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The effects of coordination number and ligand size on the gas phase dissociation and stereochemical differentiation of cobalt-coordinated monosaccharides

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Abstract

Previously we have shown that stereoisomeric hexosamines and N-acetylhexosamines can be uniquely identified by mass spectrometry when they are derivatized with $[Co(DAP)_2Cl_2]Cl$ and subjected to ES-MS^n studies. Here, we explore why the diastereomers dissociate differently by investigating the role of the auxiliary ligand. Complexes which have two, three, and four auxiliary nitrogens coordinated to the Co center are investigated. By comparing these complexes, we demonstrate that the ligand affects the dissociations by contributing to both the sterics and the electronics about the metal center. Finally, we use this information to gain new insight about a key dissociation mechanism, loss of formaldehyde, which occurs stereoselectively for the $[Co(DAP)(GalNAc–2H)]^+$ complex. (Int J Mass Spectrom 209 (2001) 171–184) © 2001 Elsevier Science B.V.

1. Introduction

Chemists have been using ligated metal centers to control the stereoselectivity of organic reactions for many years. Two examples include Noyori's $(binap)Ru^{II}$ catalysts that promote catalytic hydrogenation in an enantioselective fashion [1,2] and boron-ligated reagents designed by Brown, which are used to dictate the stereoselectivity of aldol condensations [3–6]. Although enhancing stereochemical control of synthetic reactions is certainly paramount for the organic chemist, determining the stereochemistry of unknown compounds is equally important to the analytical chemist. In pursuing the latter goal, we have followed the lead of organic chemists in using metal-ligated compounds. Specifically, we have shown that coordinating metal-ligand systems to stereoisomers provides a means for stereochemical differentiation when adducts are analyzed by tandem mass spectrometry [7–14].

For example, we have shown that glucose and mannose, two multihydroxylated, isomeric monosaccharides, could be discriminated via mass spectrometry when reacted with [Zn(diethylenetriamine)(Cl)] or $[Ni(1,3\text{-diaminopropane})_3]Cl_2$ complexes and allowed to undergo collision induced dissociation (CID) [7,10,11]. In both cases, the metal ligand systems facilitated stereoselective dissociation reactions that were not observed for the underivatized monosaccharides. Based on the different product ions formed in these stereoselective dissociations, the monosaccharides could easily be distinguished.

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We have recently applied a similar methodology to distinguishing isomeric amines and N-acetylated amines [12,13]. However, for these sets of stereoisomers, a new derivatizing reagent, [Co(1,3-Diaminopropane)$_2$Cl$_2$]Cl was required. Although the use of metal-ligated derivatizing reagents has proven quite successful in achieving stereochemical differentiation via tandem mass spectrometry, we have not yet gained a complete understanding of the fundamentals which contribute to the differentiation process or how these principles can be best applied to general classes of stereoisomers. In order to address these issues, we investigate herein the stereoselectivity of the reactions by determining the effects of ligand size and number on the observed dissociation processes.

2. Experimental

[Co(DAP)$_2$Cl$_2$]Cl was synthesized as described previously [15]. [Co(EN)$_2$Cl$_2$]Cl was prepared as described elsewhere [16]. The metal-coordinated carbohydrate complexes were prepared in the following manner.

