Solvent Effects in Tandem Mass Spectrometry: Mechanistic Studies Indicating How a Change in Solvent Conditions and pH Can Dramatically Alter CID Spectra

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Dramatically different CID (collision-induced dissociation) spectra are obtained when the complex $[Zn(dien\text{-}glucose)]^+$ is electrosprayed from acidic and basic solutions. To understand this peculiar phenomenon, an in-depth mechanistic study was performed on one of the product ions that is present when the initial complex is diluted in basic solution but absent when the complex is diluted with acidic solution. On the basis of the results of this study, the differences in the CID spectra can be rationalized by the fact that the complex electrosprayed from basic solution was kinetically trapped, with the deprotonation site distal from the metal center. Under acidic conditions, the deprotonation site is at a hydroxyl group coordinated to the metal ion. A variety of experiments support this hypothesis. The studies herein underscore the importance of using identical solvent conditions when comparing sets of CID spectra. The data also highlight a very interesting phenomenon involving deprotonation of a hydroxyl group, which was several atoms away from the Zn(II) metal center.

Introduction

The use of MS/MS spectra for structural identification of biological molecules is now a commonly used practice. Protein sequencing is routinely achieved by using tandem mass spectrometry.[1−3] The technique is also used for DNA sequencing[4] and for the structural elucidation of drug metabolites,[5,6] lipids,[7] and carbohydrates.[8−10] The advantages of using tandem mass spectrometry for structural interpretation include very low sample consumption, the speed with which data can be collected and interpreted, and the ability to analyze a single product isolated from hair,[5] sludge,[6] or similar matrixes. In addition, when properly collected, the data obtained during tandem mass spectrometric experiments are highly reproducible. In fact, several groups have demonstrated that product ion abundances obtained during MS^n experiments can be used to quantify isomeric mixtures.[11−14]

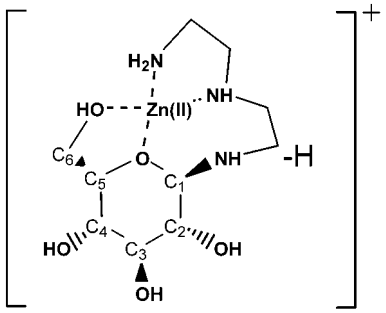

Figure 1. Depiction of the gas-phase $[Zn(dien\text{-}glucose)]^+$ complex.

Recently, we have discovered that the reproducibility of MS^n spectra in an ion trap mass spectrometer may not only be dependent on careful control of instrumental parameters such as tuning and activation conditions but also on the makeup of the electrospray solvent. When the complex $[Zn(dien\text{-}glucose)]^+$ (dien = diethylenetriamine; see Figure 1) is electrosprayed from a solution containing 10% ammonium hydroxide in methanol, it dissociates as reported previously when subjected to collision-induced dissociation (CID) experiments.[15] However, if this complex is electrosprayed from a solution containing 1% formic acid in methanol, a dramatically different CID spectrum is observed. The relative abundance of two product ions decrease substantially, and a third product ion disappears entirely. While many have demonstrated that changing the pH of a solution has changed the appearance of MS^1 spectra,[16−18] relatively few examples exist that illustrate the effect of changing solvent com-

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(1) Annan, R. S.; Carr, S. A. *Anal. Chem.* **1996**, *68*, 3413−3421.
(2) Zeng, C.; Spielman, A. I.; Vowels, B. R.; Leyden, J. J.; Biemann, K.; Preti, G. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 6620−6630.
(3) Deissler, H.; Wilm, M.; Genc, B.; Schmitz, B.; Ternes, T.; Naumann, F.; Mann, M.; Doerfler, W. *J. Biol. Chem.* **1997**, *272* (27), 16761−16768.
(4) Burlingame, A. L.; Boyd, R. K.; Gaskell, S. J. *Anal. Chem.* **1998**, *70*, 647R−716R.
(5) O'Kane, S. M.; Smyth, W. F. *J. Chromatogr., B: Biomed. Sci. Appl.* **2000**, *740* (2), 141−157.
(6) Berset, J. D.; Bigler, P.; Herren, D. *Anal. Chem.* **2000**, *72*, 2124−2131.
(7) Schneiter, R.; Grugger, B.; Sandhoff, R.; Zellnig, G.; Leber, A.; Lampl, M.; Kohlwein, S. D. *J. Cell Biol.* **1999**, *146* (4), 741.
(8) Weiskopf, A. S.; Vouros, P.; Harvey, D. J. *Anal. Chem.* **1998**, *70*, 4441−4447.
(9) Sheeley, D. M.; Reinhold, V. N. *Anal. Chem.* **1998**, *70*, 3053−3059.
(10) Gaucher, S. P.; Cancilla, M. T.; Phillips, N. J.; Gibson, B. W.; Leary, J. A. *Biochemistry* **2000**, *39* (40), 12406−12414.
(11) Desaire, H.; Sirich, T. L.; Leary, J. A. *Anal. Chem.* **2001**, *73*, 3513−3520.
(12) Zaia, J.; Costello, C. E. *Anal. Chem.* **2000**, *73*, 233−239.
(13) Seymour, J. L.; Turecek, F. *J. Mass Spectrom.* **2000**, *35*, 566−571.
(14) Tao, W. A.; Gozzo, F. C.; Cooks, R. G. *Anal. Chem.* **2001**, *73*, 1692−1698.
(15) Gaucher, S. P.; Leary, J. A. *Anal. Chem.* **1998**, *70*, 3009−3014.
(16) Wang, G.; Cole, R. B. *Org. Mass Spectrom.* **1994**, *29*, 419−427.

