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Formation of sugar radical cations from collision-induced dissociation of non-covalent complexes with *S*-nitroso thiyl radical precursors☆

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Dedicated to Prof. Ronnie Bierbaum on the occasion of her 65th Birthday and in recognition of her important contributions to gas phase ion chemistry and service to the American Society of Mass Spectrometry.

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ABSTRACT

A 'bio-inspired' method has been developed for generating sugar radical cations by multistage mass spectrometry (MS^4) experiments involving collision-induced dissociation (CID) of protonated non-covalent complexes between a sugar and an *S*-nitrosylated thiol amine, $[H_3NXSNO + M]^+$ (where $X = (CH_2)_2$, $(CH_2)_3$, $(CH_2)_4$, $CH(CO_2H)CH_2$ and $CH(CO_2CH_3)CH_2$). In the first stage of CID (MS^2), homolysis of the S–NO bond unleashes a thiyl radical to give the non-covalent radical cation, $[H_3NXS^{\bullet} + M]^+$. It was found that complexes containing *S*-nitroso cysteamine ($X = (CH_2)_2$) produced the most abundant radical cations for monosaccharides, while for larger sugars, the most abundant radical cations were generated from the *S*-nitroso derivatives of 3-amino-1-propanethiol ($X = (CH_2)_3$) and 4-amino-1-butanethiol ($X = (CH_2)_4$). CID (MS^3) of the radical cation complex resulted in the dissociation of the non-covalent complex to generate the sugar radical cation $[M^{\bullet}]^+$. Deuterium labelling studies reveal that this process involves abstraction of a hydrogen atom from a C–H bond of the sugar coupled with proton transfer to the sugar. The fragmentation reactions of the radical cation, $[M^{\bullet}]^+$, were studied by another stage of CID (MS^4). In this work, the scope of the method was established, particularly for the S–NO bond homolysis (MS^2) and $[M^{\bullet}]^+$ formation (MS^3) steps. Twenty-six different sugars were examined and radical cations could be generated for polysaccharides of varying lengths, as well as for the methyl pyranosides of a range of monosaccharides.

1. Introduction

Since the pioneering work of Finan et al. on the electron ionization (EI) of monosaccharides and small oligosaccharides [1,2], there have been numerous reports on the use of mass spectrometry to analyse sugars [3]. Electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) are now commonly used to generate even electron ions, which can then be subjected to CID to generate sequence ions (Scheme 1 shows the accepted sequence ion nomenclature [4]). In contrast, the use of gas phase radical ion chemistry to direct the fragmentation of sugars has lagged behind. Exceptions include the use of electron capture dissociation (ECD) and electron transfer dissociation (ETD), which result in radical-directed cleavages in oligosaccharides [5]; formation of radical ions by electron detachment dissociation (EDD) and the related negative electron transfer dissociation (NETD) method [6] or photo-activated electron detachment [7]; use of Siu's method [8] to form radical anions by dissociation of a ternary metal complex containing the carbohydrate [9]; and extension of Beachamp's free radical initiated peptide sequencing (FRIPS) method [10] to oligosaccharides [11].

Here, we report a new "bio-inspired" approach to generating radical cations of sugars in the gas phase. Nature exploits radical chemistry by certain enzymes to transform carbohydrate-based substrates [12]. A well-studied example involves the transformation of the sugar of RNA to DNA by the cysteinyl radical within ribonucleotide reductases. A key step in the overall transformation involves intermolecular H atom abstraction (HAT) from **1** to give radical **2** (Scheme 2) [12].

The fact that the reaction shown in Scheme 2 proceeds within a non-covalent complex inspires a MS approach that utilizes a charged non-covalent complex between a sugar and a radical to generate a charged sugar radical. The rational design of such a non-covalent complex requires: (i) ready transfer to the gas phase of a charged non-covalent complex containing a sugar and a radical precursor; (ii) formation of the radical cation non-covalent complex by unleashing the radical site from the precursor in preference to dissociation of the non-covalent complex; and

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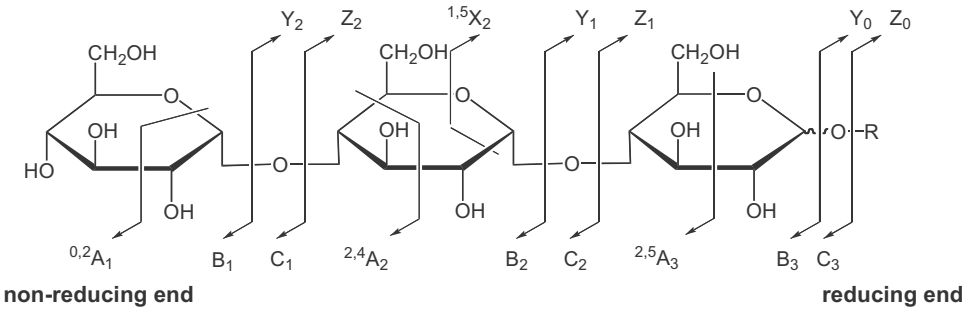


Scheme 1. Oligosaccharide sequence ion nomenclature used to assign fragment ions in mass spectrometry experiments.

(iii) transfer of the radical and charge sites to the sugar upon dissociation of the radical cation non-covalent complex.