Complexes containing 1,3-diaminopropane (DAP): A methanolic solution of the metal salt, [Co^{III}(DAP)$_2$Cl$_2$]Cl, (0.02 M, 75 μL) was reacted with an aqueous solution of the monosaccharide (0.023 M, 66 μL) and two equivalents of triethylamine was added, as described previously [13]. The reaction was heated at 60 ° C for 15 min. This synthesis generates the six-coordinate complexes [Co^{III}(DAP)$_2$(hexNH$_2$–2H)]$^+$ and [Co^{III} (DAP)$_2$(hexNAc–2H)]$^+$ in high yield. The ion representing this complex, *m/z* 384 or *m/z* 426, respectively, is the base peak in the electrospray spectrum [12,13]. The related four coordinate complexes, [Co(DAP)(hexNH$_2$–2H)]$^+$ and [Co(DAP)(hexNAc–2H)]$^+$ are generated as product ions in the quadrupole ion trap during collisional activation of the six-coordinate complexes. For example, upon activation of [Co(DAP)$_2$(hexNAc–2H)]$^+$, *m/z* 426, the product ion *m/z* 352 appears as the base peak in the CID spectrum. This ion corresponds to the loss of one of the auxiliary DAP ligands, generating the complex [Co(DAP)(hexNAc–2H)]$^+$. The product ion is then isolated in the ion trap and activated in the MS^3 experiment, *m/z* 426→ 352→, in order to probe the dissociation pathways of the four-coordinate [Co(DAP)(hexNAc–2H)]$^+$ complex.

Complexes containing ethylendiamine (EN): The EN complexes were synthesized under identical experimental conditions as the DAP complexes, with the exception that the metal salt, [Co(DAP)$_2$Cl$_2$]Cl was replaced with [Co(EN)$_2$Cl$_2$]Cl.

Complexes containing diethylenetriamine (DIEN): One molar equivalent of a methanolic solution (46 μL) containing CoCl$_2$ (0.02 M), diethylenetriamine (0.02M), and triethylamine (0.02M) was added to one molar equivalent of an aqueous solution of the monosaccharide (40 μL, 0.023M), as adapted from a prior procedure [12]. The reaction mixture was heated at 60 °C for 15 min. Under the basic conditions described, the Co is oxidized from +2 to +3 as described previously [12], and the ion representing the complex [Co^{III}(DIEN)(Hex–2H)]$^+$ appears readily in the electrospray mass spectrum. This ion was subjected to CID studies.

Mass Spectrometry: All CID spectra were acquired on a Thermoquest "LCQ" quadrupole ion trap mass spectrometer, fitted with an electrospray source (Thermoquest, San Jose, CA). Samples were diluted to 50 pmol/μL with methanol and introduced into the mass spectrometer via direct infusion at a flow rate of 5 μL/min. The heated capillary was maintained at 150 °C, and a spray voltage of 4.5 kV was applied. Identical tuning conditions were applied to each class of isomers. For CID experiments, the ion of interest was isolated using a 3 to 5 Da isolation window, and 0.36 to 0.71 V was applied for 30 ms across the endcaps of the ion trap at the axial secular frequency of the ion, in order to induce dissociation. (Each set of isomers was run under identical activation conditions.) Thirty to sixty scans were averaged for each spectrum.

3. Results and discussion

The three biologically relevant diastereomeric hexosamines, glucosamine (GlcNH$_2$), galactosamine

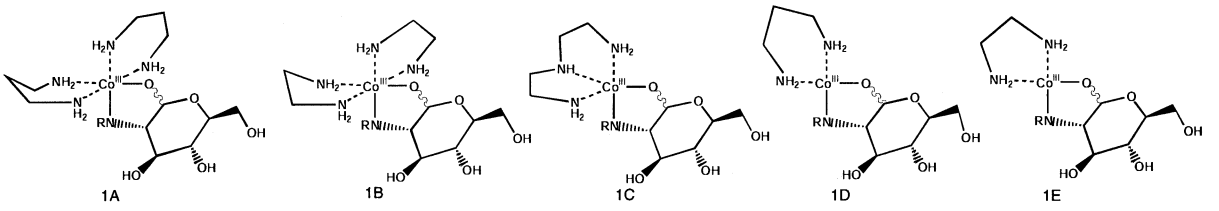

Fig. 1. Complexes studied. **1A:** $[Co(DAP)_2(Hex–2H)]^+$; **1B:** $[Co(EN)_2(Hex–2H)]^+$; **1C:** $[Co(DIEN)(Hex–2H)]^+$; **1D:** $[Co(DAP)(Hex–2H)]^+$; **1E** $[Co(EN)(Hex–2H)]^+$ where Hex may be any hexosamine or *N*-acetylhexosamine.

