Differentiation of Stereochemistry of Glycosidic Bond Configuration: Tandem Mass Spectrometry of Diastereomeric Cobalt–Glucosyl-Glucose Disaccharide Complexes

Glenn Smith and Julie A. Leary

Department of Chemistry, University of California, Berkeley, California

Configurations of glycosidic linkages (α or β) in a series of 1,3-, 1,4-, and 1,6-glucosyl-glucose disaccharides were differentiated by tandem mass spectrometry. Diastereomeric octahedral complexes, $[Co^{+3}(acac)_2/disaccharide]^+$, were generated in situ via fast-atom bombardment ionization. Mass-analyzed, ion kinetic energy spectra of the metastable complexes obtained in the absence of collision gas indicated that the major product ion results from the loss of an acetylacetonate ligand, which thus generates the ion $[Co^{+2}(acac)/disaccharide]^+$. Kinetic energy release measurements for this dissociation display a consistently greater value for complexes that possess an α-linked disaccharide relative to those that possess β-linked disaccharides, regardless of linkage position. *(J Am Soc Mass Spectrom 1996, 7, 953–957)*

The characterization of glycosidic bonds in carbohydrates is essential if one wishes to predict the overall structure and function of these ubiquitous molecules. There are several sites for glycosidic bond formation on all monosaccharides and two possible orientations (α and β) of the glycosidic bond at each linkage position in the larger oligomers [1]. The different types of glycosidic bonds result in different orientations of two connected hexose units. Therefore, knowledge of only the monosaccharide sequence in carbohydrates is insufficient for structural analysis. Carbohydrates that possess the same monosaccharide sequence, but different glycosidic bond linkages, may possess significant differences in structure as well as function.

Elucidation of the types of glycosidic bonds present in a carbohydrate traditionally has been achieved through chemical, enzymatic, or NMR techniques [2]. However, these techniques have the major disadvantage that they require large quantities of highly purified analyte. Additionally, NMR analysis typically requires many hours of instrument time. Mass spectrometry presents a technique with sensitivity that is generally many times greater than that of the traditional procedures and data acquisition usually can be performed in minutes, which lowers the demand for analyte and instrument time. Also, low sample purity need not hinder carbohydrate analysis by mass spectrometry. Various tandem mass spectrometry experiments allow for the selective analysis of ions produced from the molecule of interest, while those due to sample impurities are ignored [3].

Traditionally, mass spectrometry has not been thought of as a technique that can be used to differentiate stereoisomers; such differentiation has long been the domain of NMR spectroscopy. However, many reports have demonstrated the capability of mass spectrometry to differentiate molecules that possess different stereochemical features [4]. Indeed, several studies have attempted to characterize the glycosidic bond in a series of glucosyl-glucose disaccharides via mass spectrometry. The use of negative ion fast-atom bombardment (FAB) in conjunction with either collision-induced dissociation tandem mass spectrometry (CID-MS/MS) or metastable ion dissociation tandem mass spectrometry has shown some success in differentiation of both linkage position and glycosidic bond configuration in underivatized disaccharides [5, 6]. More recently, negative ion electrospray ionization–mass spectrometry (ESI-MS) also has been shown to be capable of characterization of glycosidic bonds in underiva-

Address reprint requests to Dr. Julie Leary, Department of Chemistry, University of California, Berkeley, CA 94720.

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tized disaccharides [7]. However, positive ion mass spectrometry has not been as successful. Positive ion chemical ionization–mass spectrometry, desorption-electron ionization, and fast-atom bombardment–mass spectrometry (FAB-MS) of peracetylated disaccharides following collision-induced dissociation (CID) have shown only small differences in the product ion spectra of α- and β-linked disaccharides [8, 9]. Also, Dallinga and Heerma [10] have shown that positive ion fast atom bombardment tandem mass spectrometry (FAB-MS/MS) along with CID of underivatized disaccharides can provide linkage position information on the glycosidic bond, but again the glycosidic configuration could not be discerned. Hofmeister et al. [11] also demonstrated the ability of positive ion FAB-MS/MS of lithium-cationized disaccharides in the determination of linkage position, although no differentiation of glycosidic bond configuration was mentioned. Despite its previously reported lack of sensitivity toward the configuration of the glycosidic bond, this work will demonstrate that positive ion tandem mass spectrometry is capable of distinguishing between α- and β-linked disaccharides. Various disaccharides were combined with cobalt trisacetylacetonate [$Co(acac)_3$] and the resulting solutions were analyzed by FAB-MS. Octahedral complexes that resulted from the substitution of a disaccharide onto $Co(acac)_2$ were detected and chosen for subsequent mass-analyzed ion kinetic energy (MIKE) spectrometry and kinetic energy release (KER) studies. A similar methodology was reported to be capable of distinguishing enantiomeric dialkyl tartrates [12].