Regarding requirement (i), there are numerous reports that demonstrate that ESI readily affords non-covalent complexes of sugars, particularly those involving ammonium ions, $[M + RNH_3]^+$ [13]. Requirement (ii) can be met by a consideration of bond dissociation energies to unleash radical sites [14] versus binding energies of non-covalent complexes [15]. An ideal radical precursor is *S*-nitrosocysteine, since it has a weak S–NO bond (BDE ~113 kJ mol^{-1}) [16]. Indeed, we have been able to cleanly generate thiyl radicals of cysteine (Eq. (1), where X = $CH(CO_2H)CH_2$), its derivatives and peptides to study their gas phase structure and fundamental bimolecular and unimolecular reactivity [17]. Aside from the example of ribonucleotide reductase discussed earlier (Scheme 2), there is evidence in the solution phase literature that shows requirement (iii) can be met, since thiyl radicals have been shown to abstract hydrogen atoms from carbohydrates [18].

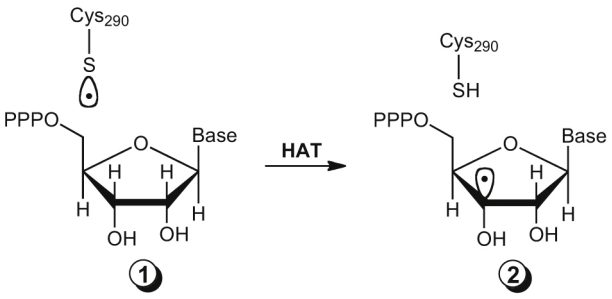
$$[H_3NXSNO]^+ \rightarrow [H_3NXS]^{\bullet +} + NO^{\bullet} \quad (1)$$

Recently, we have shown that all three requirements can indeed be met [19]. As a "proof of concept", the non-covalent complex between protonated *S*-nitrosocysteine and 18-crown-6 (18-C-6) was formed by ESI and shown to fragment via $NO^{\bullet}$ loss to produce the radical cation non-covalent complex (Eq. (2)). The subsequent CID spectrum of $[CysS + H + (18\text{-C-}6)]^{\bullet +}$ shows losses from the cysteine as well as from the crown ether ring, suggesting that intramolecular HAT can occur within the non-covalent complex.

$$[CysSNO + H + (18\text{-C-}6)]^+ \rightarrow [CysS + H + (18\text{-C-}6)]^{\bullet +} + NO^{\bullet} \quad (2)$$

Here, we explore the use of Siu's method (Eq. (3)) [8] and protonated *S*-nitrosothioamine non-covalent complexes for forming radical cations of sugars. The sugars and *S*-nitrosothioamines studied are shown in Scheme 3.

$$[Cu(typ)(M)]^{2+} \rightarrow [M]^{\bullet +} + [Cu(typ)]^+ \quad (3)$$



Scheme 2. Hydrogen atom transfer (HAT) from a sugar cysteinyl radical is implicated in the mechanism of ribonucleotidereductase, and provides motivation for a 'bio-inspired' approach to the generation of sugar radical cations.

2. Materials and methods

2.1. Materials

Cysteine, cysteine *O*-methyl ester, cysteamine, 3-amino-1-propanethiol, and *tert*-butyl nitrite were purchased from Sigma–Aldrich Chemical Co. and used as received. 4-Amino-1-butanethiol was synthesized by Otava Chemical Co. (Ontario, CA). The sugars: methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, D-glucose, methyl α-D-galactopyranoside, methyl β-D-galactopyranoside, D-galactose, methyl α-D-mannopyranoside, methyl β-D-mannopyranoside, D-mannose, D-mannitol, phenyl β-D-glucopyranoside, 4-hydroxyphenyl β-D-glucopyranoside, *myo*-inositol, L-arabinose, D-ribose, D-xylose, methyl β-cellobioside, cellobiose, trehalose, lactose, sucrose, maltotriose, maltotetrose, maltopentose, maltohexose, and maltoheptose were purchased from Sigma–Aldrich Chemical Co. and Carbosynth Ltd. (Compton, UK) and used as received.

The $[Cu(tpy)(M)]^{2+}$ complexes were formed by mixing the (2,2′:6′,2″-terpyridine)copper(II)nitrate monohydrate complex ($[Cu(II)(tpy)(NO_3)_2]\cdot H_2O$) [8b] with the sugar of interest (M) in a 1:1 ratio in 50/50 methanol/water with 1% acetic acid. The solution was then diluted to 50 μM.

The *S*-nitroso derivatives were generated by allowing a 1.5:1 mixture of *tert*-butyl nitrite and a 1 mM solution of the thiol (in 1:1 methanol:water with 1% acetic acid) to react for 10 min at room temperature. The reaction mixture was then diluted 100-fold using 50/50 methanol:water with 1% acetic acid.

2.2. Mass spectrometry

Complexes containing the sugar non-covalently bound to the *S*-nitroso derivative were made by mixing 2 equivalents of the sugar with 1 equivalent of the *S*-nitroso derivative in 1:1 methanol: water with 1% acetic acid to give a final concentration of 1 μM.

Electrospray ionization (ESI) mass spectra were collected using a Thermo Scientific LTQ-FT-ICR hybrid mass spectrometer (Bremen, Germany), consisting of a linear ion trap coupled to a 7T FT-ICR mass spectrometer. Analyte solutions were injected onto the ion trap mass spectrometer using a syringe pump with a flow rate of 5.0 μL min^{-1}. The instrument settings were optimized for maximum $[H_3NXSNO + M]^+$ signal intensity by using the auto-tune routine within the Tune program. The spray needle voltage, nitrogen sheath gas flow rate, and the capillary temperature were maintained at +5 kV, 5 arbitrary units, and 200 °C, respectively. Helium gas was used as the collision gas for CID experiments.

CID studies were performed by mass selecting the precursor ion and subjecting it to collisional activation. The normalized collision energy (RCE), which sets the amplitude of the RF energy applied to the end-cap electrodes, was set between 16 and 18% (arbitrary units) depending on the complex. The activation *Q* was set at 0.25