($GalNH_2$), and mannosamine ($ManNH_2$) and their acetylated counterparts, (GlcNAc, GalNAc, and ManNAc), all produce unique product ion spectra when derivatized with $[Co(DAP)_2(Cl)_2]Cl$ and subjected to CID in a quadrupole ion trap mass spectrometer. Because diastereomeric differentiation is not achieved in the absence of the metal ligand system, systematic studies were undertaken to explore why the metal-ligand system promoted stereoselective dissociation reactions. Specifically, we investigated the effects of ligand size and number on both the cobalt-coordinated hexosamine and *N*-acetylhexosamine complexes. In order to achieve this, the series of complexes in Fig. 1 were analyzed. For each complex depicted in Fig. 1, a total of six structures were compared; these structures correspond to the metal-ligand complex coordinated to one of the three *N*-acetylhexosamines or the three hexosamines depicted in Fig. 2. Thus, since there are five complexes in Fig. 1, and each represents one of six structures analyzed, a total of 30 compounds were investigated. These complexes varied in the size of the ligand, the number of coordinating nitrogens, and the number of ligands present. Two complexes with auxiliary ligands containing four coordinating nitrogens $[(Co(DAP)_2(Hex–2H)]^+$, **(1A)** and $[Co(EN)_2(Hex–2H)]^+$**(1B)** were analyzed. ("Hex" may be any monosaccharide in Fig. 2). Those results were compared to a complex containing a ligand with three coordinating nitrogens $[Co(DIEN)(Hex–2H)]^+$**(1C)** and two complexes containing ligands with two coordinating nitrogens, $[Co(DAP)(Hex–2H)]^+$**(1D)** and $[Co(EN)(Hex–2H)]^+$**(1E)**.

For the purposes of clarity and ease of understanding, we have used an abbreviated designation for each complex, based on ligand type and coordination number. For example, compound **1A** in Fig. 1, $[(Co(DAP)_2(HexNH_2–2H)]^+$, is designated as DAP_2/$HexNH_2$/4/6. This indicates that the complex contains two DAP ligands, a hexosamine monosaccharide, a total of four auxiliary nitrogens coordinating to the

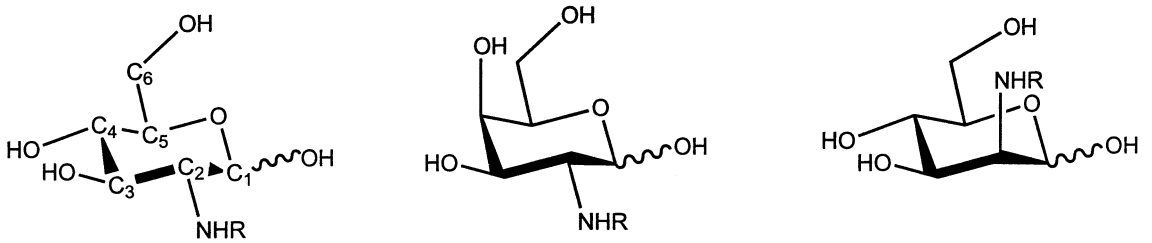

Fig. 2. Stereoisomeric hexosamines and *N*-acetylhexosamines.

Table 1
Comparing CID spectra for the HexNAc complexes with 4 auxiliary nitrogens

EN_2/HexNAc/4/6 Complexes			
Product Ions	*m/z* 267	*m/z* 278	*m/z* 338
Neutral loss	$C_4H_9NO_3$	$C_4H_8O_4$	EN
GlcNAc	5%	15%	100%
GalNAc	. . .[a]	5%	100%
ManNAc	. . .[a]	25%	100%
DAP_2/HexNAc/4/6 Complexes			
Product Ions	*m/z* 295	*m/z* 306	*m/z* 352
Neutral loss	$C_4H_9NO_3$	$C_4H_8O_4$	DAP
GlcNAc	15%	4%	100%
GalNAc	. . .[a]	—	100%
ManNAc	. . .[a]	20%	100%

[a] % Relative abundances are reported. Ions with <3% relative abundance are considered absent and indicated with "· · ·".