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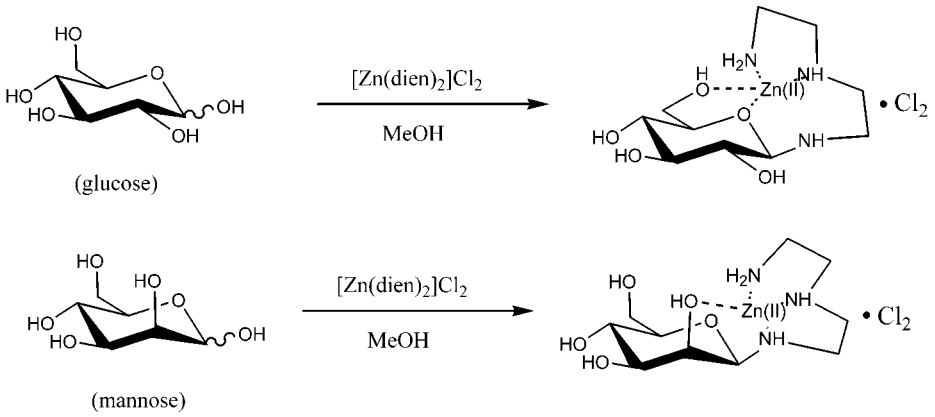
Scheme 1



position on MS/MS spectra.[19] This is a significant distinction since a change in the abundance of certain ions in MS can easily be attributed to a change in the ionization efficiency, or preference for a particular charge state, when the pH is altered. However, neither of these phenomena explains why the MS/MS spectrum would change for a given ion depending on the solution acidity or bacisity. This report illustrates the critical importance of using identical solvent conditions when comparing MS/MS spectra of diastereomers.

By studying how and why MS/MS spectra are altered when the acidity or bacisity of the electrospray solution is changed, we believe a better understanding of the process of collision-induced dissociation will result. Furthermore, this information will be applicable to those using tandem mass spectrometry to obtain structural information on compounds, such as carbohydrates, proteins, DNA adducts, lipids, and drug metabolites. Finally, because researchers often rely on the reproducibility of CID spectra to quantify isomeric mixtures, knowing that these spectra may be altered when the compounds originate in different solutions is important.

Experimental Section

Synthesis of the Metal−Ligand Complexes. The $[Zn(dien\text{-}hexose)]^+$ complexes were synthesized as described earlier.[15] Briefly, 0.5 mg of $[Zn(dien)_2]Cl_2$ was added to 0.5 mg of the monosaccharide in 50 μL of MeOH. The reaction was refluxed for 30 min at 70 °C. For mass spectrometric analysis, 1 μL of this solution was diluted to 500 μL with either MeOH (for "normal" conditions) or 500 μL of a solution containing 90% MeOH and 10% aqueous (0.7 M) ammonium hydroxide for "basic conditions". The solvent composition is 99% MeOH with 1% formic acid for acidic conditions. All the complexes were analyzed immediately after dilution. No changes were made to the synthetic procedure when the various glucose analogues were investigated. The $[Zn(dien)(OH)]^+$ complex forms readily during the electrospray process if $[Zn(dien)_2]Cl_2$ is dissolved in water and diluted to a 100 μM solution with 90% methanol.

Mass Spectrometry. All complexes were analyzed on a quadrupole ion trap mass spectrometer purchased from Thermo-Finnigan (San Jose, CA). They were infused directly via a syringe pump at 5 μL/min. Electrospray ionization was employed, using a spray voltage of 4.5 kV. Initially, the $[Zn(dien\text{-}glucose)]^+$ complex was tuned to maximize the signal for the ion of interest, *m/z* 328, and these tuning parameters were used for each subsequent experiment. Once isolated in the ion trap, collision-induced dissociation (CID) was carried out using the He gas present in the ion trap. All MS/MS experiments utilized an isolation width of 1 Da and end-cap voltages of 0.48−0.72 V. (All isomeric complexes were run under identical activation conditions.) For MS^3 experiments conducted on the chlorinated complex, *m/z* 364, an initial isolation width of 5 Da and an activation amplitude of 0.76 V were used for the MS^2 stage, and a subsequent isolation width of 1 Da and activation amplitude of 0.48 V were used for the MS^3 portion of the experiment.