Experimental

Instrumentation

Fast-atom bombardment ionization mass spectrometry (FAB-MS) was performed using a ZAB2-EQ mass spectrometer (Fisons/VG Analytical, Manchester, UK) of BEoQ geometry. (o is an rf-only octapole collision cell fabricated in our laboratory and it replaces the conventional quadrupole in this particular instrument.) This instrument is equipped with three reaction regions. Only the second reaction region, which is used for MIKES scanning, was utilized.

The FAB-MIKES spectrometry studies were carried out in the second reaction region between the magnet (B) and the electric sector (ESA). Spectra were obtained by using an 8-kV accelerating voltage. The precursor ion was transmitted into the second field-free region and allowed to undergo unimolecular decomposition at a pressure of 1×10^{-8} torr (no collision gas was added). The MIKES cell was at ground potential. The product ion spectra were generated by scanning the ESA while the magnet was set to pass only the mass of the precursor ion. The product ion spectra resulted from the signal averaging of 20–25 scans.

Kinetic Energy Release Determination

The KER values were calculated according to the following equation [13]:

$$\mathrm{KER} = \frac{y^2 m_1^2 E}{16 m_2 m_3}\left(\frac{\Delta E}{E}\right)^2 \quad (1)$$

where m_1 is the mass of the precursor ion, m_2 is the mass of the product ion, m_3 is the mass of the neutral loss, y is the charge of the product ion, E is the accelerating voltage (8000 kV); $\Delta E = [\Delta E_{50\%}^2 - \Delta E^2(m_1)]^{1/2}$, $\Delta E_{50\%}^2$ is the width (in volts) of the product ion peak at half height, and $\Delta E^2(m_1)$ is the width (in volts) of the precursor ion peak at half height. All half-height widths were measured from the signal-averaged product ion spectra. The precursor ion peak was not allowed to saturate the detector to avoid overestimation of $\Delta E^2(m_1)$.

All MIKE spectra underwent smoothing and background subtraction prior to KER determination. Because of the dependence of the KER values upon the accurate measurement of peak widths and heights, alterations to the peak shape that resulted from data processing were avoided. To ensure that excessive peak shape deformation did not occur, all processed data were compared carefully to the raw data. Figure 1 presents a portion of a typical raw and processed signal-averaged MIKE spectrum.

Reported KER values result from the averaging of eight different measurements obtained under identical experimental conditions. Standard deviations were calculated for the mean KER values to determine the precision with which the measurements were obtained. All standard deviations were less than 4% of the mean KER value.

High-Resolution Mass Measurements

High-resolution FAB-MS was performed on all precursor ions prior to MIKE spectrometry studies to determine the presence of isobaric ions. Accurate mass measurements were made at a resolution of 1:10,000 by peak matching the m/z 599 ion against the internal standard β-casomorphine fragment Tyr-Pro-Phe-Pro-Gly · HCl (m/z 580.2771).