Co; and the complex is six-coordinate. Using this nomenclature "HexNAc" will be used to indicate *N*-acetylhexosamine. Hex will be used to indicate both $HexNH_2$ and HexNAc when discussing the general attributes of the complexes. Specific cases will be denoted by the abbreviation of the specific monosaccharide. For all the complexes, cobalt is in the +3 oxidation state, so there are two sites of deprotonation on the complex, allowing for an overall +1 charge. For each complex in Fig. 1, the monosaccharide is depicted as a bidentate ligand, binding at the C1 hydroxyl group and the nitrogen. This is not meant to represent the only coordination site of the sugar, as previous experimental studies on the $[Co(DAP)_2(HexNH_2\text{-}2H)]^+$ complexes show that a number of different binding sites are possible [17].

3.1. Stereochemical differentiation

In order for CID spectra to be useful in differentiating groups of isomers, we have established the following criteria: Each diastereomer must be identified by the unique presence or absence of at least one product ion. For an ion to be clearly present, its relative abundance must consistently be greater than 10%. In order for an ion to be determined absent, it's relative abundance must consistently be less than 3%.

For the five different types of complexes studied (Fig. 1) the metal-ligand systems that provided clear stereochemical differentiation based on the criteria described previously were the DAP_2/Hex/4/6 and DIEN/Hex/3/5 complexes **(1A and 1C)**. These complexes could be used to differentiate either the hexosamines or the *N*-acetylhexosamines. The product ions from the $EN_2/HexNH_2/4/6$ **(1B)** complexes were similar to those obtained for $DAP_2/HexNH_2/4/6$, so the six-coordinate EN complexes could be used to identify the hexosamine diastereomers as well. The EN_2/HexNAc/4/6 complexes did not meet the criteria for stereochemical differentiation (Table 1). Finally, the DAP/$HexNH_2$/2/4 complexes **(1D)** and the EN/$HexNH_2$/2/4 **(1E)** complexes could not be used to differentiate the diastereomers based on presence or absence of product ions because the CID spectra of the $GlcNH_2$ and $ManNH_2$ analogs had the same product ions (Table 2). Likewise, for the *N*-acetylhexosamines, the GlcNAc and GalNAc complexes produced the same product ions (Table 3). However, the product ion intensities for the DAP/HexNAc/2/4 samples were remarkably different for the three diastereomers, and we have recently quantified mixtures of *N*-acetylhexosamines using DAP/HexNAc/2/4 product ion spectra [18]. Since the EN/HexNAc/2/4 spectra were essentially identical to the DAP/HexNAc/2/4 spectra, it is likely that they could be used to distinguish the diastereomers based on product ion intensi-

Table 2
Comparing CID spectra for the $HexNH_2$ complexes with 2 auxiliary nitrogens

EN/$HexNH_2$/2/4 Complexes								
Product Ions	*m/z* 176	*m/z* 189	*m/z* 190	*m/z* 206	*m/z* 207	*m/z* 209	*m/z* 236	*m/z* 266
Neutral loss	$C_4H_8O_4$	$C_3H_9NO_3$	$C_3H_6O_4$	$C_3H_6O_3$	$C_3H_7NO_2$	$C_3H_5NO_2$	$C_2H_4O_2$	CH_2O
$GlcNH_2$	47%	65%	57%	30%	100%	22%	...[a]	5%
$GalNH_2$	...[a]	...[a]	8%	...[a]	...[a]	...[a]	40%	100%
$ManNH_2$	27%	80%	100%	30%	32%	60%	...[a]	20%
DAP/$HexNH_2$/2/4 Complexes								
Product Ions	*m/z* 190	*m/z* 203	*m/z* 204	*m/z* 220	*m/z* 221	*m/z* 223	*m/z* 250	*m/z* 280
Neutral loss	$C_4H_8O_4$	$C_3H_9NO_3$	$C_3H_6O_4$	$C_3H_6O_3$	$C_3H_7NO_2$	$C_3H_5NO_2$	$C_2H_4O_2$	CH_2O
$GlcNH_2$	10%	20%	15%	15%	100%	5%	...[a]	...[a]
$GalNH_2$	...[a]	...[a]	...[a]	...[a]	...[a]	...[a]	42%	100%
$ManNH_2$	10%	40%	100%	20%	15%	35%	...[a]	5%