For some experiments, the ion was allowed to remain in the ion trap for 50−5000 ms prior to activation. For these experiments, the instrument was programmed to perform an MS^3 experiment in which the MS^2 stage isolation width was set to 1 Da, the activation amplitude was 0.0 V, and the isolation time ranged from 50−5000 ms. The MS^3 conditions used an isolation width of 1 Da, an end-cap voltage of 0.48 V, and an activation time of 30 ms. For all other CID experiments, the activation time for each stage was 30 ms and q_z was maintained at 0.25 for all CID experiments.

Results and Discussion

When isomeric monosaccharides are reacted with $[Zn(dien)_2]Cl_2$ in methanol, the resulting product is an *N*-glycoside coordinated to Zn (Scheme 1). Previous studies have shown that CID of this compound results in unique product ion spectra for four stereoisomeric hexose monosaccharides.[15] However, we have recently discovered that spraying these complexes out of a solution containing 1% formic acid dramatically changes the CID spectrum for the glucose diastereomer. Figure 2A,B shows the glucose and mannose complexes sprayed from acidic solution while Figure 2C,D shows these complexes sprayed under basic conditions. The product ion intensities for the ions with *m/z* 268 and 280 decrease dramatically, and the product ion *m/z* 226 disappears completely when comparing the spectrum for the glucose diastereomer sprayed from a basic solution (Figure 2C) to the same complex sprayed from acidic solution (Figure 2A). In addition, the spectra obtained for the two diastereomers under acidic conditions (Figure 2A,B) are substantially less useful in discriminating glucose and mannose than the spectra obtained under basic conditions (Figure 2C,D). Knowing precisely how the three product ions, *m/z* 268, 280, and 226, were generated under basic conditions would likely allow for an explanation as to why they do not appear when sprayed out of acidic solution. Since the ion *m/z* 226 is the only product ion that is eliminated

(17) Le Blanc, J. C. Y.; Wang, J.; Guevremont, R.; Siu, K. W. M. *Org. Mass Spectrom.* **1994**, *29*, 587−593.
(18) Li, J.; Taraszka, J. A.; Countermann, A. E.; Clemmer, D. E. *Int. J. Mass Spectrom.* **1999**, *185/186/187*, 37−47.
(19) Collette, C.; Meunier, C.; De Pauw, E.; Dumont, A.; Desreux, J. F. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1521−1528.

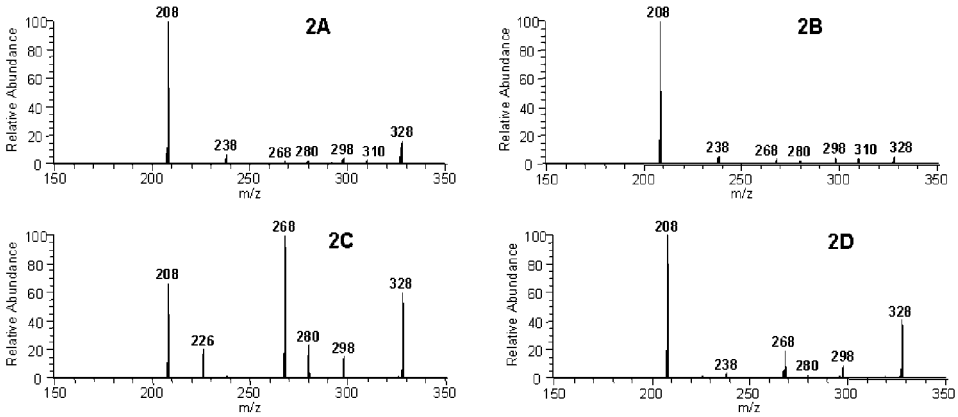


Figure 2. MS/MS spectra of *m*/*z* 328: (A) glucose complex in acidic solution; (B) mannose complex in acidic solution; (C) glucose complex in basic solution; (D) mannose complex in basic solution.

