent renaissance of mass spectrometry-based studies of amino acid and peptide radical ions, the effects of hydrogen bonding on radical reactivity have not been widely studied. A rare example ascribed the differences between the gas-phase reactivity of distonic radical cations of cysteine and homocysteine to hydrogen bonding effects arising from the difference in the distances between the N-terminal hydrogen atom and the sulfur radical (Scheme 2) [37].

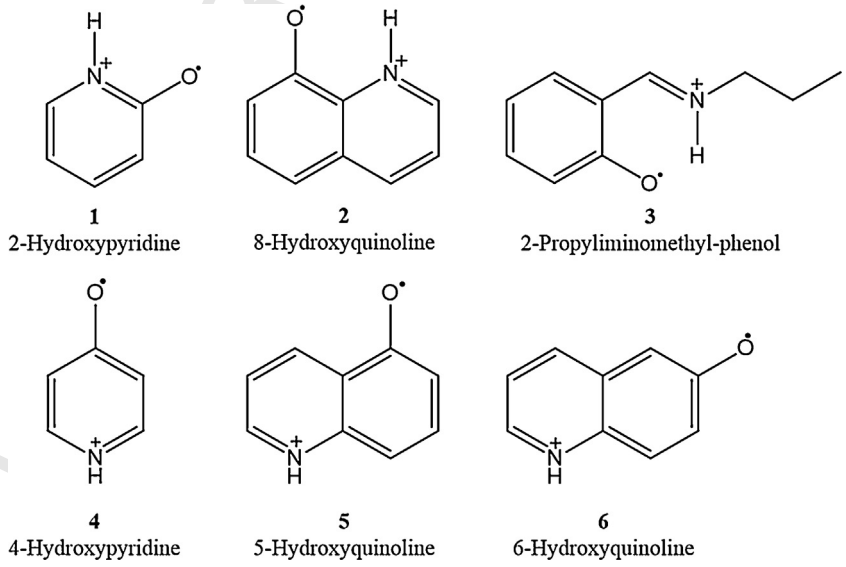
Mass spectrometry has been used to study gas-phase chemistry of tyrosyl radicals [38–40]. Siu and co-workers initially demonstrated the ability to form radical cations of peptides using the ternary copper (II) complex dissociation method in peptides containing tyrosine and an assisting basic amino acid [39]. This method was later utilized to form radicals and study a wide variety of tyrosine-containing peptides [40–43]. Covalent chemical modification of the tyrosine side chain and subsequent homolytic cleavage of a labile bond has been another productive route to formation of Tyr-based radical cations. This method has been successful in generating radical cations of iodotyrosine-containing peptides through photo-irradiation by Julian and co-workers [44,45]. They postulated that the initial phenyl carbon radical can quickly rearrange into the oxygen-based phenoxyl radical species [45,46]. However, because the tyrosyl radical easily loses its side chain in the gas phase, resulting in the captodatively stabilized glycine radical, forming and studying the oxygen-based radical cation of tyrosine is a challenge [47–49]. Siu and co-workers were able to form small amounts of the tyrosyl radical through collision-induced dissociation (CID) of $[Cu(Tyr)_2]^{2+}$ complex [47]. However, these ions dissociated rapidly to yield the p-hydroxybenzyl and p-cresol radical cations, which indicated the dissociation of the α–β bond and loss of the side chain [47]. Similarly, radical generation at tyrosine residues in peptides is known to result in characteristic side chain losses under mild CID conditions [48,50].

In this study, we circumvent this problem by using model nitrogen bases **1–3** (Scheme 3) that possess a phenoxyl radical site but lack facile elimination channels as seen with the loss of the Tyr side chain. Choosing the position of the nitrogen atom in the molecule allows us to explore the possibility and vary the extent of hydrogen bonding in the resulting radical cation. The effects of spin density at the oxygen atom on these reactions are also investigated using compounds **4–6**. We utilize gas-phase ion-molecule reactions (IMRs) to probe the reactivity of these radical species and density functional theory (DFT) calculations to complement the experimental data. We also examine the chemistry of the radical cation of the dipeptide, $[LysTyr(O^{\bullet})]^{+}$.

2. Experimental

2.1. Materials

All chemicals and reagents were used as received without any further purification. All model compounds, or their precursors, including 2-hydroxypyridine, 8-hydroxyquinoline, 4-methoxypyridine, 2-methoxypyridine, 6-methoxyquinoline, salicylaldehyde, and propylamine, were purchased from Sigma-Aldrich (Milwaukee, WI). The remaining reagents, $CuSO_4$, 2,2′:6′,2″-terpyridine, *n*-propyl thiol, potassium carbonate, dimethylformide (DMF), dimethylsulfate, diethylether, and sodium sulfate, were



Scheme 3.