[a] % Relative abundances for abundant ions are reported. Ions with <3% relative abundance are considered absent and indicated with "...".

ties as well. As a first step to understanding the stereoselective dissociation processes involved in these complexes, the role of the ligand is carefully evaluated.

3.2. Effects of ligand

3.2.1. Complexes with four auxiliary nitrogens

For the complexes with four auxiliary nitrogens, **1A** and **1B**, changing the ligand from DAP to EN had very little effect on the presence of product ions for a given diastereomer. This trend was observed for both the hexosamine and N-acetylhexosamine complexes. For example, when the complex $DAP_2/GlcNH_2/4/6$ was subjected to CID, [Fig. 3(a)] the product ions, *m/z* 366, 326, 310, and 295 are generated. These ions correspond to neutral losses of H_2O, C_2H_4NO, DAP, and $C_3H_7NO_2$ as previously determined by exact mass measurements [17]. Replacing the DAP ligand with EN does not affect the monosaccharide dissociations. The CID spectrum of $EN_2/GlcNH_2/4/6$ is depicted in Fig. 3(b). Again, the neutral losses detected include loss of H_2O (*m/z* 338), C_2H_4NO (*m/z* 298), EN (*m/z* 296), and $C_3H_7NO_2$ (*m/z* 267). Al-

Table 3
Comparing CID spectra for the HexNAc complexes with 2 auxiliary nitrogens

EN/HexNAc/2/4 Complexes					
Product Ion	*m/z* 207	*m/z* 218	*m/z* 248	*m/z* 278	*m/z* 308
Neutral loss	$C_4H_9NO_3$	$C_4H_8O_4$	$C_3H_6O_3$	$C_2H_4O_2$	CH_2O
GlcNAc	...[a]	5%	100%	40%	20%
GalNAc	...[a]	...[a]	30%	45%	100%
ManNAc	15%	90%	100%	90%	35%
DAP/HexNAc/2/4 Complexes					
Product Ion	*m/z* 221	*m/z* 232	*m/z* 262	*m/z* 292	*m/z* 322
Neutral loss	$C_4H_9NO_3$	$C_4H_8O_4$	$C_3H_6O_3$	$C_2H_4O_2$	CH_2O
GlcNAc	...[a]	...[a]	100%	16%	4%
GalNAc	...[a]	...[a]	25%	50%	100%
ManNAc	30%	35%	100%	23%	16%

[a]% Relative abundances for abundant ions are reported. Ions with <3% relative abundance are considered absent and indicated with "...".