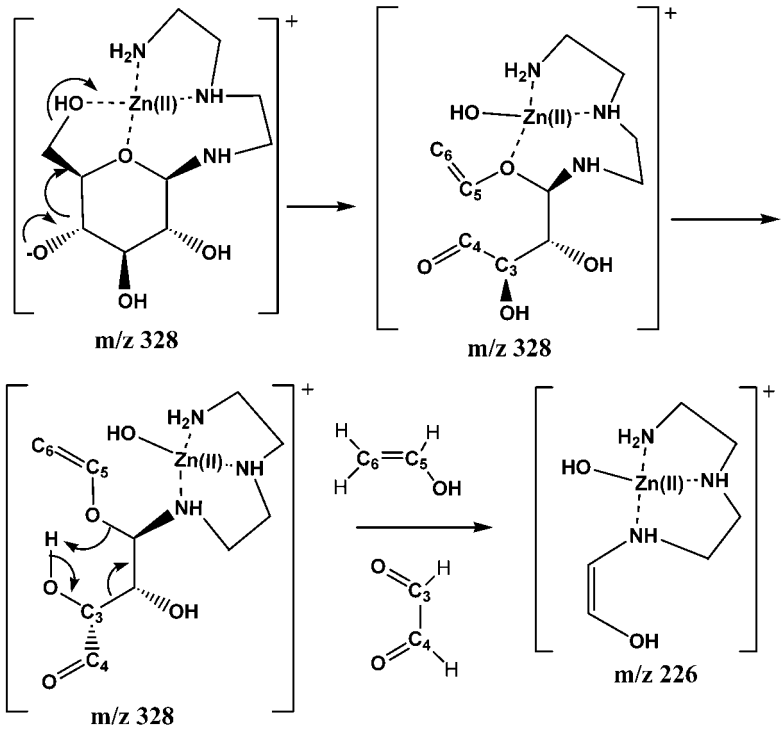


Figure 3. Proposed dissociation mechanism for the loss of $C_4H_6O_3$.

upon addition of acid, its dissociation pathway was chosen to be examined in depth.

Dissociation Mechanism. Previous studies have provided a substantial amount of information regarding the origin of the product ion *m*/*z* 226. For example, through isotopic labeling studies, it was determined that the loss of $C_4H_6O_3$ contained carbons 3−6.[15] In addition, the dissociation mechanism was not observed for 3-deoxyglucose or 6-deoxyglucose.[15] On the basis of this information, a mechanism is postulated for the dissociation and is displayed in Figure 3. In the mechanism, the C4 hydroxyl group starts out deprotonated, and the first step involves transfer of the C6 hydroxyl group to Zn. This step is simply a rearrangement, as no fragments are lost. The dissociation step of the mechanism involves a proton transfer from the C3 hydroxyl group to the ring oxygen and simultaneous loss of ethenol and 2-oxoethanal. To validate this mechanism three studies were undertaken. First, glucose analogues were probed to confirm which functional groups were involved in the dissociation. Second, MS^3 experiments were performed on the product of the dissociation (i.e. $[M - H]^+ \rightarrow [M - H - C_4H_6O_3]^+ \rightarrow$) and a synthetic analogue to demonstrate that the product ion resulting from the neutral loss of $C_4H_6O_3$ contained a hydroxyl group attached to Zn. Finally, experiments were undertaken to ascertain information about the site of deprotonation.

(A) Glucose Analogues. Several commercially available derivatives of glucose were reacted with the Zn complex and analyzed to obtain as much information about the loss of $C_4H_6O_3$ as possible. Specifically, in addition to the previously mentioned 3-deoxyglucose and 6-deoxyglucose complexes, the following compounds were also investigated: 2-deoxy-2-aminoglucose; 2-deoxy-2-fluoroglucose; 4-deoxy-4-fluoroglucose; 6-deoxy-6-fluoroglucose. These results are compiled in Table 1. As seen from the results of this study, substitution at the 2 position did not effect the dissociation. For both 2-deoxy-2-aminoglucose and 2-deoxy-2-fluoroglucose, the $C_4H_6O_3$ neutral loss was observed. For 4-deoxy-4-fluoroglucose,

