Rapid Identification of Disaccharides by Tandem Mass Spectrometry

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Carbohydrates are the important class of biological molecules. The wide variety of positional and anomeric structures makes it possible for saccharides to form plenty of compounds. It is well known that oligosaccharides attached to proteins or lipids (forming glycoconjugates) and provide important biological functions. Furthermore, oligosaccharide units can also be found in flavonoid glycosides, and their mass spectrometric behaviors have been investigated in detail. The function of the disaccharides is strongly influenced by their structures. Therefore it is necessary to determine the stereochemistry of the monosaccharide units, the linkage position and anomeric configuration of the isomeric disaccharides. Tandem mass spectrometry combined with soft ionization methods such electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) is an effective tool for the structural analysis of oligosaccharides. Determination of the linkage position and anomeric configuration has been demonstrated both in the negative and positive ion modes. For protonated oligosaccharides cleavage occurs almost exclusively at the non reducing side of the glycosidic oxygen (referred as B and Y-type ions). For alkali metal adducts cross ring fragmentation can also be observed besides the abundant B- and Y-type ions. The effect for the destabilization of the glycosidic bond is decreasing with the size of the cations. Hence higher energy required to fragment the disaccharides cationized by larger ions. In the negative ion mode the collision induced dissociation (CID) spectra of the deprotonated disaccharides show distinguishable fragmentation patterns with C- and A type ions for the different isomeric and anomeric configurations. Moreover, derivatization (e.g. permethylation) can be applied to increase the extent of fragmentation, and negative ion adducts are also used for the analysis of disaccharides Glucose disaccharides varying solely in their anomeric configuration were clearly distinguished on the basis of the relative abundance of the characteristic product ions arising from the cleavage of the glycosidic bond of the lithiated adduct ion at *m/z* 169 and 187 using wavelength-tunable infrared multiple-photon dissociation-mass spectrometry. Very recently, Domingues et al. proposed that the ratio of *m/z* 169/187 for $[M + Li]^+$ ions and *m/z*

185/203 for $[M+Na]^+$ ions seems to be a simple criterion for the differentiation of isomeric hexose disaccharides with β-(1→4) linkage but distinct monosaccharide composition.

In this work, we have examined the use of ESI-MS/MS in positive ion mode of the lithiated, sodiated and ammoniated adducts for the differentiation of four glucose disaccharides: maltose (Glcα1-4Glc), cellobiose (Glcβ1-4Glc), isomaltose (Glcα1-6Glc) and gentiobiose (Glcβ1-6Glc). According to our best knowledge, no detailed report on the fragmentation of the ammoniated adducts of the glucose disaccharides has been issued. Our goal was to define simple criteria for distinguishing between the four disaccharides. Herein we also report a highly automated data acquisition and processing method for the identification of these disaccharides by ESI-MS/MS thereby considerably reducing the analysis time.