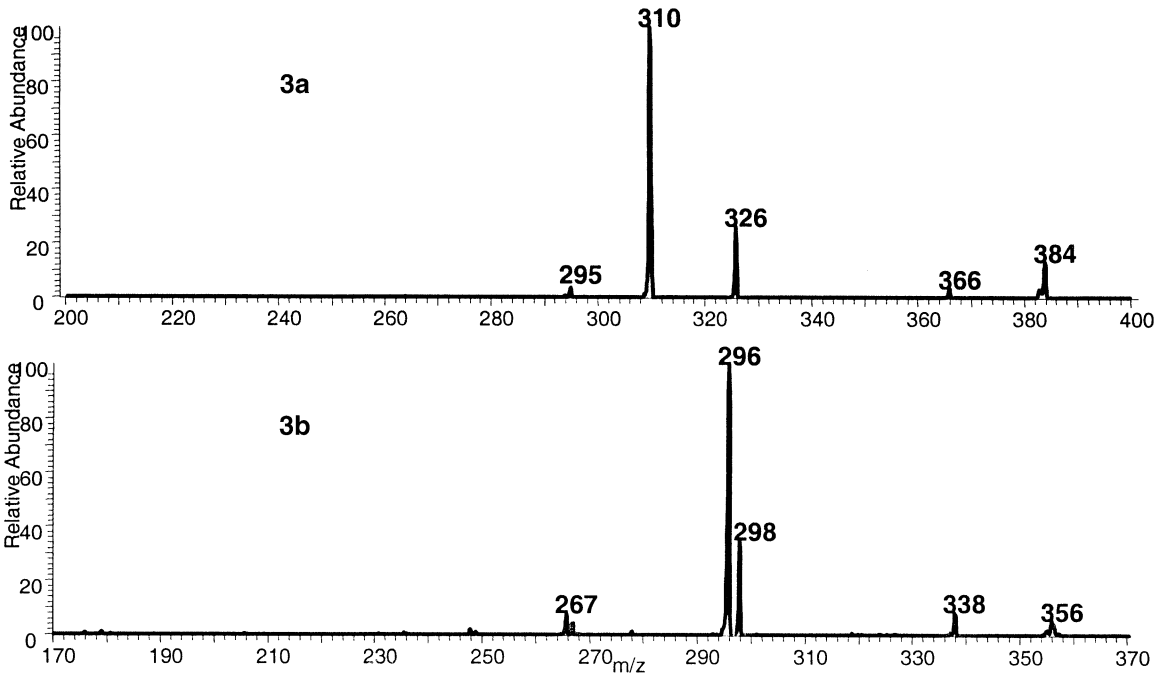

Fig. 3. CID spectra of DAP_2/$GlcNH_2$/4/6 (a) and EN_2/$GlcNH_2$/4/6 (b). The product ions observed correspond to a loss of H_2O (*m/z*366 and 338) C_2H_4NO (*m/z* 326 and 298), DAP or EN (*m/z* 310 and 296), and $C_3H_7NO_2$ (*m/z* 295 and 267) for (a) and (b), respectively.

though in almost every case the DAP and EN complexes with four auxiliary nitrogens generated identical CID spectra for a given monosaccharide, one exception to this generality is the presence of two ions *m/z* 278 and *m/z* 266 in the CID spectrum of EN_2/$GalNH_2$/4/6 [Fig. 4(a)]. These ions are not present in the corresponding DAP_2/$GalNH_2$/4/6 spectrum [Fig. 4(b)]. These neutral losses have a composition of M-(H_2O/EN), for *m/z* 278, and M-(CH_2O/EN), for *m/z* 266).

For the *N*-acetylated hexosamines, the CID spectra for DAP_2/ManNAc/4/6 and EN_2ManNAc/4/6 [Figs. 5(a) and 5(b), respectively] contain similar neutral losses including loss of an auxiliary ligand (DAP at *m/z* 352 or EN at *m/z* 338), and loss of $C_4H_8O_4$ [*m/z* 306 and 278 for Figs. 5(a) and 5(b), respectively]. Changing the ligand from DAP to EN also had little effect on the CID spectra for the GlcNAc complexes (Table 1). CID of the DAP_2/GalNAc/4/6 complex produced only a loss of ligand, whereas CID of the EN_2/GalNAc/4/6 complex produced loss of ligand and a very small loss of $C_4H_8O_4$ (Table 1).