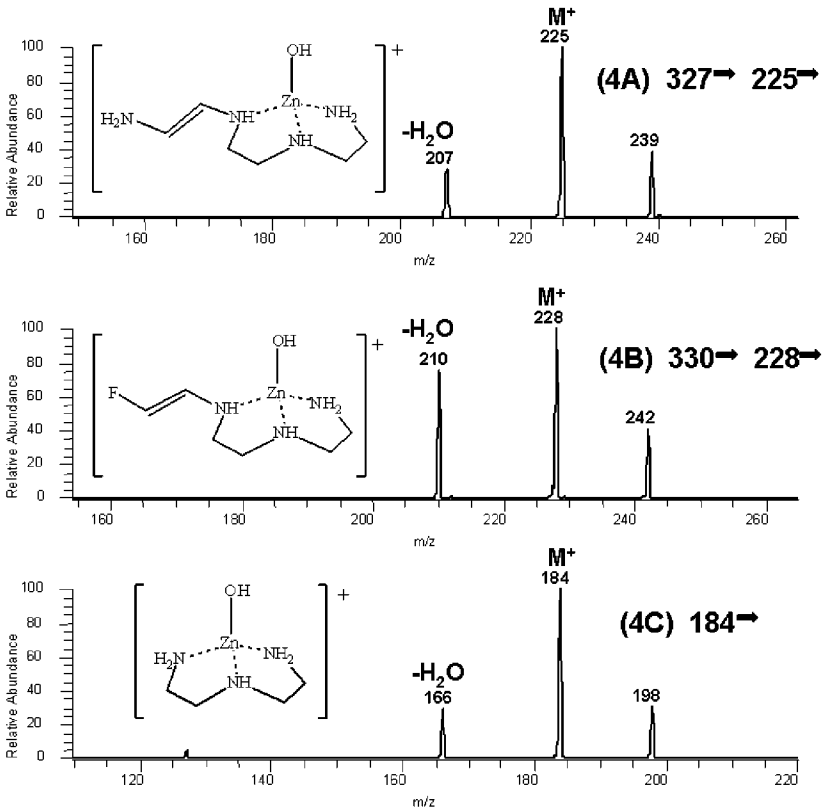


Figure 4. Spectra comparing the proposed products of the $C_4H_6O_3$ dissociation with the CID spectrum of a synthetic analogue: (A) MS^3 spectrum acquired after activating the product ion, *m/z* 225, which corresponds to a neutral loss of $C_4H_6O_3$ from the glucosamine complex; (B) MS^3 spectrum acquired after activating the product ion, *m/z* 228, which corresponds to a neutral loss of $C_4H_6O_3$ from the 2-flouro-2-deoxyglucose complex; (C) MS/MS spectrum of $[Zn(dien)(OH)]^+$ (see text for explanation of ions).

Table 1. Effects of Substitution on the Dissociation of $C_4H_6O_3$

analog	loss of $C_4H_6O_3$ during CID
2-deoxy-GLC[a]	yes
2-fluoro-2-deoxy-GLC	yes
2-amino-2-deoxy-GLC	yes
3-deoxy-GLC[a]	no
4-fluoro-4-deoxy-GLC	no
6-deoxy-GLC[a]	no

[a] Previously published results. See ref 15.

the dissociation was absent, and only 2 product ions were present in the CID spectrum. One product ion corresponded to the loss of $C_4H_7FO_3$, and the other loss was $C_2H_4O_2$. For the 6-deoxy-6-fluoroglucose complex, the $C_4H_6O_3$ neutral loss was apparent. Additional cross-ring cleavages for this complex were similar to those observed for the 4-deoxy-4-fluoroglucose complex.

These data support the dissociation mechanism proposed in Figure 3. All of the glucose analogues substituted at C2 underwent the $C_4H_6O_3$ loss, and in the proposed mechanism, the functional group at C2 plays no part in the dissociation. The loss of $C_4H_6O_3$ does not occur if 3-deoxyglucose is used; this is consistent with the final step of the mechanism where a proton from C3−OH must be transferred to the ring oxygen. This step obviously cannot occur with 3-deoxyglucose. The dissociation is also blocked if C4 contains fluorine instead of a hydroxyl group. Since the F and OH groups are approximately isosteric and have similar electronegativity,[20] the fact that the dissociation does not occur with the fluorine analogue indicates that the deprotonation of the C4 hydroxyl group is essential. In the proposed mechanism, the C4 hydroxyl group starts out deprotonated, and this deprotonation drives the rearrangement.

Finally, the results from the C6-fluorinated glucose also contribute significantly to understanding the mechanism. For the 6-fluoro-6-deoxyglucose complex, fluorine is retained with the metal complex during the $C_4H_6O_3$ loss. Therefore, the C6 hydroxyl group (or fluorine) is not part of the dissociation, even though C6 is lost. This is consistent with the first step of the mechanism, which involves transfer of the C6 functional group to Zn.

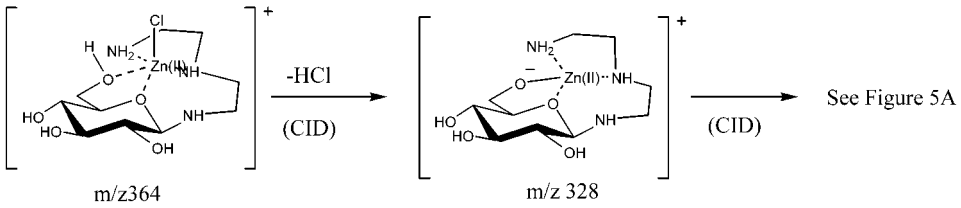
(B) Confirming the Starting Structure and Product of the Dissociation. The coordination site in the initial structure shown in Figure 3 is strongly supported by recent ion mobility and computational studies which have shown that the chlorinated adduct of the glucose complex, $[Zn(dien\text{-}glucose)(Cl)]^+$, has a dative bond between the C6 hydroxyl group and the Zn.[21]

To confirm that the dissociation mechanism results in a product ion that contains a hydroxyl group coordinated to Zn, as depicted in Figure 3, MS^3 studies were performed on the product of the $C_4H_6O_3$ dissociation (i.e. $[M - H]^+ \rightarrow [M - H - C_4H_6O_3]^+ \rightarrow$). These results are compared to the CID spectrum of a synthetic analogue. The MS^3 spectra of the 2-amino-2-deoxyglucose complex (*m/z* 327 → 225 →) and the 2-fluoro-2-deoxyglucose complex (*m/z* 330 → 228 →) are depicted in Figure 4A,B. The proposed structures for the ions *m/z* 225 and 228 are

(20) Hoffmann, M.; Rychlewski, J. *J. Am. Chem. Soc.* **2001**, *123* (10), 2308−2316.

