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Radical mediated dissection of oligosaccharides

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ABSTRACT

Radical chemistry continues to play an increasingly important role in tandem mass spectrometry based experiments on biomolecules. Oligosaccharides represent a very important class of target molecules that require structural characterization in terms of both monosaccharide identity and overall connectivity. Herein, two methods that generate radical oligosaccharides for subsequent activation are described. In one approach, a radical precursor is covalently attached to the oligosaccharide by reductive amination. Radicals can then be generated by homolytic bond cleavage of specific carbon–iodine bonds in protonated systems by either collisional activation or photodissociation. Subsequent activation of the radical species generates information rich spectra including numerous cross-ring fragments. Alternatively, noncovalent complexation with iodophthalic acid can be used to generate radical disaccharides by photoactivation. Subsequent radical transfer, loss of the radical precursor adduct, and collisional activation of the radical disaccharide results in characteristic glycosidic bond cleavage and cross-ring cleavage products that can easily distinguish isomeric species. Radical chemistry is demonstrated to have several advantages for the characterization of oligosaccharides relative to other approaches, including the identification of isomeric molecules of various sizes or analysis of various charge states.

1. Introduction

Tandem mass spectrometry is a fast, accurate, and sensitive approach for structural characterization of oligosaccharides. Various ionization and dissociation methods have been used to obtain structural information from oligosaccharides including the compositions of substructures, the linkage positions, and even the epimeric nature. Collision induced dissociation (CID) is the most commonly used way to produce tandem mass spectra for oligosaccharides [1–6]. Low energy CID of even electron oligosaccharide ions generally results in abundant glycosidic bond cleavage but cross-ring cleavage can be limited. The advantage of cleavage at glycosidic bonds is that the generic monomeric substructure is revealed; however, linkage site information is often lost. In contrast, cross-ring cleavage is frequently observed in many activated ion dissociation methods including electron capture dissociation (ECD) [7,8], electronic excitation dissociation (EED) [9], electron transfer dissociation (ETD) [10,11], electron detachment dissociation (EDD) [12] and high energy photodissociation [13–15]. Odd-electron radical species and fragments are generated in most of these methods, which facilitate homolytic bond cleavage and ring-opening. For example, ECD of multiply charged oligosaccharides generates charge reduced radical species. Compared with even-electron methods, radical directed dissociation is more likely to initiate cross-ring cleavage because cleavage of one bond frequently yields an equally reactive radical fragment as one of the products that can easily initiate a second bond fracture.

Direct capture of an electron in multiply charged molecules (e.g., peptides, proteins, saccharides) generates a hydrogen abundant species, which can convert to hydrogen deficient radicals [16]. Hydrogen deficient radical species can also be generated directly by homolytic bond cleavage. Various methods have been used to create hydrogen deficient radical peptides in the gas phase [17–21]. Subsequent radical migration and dissociation of peptides can provide structural information such as identification of isomeric residues [22] or investigation of peptide conformation [23]. In comparison, generation and dissociation of radical oligosaccharides by direct bond cleavage has been less studied. There are several advantages for studying oligosaccharide fragmentation with hydrogen deficient radical chemistry. First, the initial radical site can be controlled if the radical is generated by homolytic bond cleavage from a known position, providing an avenue for fragmentation mechanisms to be examined more easily. Second, most carbon atoms in oligosaccharides can donate a hydrogen atom and become free radical sites, which facilitates radical migration and increases the

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potential for backbone cleavage and generation of cross-ring fragments. Third, unlike ECD and ETD, singly charged ions can also be analyzed because generation of radicals does not involve charge reduction. Recently, Beauchamp and co-workers utilized collisional activation of TEMPO based radical precursors to generate radicals in covalently modified oligosaccharides [24]. Glycoside bond cleavage as well as two types of cross-ring fragments, $^{1,5}X$ and $^{0,2}X$, were observed in this study, suggesting that radical chemistry may be advantageous for the study of oligosaccharides.

Herein, we have developed two radical directed dissociation methods for characterizing oligosaccharides based on photodissociation mass spectrometry. Radicals are introduced to saccharides by covalent derivatization or noncovalent attachment with highly reactive phenyl radical precursors. The radical precursors used in this study are iodoaniline or iodophthalic acid based, but both have a carbon–iodine bond on the benzene ring. The benzene ring serves as a UV chromophore to absorb a photon, leading to homolytic dissociation of the carbon–iodine bond and generation of a highly reactive radical. The phenyl radical can subsequently migrate to other positions and initiate radical directed dissociation (RDD) of the molecule. More types of fragmentation are generated by RDD compared with CID of protonated species, which provides more information about structure. For example, positional isomers of fucopentaose can be easily distinguished based on RDD mass spectra. In addition, $^{1,5}X$ ions are the dominant cross-ring fragments generated in RDD. $^{0,2}X$ ions are also observed with a lower abundance.