Chemicals and Solutions

Solutions for FAB-MS were prepared by mixing 3 μL of 0.047 M (aqueous) disaccharide, 1 μL of 0.025 M (methanol) Co(acac)3, and 3 μL of neat DTT/DTE (a 3:1 mixture of dithiothreitol/dithioerythritol) as matrix compound. Final concentrations of the disaccharide and Co(acac)3 were 20 and 3 nmol/μL, respectively. Approximately 1.5 μL of the sample mixture was

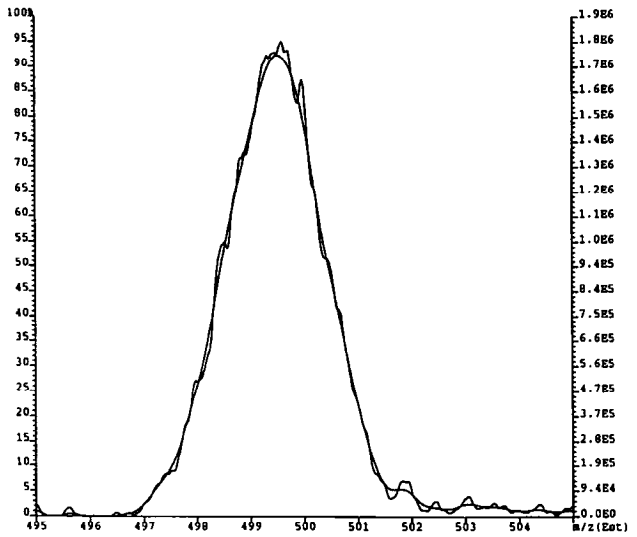

Figure 1. Overlay of raw and processed partial MIKE spectra of the metastable $[Co^{+3}(acac)_2/maltose]^+$ (m/z 599) generated from a mixture of $Co(acac)_3$ and maltose with DTT/DTE matrix. The product ion at m/z 500 represents $[Co^{+2}(acac)/maltose]^+$ for which KER measurements were made.

deposited onto the FAB probe tip. The sample was ionized with 20-kV Cs ions at a current of ~ 1.0 μA.

Laminaribiose, nigerose, gentiobiose, maltose, and isomaltose were obtained from Sigma Chemical Co. (St. Louis, MO). Cellobiose was obtained from ICN Biomedical, Inc., Costa Mesa, CA. Dithiothreitol, dithioerythritol, and cobalt trisacetylacetonate were obtained from Aldrich Chemical Co. (Milwaukee, WI). All materials were used as received without further purification.

Results and Discussion

Six α and β isomeric glucose disaccharides (1,3-linked nigerose and laminaribiose, 1,4-linked maltose and cellobiose, and 1,6-linked gentiobiose and isomaltose) were dissolved with $Co(acac)_3$ in methanol and the in situ mixture was analyzed by FAB ionization. Nigerose and laminaribiose also were analyzed by electrospray ionization (ESI). In addition to other ions formed, one ion that corresponded to $[Co(acac)_2/disaccharide]^+$ was generated in the mass spectrometer and subjected to unimolecular MIKE spectrometry. Kinetic energy release values then were measured for the ensuing product ion that corresponded to $[Co(acac)/disaccharide]^+$.

Before presenting the data for this study, a brief explanation of the charge states of the metal and ligands is obligatory. The $Co(acac)_3$ as purchased and used is a neutral compound: that is, each acetylacetonate (acac) ligand carries a -1 charge while cobalt is in the $+3$ oxidation state. All of the disaccharides studied are neutral molecules. Thus for the octahedral complex of interest, $[Co(acac)_2/disaccharide]^+$ observed at m/z 599, Co is still $+3$, each acac ligand is -1, and the disaccharide is neutral, which thus provides an ion with an overall $+1$ charge.

Low- and High Resolution Fast-Atom Bombardment–Mass Spectrometry

Low resolution FAB mass spectra were obtained for each of the six $Co(acac)_3$/disaccharide solutions. Figure 2 is a representative spectrum collected for the $Co(acac)_3$/maltose mixture and Table 1 lists the compositions of the major ions detected. Low resolution mass spectra of the other $Co(acac)_3$/disaccharide mixtures were identical to that shown in Figure 2 except for small differences in relative abundances of some ions. As can be seen from the data, various oxidation states of cobalt exist with differing acac ligand substitution patterns. The most interesting ion in terms of stereochemical differentiation is m/z 599, which represents the six-coordinate octahedral configuration of cobalt with two acac ligands and one disaccharide. This complex is equivalent to that studied previously in our tartrate experiments [12].