(21) Leavell, M. D.; Taraszka, J. A.; Gaucher, S. P.; Clemmer, D. E.; Leary, J. A. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 284−293.

Scheme 2



also shown. When the product ions, *m*/*z* 225 and 228, respectively, are subjected to MS^3 studies, a loss of H_2O, and an *addition* of 14 mass units is observed. (Note: these analogues were chosen because they do not contain a hydroxyl group at C2, so there is only one possibility for the origin of the loss of H_2O.) If this water loss were due to a hydroxyl group coordinated to Zn, then the spectra should match that of a synthetic analogue which also has a hydroxyl group attached to Zn. Figure 4C corresponds to the CID spectrum of the complex $[Zn(dien)(OH)]^+$, and the neutral losses are identical to those observed in Figure 4A,B. The addition of 14 mass units in all three spectra most likely results from the adduction of methanol to the Zn, after H_2O is lost. These types of neutral adductions are quite common among coordinatively unsaturated transition metal complexes undergoing CID in ion traps.[22,23]

(C) Investigating the Deprotonation Site. The proposed mechanism in Figure 3 has the C4 hydroxyl group deprotonated. While the C6 hydroxyl group is clearly more acidic, we hypothesize that a population of complexes deprotonated at C4−OH is present in basic solution and the C4 hydroxyl group remains deprotonated when the complex enters the gas phase. This hypothesis is consistent with the CID results of the fluorinated glucose analogues. The 4-deoxy-4-fluoroglucose complex did not undergo the $C_4H_6O_3$ dissociation, but the 6-fluoro-6-deoxyglucose complex did. Therefore, a hydroxyl group (which may become deprotonated) is required at C4, but deprotonation at C6−OH is not required in the $C_4H_6O_3$ dissociation.

Because the C6 hydroxyl group is coordinated to Zn in the glucose complex, C6−OH is presumably much more acidic than the C4 hydroxyl group. To rule out the possibility that the C6−OH is the initial deprotonation site, we conducted a CID experiment in which C6−OH *must* be the sole deprotonation site and compared those results to the spectra in Figure 2. To achieve deprotonation exclusively at C6−OH, the ion *m*/*z* 328 was generated in an MS^2 experiment, starting with the chlorinated adduct, *m*/*z* 364, $[Zn(dien\text{-}glucose)(Cl)]^+$. (See Scheme 2.) In this case, there is no deprotonation site for the parent ion. Once this complex is in the gas phase, it can be isolated inside the ion trap and activated to lose HCl. This experiment generates the deprotonated $[Zn(dien\text{-}glucose)]^+$ complex, *m*/*z* 328, as shown in Scheme 2. In this case the deprotonation site must be C6−OH, because recent studies have shown that this is the only hydroxyl group within hydrogen-bonding distance of the chloride.[21] After loss of HCl in an MS^2 experiment, the ion *m*/*z* 328 is activated, and its CID spectrum is shown in Figure 5. Note Figure 5A is the CID spectrum for the glucose complex and Figure 5B is that of the mannose complex. Under these conditions, the $C_4H_6O_3$ neutral loss (*m*/*z* 226) is absent after activation of both the glucose and mannose complexes. Therefore, C6−OH must not be the site of deprotonation which leads to the $C_4H_6O_3$ neutral loss.

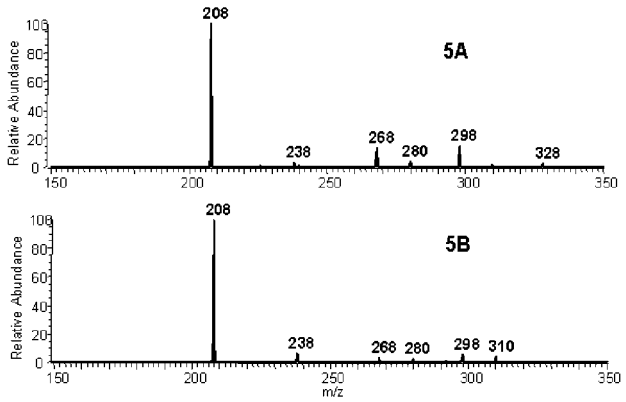


Figure 5. MS^3 spectra acquired after activating the product ion *m*/*z* 328, which corresponds to a neutral loss of HCl from the previously characterized[21] $[Zn(dien\text{-}hexose)(Cl)]^+$ complexes (*m*/*z* 364): (A) CID spectrum for $[Zn(dien\text{-}glucose)]^+$; (B) CID spectrum for $[Zn(dien\text{-}mannose)]^+$.