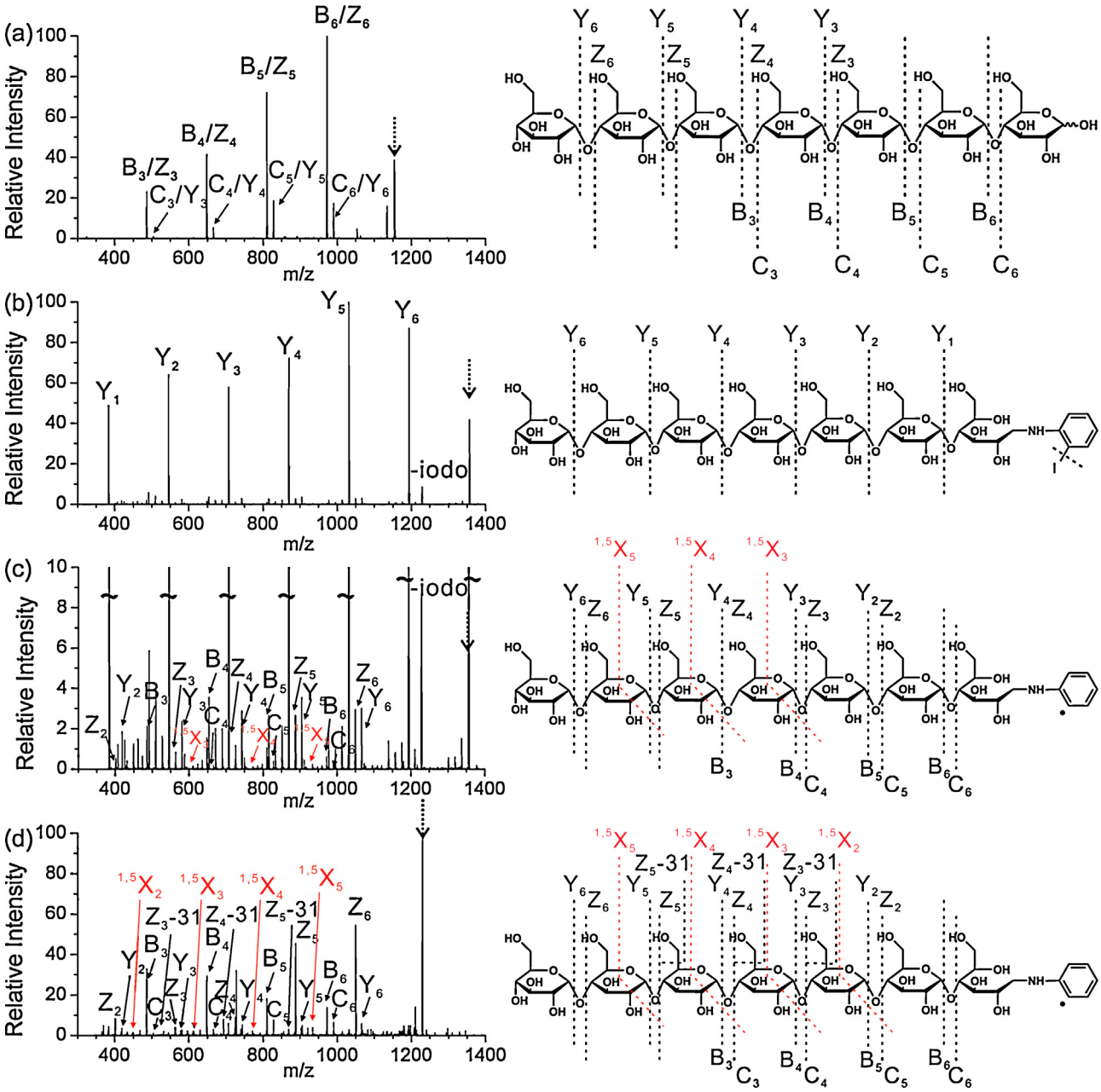

Fig. 1. (a) CID of protonated maltoheptaose. Assignment of peaks is ambiguous due to symmetry. (b) CID of protonated maltoheptaose modified with 2-iodoaniline. (c) Zoom in of Fig. 1(b), X, Y, Z fragments are assigned based on radical precursor with the structure shown on the right. (d) CID of protonated radical maltoheptaose, which was generated by photodissociation of 2-iodoaniline modified maltoheptaose.