Before the unimolecular MIKE experiments and KER measurements were performed, high resolution mass measurements were conducted. Exact mass measurements were made to identify the precursor ion unambiguously at m/z 599 and to establish its purity; that is, to eliminate the question of isobaric ions that might interfere with the KER measurements. The ion generated from the laminaribiose complex was measured at 1:10,000 resolution and experimentally determined to be 599.1390. This value is -0.4-millimass units (mmu) deviation from the theoretical value of 599.1386 and corresponds to $C_{22}H_{36}O_{15}Co$. Assignment of m/z 599 as $[Co(acac)_2/laminaribiose]^+$ was thus supported by the high-resolution data. Signals at m/z 599 appeared

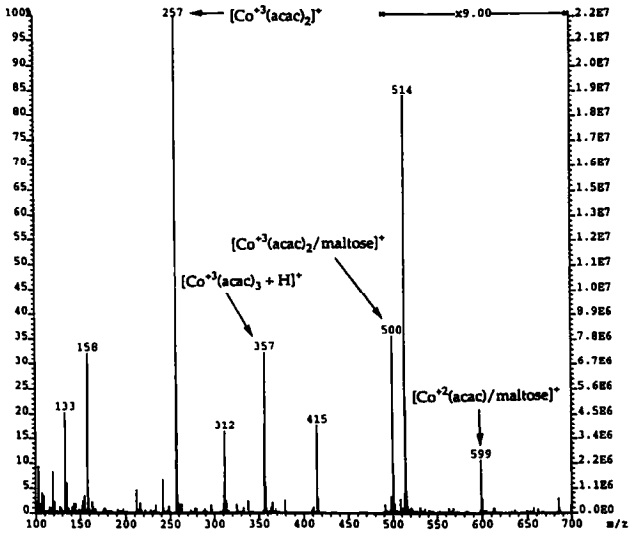

Figure 2. FAB mass spectrum of a mixture of $Co(acac)_3$ and maltose with DTT/DTE matrix.

Table 1. Compositions of the major ions detected in the FAB mass spectrum of a mixture of $Co(acac)_3$ and maltose with DTT/DTE matrix (see Figure 2)

Mass-to-charge ratio	Composition
158	$[Co^{+2}(acac)]^+$
257	$[Co^{+3}(acac)_2]^+$
312	$[matrix + Co^{+2}(acac)]^+$
357	$[Co^{+3}(acac)_3 + H]^+$
415	$[Co_2^{+2}(acac)_3]^+$
500	$[Co^{+2}(acac)/maltose]^+$
514	$[Co^{+3}Co^{+2}(acac)_4]^+$
599	$[Co^{+3}(acac)_2/maltose]^+$

Table 2. KER values of the m/z 500 product ion for all six diastereomers analyzed

Disaccharide	Glycosidic linkage	KER (meV)[a]
Nigerose	α-1,3	48 ± 2
Laminaribiose	β-1,3	37 ± 1
Maltose	α-1,4	48 ± 1
Cellobiose	β-1,4	39 ± 1
Isomaltose	α-1,6	39 ± 1
Gentiobiose	β-1,6	31 ± 1

[a]KER values are mean values for nine experiments. Error values are standard deviations of the mean.

as single peaks for all complexes studied when measured at 1:10,000 resolution.

Fast-Atom Bombardment–Tandem Mass Spectrometry

The ions at m/z 599 generated for each of the six complexes were transmitted into the MIKES spectrometry cell and allowed to undergo unimolecular decomposition. Figure 3 is the resulting MIKE spectrum from the $[Co(acac)_2/maltose]^+$ complex. Each of the complexes produced identical MIKE spectra and the major ion observed at m/z 500, which resulted from the loss of an acac ligand, was used to measure the kinetic energy release from each of the complexes. The KER values measured for m/z 500 of all six complexes are provided in Table 2. As seen from Table 2, the KER values measured between α and β isomers of similar linkages were consistently *different*. Of equal importance is the fact that the complexes that contained the α isomer always produced higher KER values than the corresponding β isomer.