Comparing the CID spectra in Figure 5 with those in Figure 2, another crucial, yet somewhat serendipitous, piece of information is obtained. The CID spectra generated in Figure 5, in which the complex was exclusively deprotonated in the gas phase, are identical to the CID spectra generated when formic acid is added to the solution. That is, the CID spectra for the glucose complexes in Figures 2A and 5A are identical, as are those of the mannose complexes in Figures 2B and 5B. This observation helps explain why the glucose complex diluted in acidic solution (Figure 2A) provides a CID spectrum that is different from when the same complex is diluted in basic solution (Figure 2C). For the acidic complex, deprotonation must occur at C6−OH, as the spectrum is identical to the glucose complex in Figure 5A. In basic solution, different sites of deprotonation must be present, since the CID spectra are different from those in Figures 2A and 5A.

Thus, there is strong evidence that the $C_4H_6O_3$ loss is dependent on the acidity or bacisity of the solution. This is due to the pH of the electrospray solution, which will effect the sites of deprotonation. In a basic solution, a small population of complexes exists where the C4 hydroxyl group is deprotonated. When this population gets transferred to the gas phase, it can undergo the $C_4H_6O_3$ dissociation. In an acidic solution, it is highly unlikely that C4−OH is deprotonated. Rather, the site of deprotonation is probably generated in the electrospray process, at C6−OH. As the molecule is entering the gas phase, there are far fewer intermolecular interactions that will stabilize a deprotonation site at a hydroxyl group that is not coordinated to the metal. Intramolecular

(22) Vachet, R. W.; Hartman, J. A. R.; Callahan, J. H. *J. Mass Spectrom.* **1998**, *33*, 1209−1225.
(23) Lee, Y. I.; Jo, S. C.; Tao, W. A.; Cooks, R. G. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 484−488.

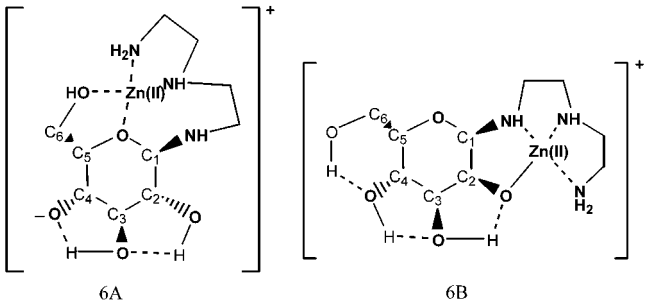


Figure 6. Hydrogen-bonded complexes: (A) [Zn(dien-glucose)]$^+$; (B) [Zn(dien-mannose)]$^+$.

interactions become much more important. Therefore, deprotonation will occur exclusively at a functional group that is coordinated to Zn, namely C6−OH for glucose.

Initially, the idea that changing the acidity or bacisity of the solution would cause different sites of deprotonation to occur and that these sites are then "frozen" seemed counterintuitive, since it is a well-known fact that protons can migrate in the gas phase.[24,25] It would seem that even if different sites of deprotonation were available initially, a series of proton transfers should be available that cause the deprotonation site to be predominately at the C6 hydroxyl group.

The structures in Figure 6 provide a possible explanation for the lack of proton transfer to the C4 hydroxyl group.[21] Evidence for this structure was found from a study involving ion mobility measurements in combination with DFT calculations.[21] For the glucose complex (Figure 6A), the ion deprotonated at the C4 hydroxyl group can be stabilized by the hydrogen bonding network between the C2, C3, and C4 hydroxyl groups; however, the C4 hydroxyl group is not hydrogen bonded to C6−OH. To transfer the proton from C6−OH to C4−OH, the bond between C6−OH and the metal must first be broken, since the C6 hydroxyl group is coordinated to the Zn.[21] The transition state for this process is very high in energy, with an energy barrier of approximately 40 kcal/mol. (This is the amount of energy needed for one water molecule to dissociate from a tetravalent zinc ion.[26]) Thus, the energy barrier of transferring the deprotonation site to the metal center is simply higher than the $C_4H_6O_3$ dissociation. In essence, the ion with a deprotonation site away from the metal center is kinetically trapped.

To investigate this further, a series of experiments were performed in which the precursor ion, *m*/*z* 328, was allowed to remain in the ion trap, unactivated for varying amounts of time, prior to CID. This was accomplished by providing an activation energy of 0 V during the MS^2 stage of an MS^3 experiment (see Experimental Section for details). The ion at *m*/*z* 328 was stored, unactivated, in the ion trap for 50−5000 ms. This ion was then activated under the "usual" conditions (i.e. 0.48 V activation amplitude, 30 ms activation time) as a MS^3 experiment. Under these conditions, the precursor ion could be stored, unactivated, in the ion trap for 50 ms−5 s without substantial loss of ion signal. When this same precursor ion is activated, even after a storage time of five seconds, the product ion *m*/*z* 226 is still present at approximately the same abundance as in Figure 2C, in which the ion is not stored in the ion trap prior to activation. Therefore, the deprotonation site is not transferred to the C6−OH, even at these longer times.