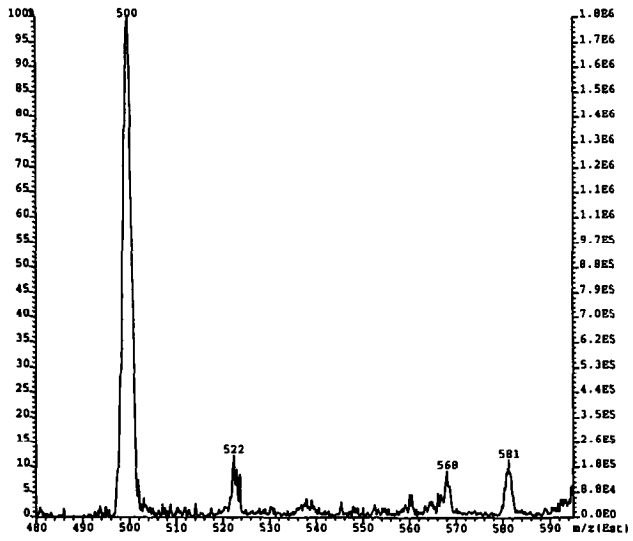

Figure 3. Partial MIKE spectrum of the metastable $[Co^{+3}(acac)_2/maltose]^+$ (m/z 599) generated from a mixture of $Co(acac)_3$ and maltose with DTT/DTE matrix. The ion at m/z 500 represents $[Co^{+2}(acac)/maltose]^+$ for which KER measurements were made.

The KER differences between the α and β isomeric complexes of the same linkage are significant. Because the standard deviations relative to the mean are so small (~ 4%), all of the reported values were reproducible and narrowly distributed around the mean value. Indeed, even when the distributions of the KER values are considered, the ranges of the KER values of $[Co(acac)_2/α\text{-disaccharide}]^+$ and $[Co(acac)_2/β\text{-disaccharide}]^+$ never overlap. The KER values for the two complexes are statistically distinct. Although differences in KER may reflect differences in internal energy [14, 15], it is also well known that the kinetic energy released in metastable ion transitions is less sensitive to internal energy and more sensitive to the structure of the reacting configuration and product ions [13]. Therefore, it is reasonable to pose that differences in the KER values observed here reflect differences in ion structures. In fact, recent work in our laboratory with similar octahedral $[Co(acac)_2/dialkyl\ tartrate]^+$ complexes has shown that KER values are sensitive to chirality differences between diastereomers [12]. It is not unreasonable to accept that the observed KER differences between the diastereomeric α and β complexes reflect actual structural differences. It is also conceivable, based on this data, that the α versus β configuration of the glycosidic bond dictates how the saccharide is substituted onto the octahedral cobalt complex and, therefore, how the complex dissociates.

Without detailed information on the geometry of how the disaccharide is complexed to the cobalt bis acetylacetonate, it is difficult to correlate the KER data with structural characteristics of the individual $Co(acac)_2$/disaccharide complexes. However, some factors can be inferred from the MIKE spectrometry and KER studies if a few basic assumptions are made. The first assumption is that the original six-coordinate octahedral geometry of $Co(acac)_3$ is retained after disaccharide substitution; that is, the disaccharide coordinates as a bidentate ligand. The second assumption is that greater steric crowding is present in one of the diastereomeric complexes and that this increased crowding is also present in the reacting configuration

that leads to the loss of an acac ligand. Dissociation of the complex to the four-coordinate product ion results in the relief of steric crowding. Internal energy of the reacting configuration that results from steric crowding will be converted at least partially into kinetic energy of the product ion upon dissociation of the complex. If this is the case, then the data suggest that greater KER values can be associated with greater ligand crowding in the α-linked disaccharide complex and, conversely, the lower KER values are indicative of less steric crowding in the β-linked disaccharide complex.

At the present time, these results demonstrate that diastereomeric glucosyl-glucose disaccharides can be differentiated based on their glycosidic bond configuration by using tandem mass spectrometry and KER measurements. Future and concurrent studies involve generation of these diastereomeric complexes by electrospray ionization as well as molecular modeling studies, which may provide useful information on how the disaccharide coordinates to the cobalt complex. It is anticipated that this methodology eventually may be adapted to higher molecular weight oligomers through the use of different metal–ligand complexes of different size and configuration.

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