The mannose complex, *m*/*z* 328, was also subjected to the experiments described above. That is, the ions were allowed to remain in the ion trap, unactivated, for 50−5000 ms and then allowed to undergo CID as described previously. Again, even after a storage time of 5 s, the CID spectrum for the mannose complex was not altered. Specifically, no product ion at *m*/*z* 226 was observed under any circumstances for the mannose complex.

The mannose complex does not undergo $C_4H_6O_3$ dissociation because it does not have the same gas-phase structure as the glucose complex. Ion mobility data and computational studies[21] have predicted that, unlike glucose, the mannose complex coordinates to the Zn through the C2 hydroxyl group.[27] In this case, a hydrogen-bonding network can be established among C2−OH, C3−OH, C4−OH, and C6−OH (Figure 6B). With the C2 hydroxyl group bound to the Zn, any deprotonation site generated away from the metal center may be very rapidly transferred to C2−OH through this hydrogen-bonding network, without C2−OH breaking its bond with the metal (Figure 6B). Therefore, kinetically trapped ions will not be generated. Experimental evidence obtained in this study supports this structure (Figure 6B). First, all the CID spectra for the mannose complex are the same, including those obtained under acidic and basic conditions (Figure 2B,D), and the CID spectrum originating from the MS^3 experiment *m*/*z* 364 → *m*/*z* 328 → (Figure 5B). Since all the CID spectra are identical, the gas phase complexes undergoing CID are most likely identical; therefore, all of the structures have the same site of deprotonation, presumably at the metal center. In addition, even when the mannose complex is subjected to the CID experiments where it is first stored in the ion trap unactivated, the CID spectra do not contain a $C_4H_6O_3$ dissociation. These experiments are consistent with all the evidence presented here that suggests mannose is not binding to Zn at C6−OH, but at C2−OH.

Conclusion

The [Zn(dien-glucose)]$^+$ complex produced different CID spectra depending on the acidity or bacisity of the electrospray solution because under basic conditions deprotonation of the carbohydrate may occur, to some extent, in solution. This deprotonation site, which is not adjacent to the metal center, may lead to unique dissociations that are not observed when deprotonation occurs at the metal center. This conclusion was based on a well-defined mechanistic study of one of the dissociations that only occur under basic conditions. It is supported by corroborating data from the [Zn(dien-hexose)(Cl)]$^+$ complexes which, upon loss of HCl, produce deprotonation sites exclusively at the metal center. In addition, because the gas-phase conformation of the

(24) Dongre, A. R.; Jones, J. L.; Somogy, A.; Wysocki, V. H. *J. Am. Chem. Soc.* **1996**, *118*, 8365−8374.

(25) Tsaprailis, G.; Nair, H.; Somogyi, A.; Wysocki, V. H.; Zhong, W.; Futrell, J. H.; Summerfield, S. G.; Gaskell, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 5142−5154.

(26) Pavlov, M.; Siegbahn, P. E.; Sandstrom, M. *J. Phys. Chem. A* **1998**, *102*, 219−228.

(27) These studies were conducted on the [Zn(dien-Mannose)(Cl)]$^+$ adduct. Yet, since the CID spectra for the ion *m*/*z* 328, the deprotonated adduct, are identical when originating from the chlorinated adduct (Figure 5B) or from that generated directly from the electrospray process (Figure 2B,D), the Zn must be binding at the C2 hydroxyl group in all cases.

$[Zn(dien\text{-}mannose)(Cl)]^+$ structure is known, we were able to explain why the mannose complexes did *not* produce kinetically trapped ions.

These findings are significant for a number of reasons. First, they demonstrate one more variable that may be explored in attempting to differentiate stereoisomers. While the glucose and mannose complexes showed only modest differences under acidic conditions, the diastereomers were quite easily differentiated under basic conditions. Thus, when attempting to design mass spectrometric conditions that differentiate stereoisomers, one should explore changing the solvents as well as the acidity and basicity of the electrospray solution.

In addition, solvent composition must be taken into consideration when trying to identify unknown compounds from spectral databases. These databases attempt to match MS/MS spectra of an unknown compound to an assortment of MS/MS spectra from a given library. If the library compounds are run using solvent conditions different from those of the unknown compound, the CID spectra may not match properly. In fact, the effect which we have observed herein may be one of the *most important* reasons CID spectra are not identical when run under the same activation conditions. Several laboratories have previously demonstrated that when identical activation conditions are used, and the pH of the electrospray solutions not changed, MS^2 and MS^3 spectra deviate only very slightly, even over substantial periods of time. But, as demonstrated here, identical compounds in solutions with different acidity or bacisity may have kinetically trapped sites of deprotonation (or protonation) and these ions produce dramatically different CID spectra. Thus, maintaining a constant pH in the electrospray solution is a very critical factor when collecting and comparing CID spectra.

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