Considering all the data for the complexes containing four auxiliary nitrogens, changing the ligand size had little effect on the presence of any product ions; however, the relative abundances of some of the product ions were altered. This trend can be demonstrated by comparing the product ion spectra for the two sets of complexes, EN_2/HexNAc/4/6 and DAP_2/HexNAc/4/6. These spectra are presented in tabular form in Table 1. Specifically, the larger ligand, diaminopropane, provided more-readily distinguishable spectra than the complexes with ethylenediamine. For example, both complexes containing GlcNAc display a unique loss of $C_4H_9NO_3$. For the DAP_2/GlcNAc/4/6 complex, this neutral loss was 15% relative abundance, whereas the same neutral loss was only 5% relative abundance for the EN_2GlcNAc/4/6 complex. Because the distinguishing product ion for GlcNAc was smaller in the CID spectra of EN_2/HexNAc/4/6 it is more difficult to discern GlcNAc from GalNAc or ManNAc using the CID spectra of the metal complexes ligated with ethylenediamine. For the metal-coordinated hex-

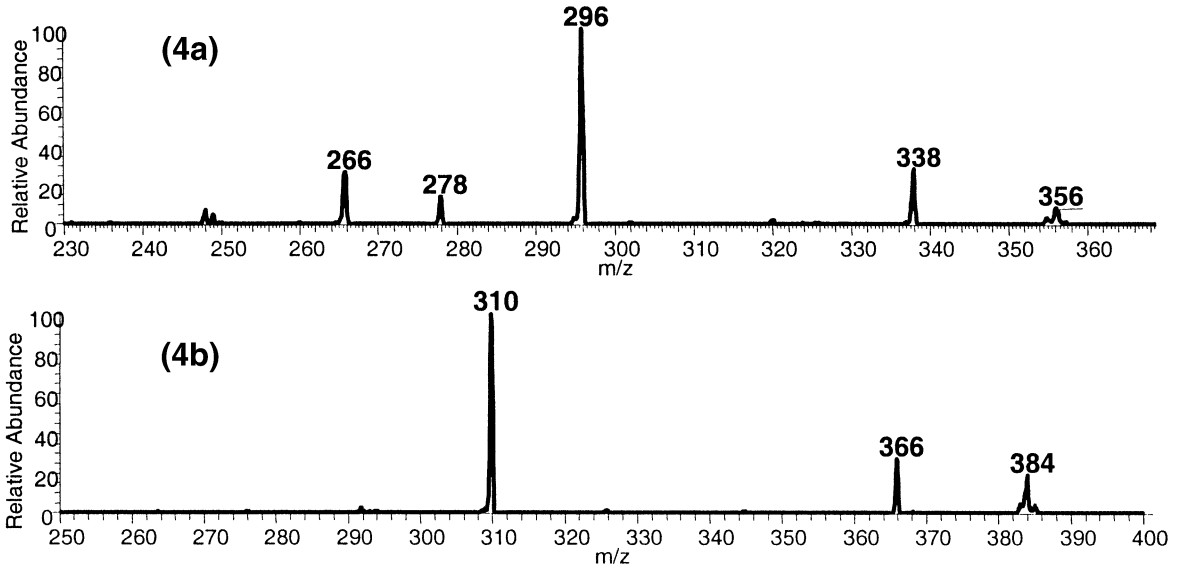

Fig. 4. CID spectra of EN_2/$GalNH_2$/4/6 (a) and DAP_2GalNH_2/3/6 (b). The product ions observed correspond to a loss of H_2O (*m/z* 338 and 366) and DAP or EN (*m/z* 296 and 310) for (a) and (b), respectively. In addition the product ions *m/z* 278 and 266 in (a) correspond to a loss of (EN and H_2O) and (EN and CH_2O).

osamine complexes, changing the ligand from DAP to EN did not diminish the stereoselective product ions generated.

3.2.2. Complexes with two coordinating nitrogens

For the complexes with two coordinating nitrogens, corresponding to **1D** and **1E**, the same general trends as described previously were also observed. Both the DAP/Hex/2/4 and EN/Hex/2/4 complexes produced very similar product ion spectra for a given diastereomer. This trend was observed for both the hexosamines and *N*-acetylhexosamines, and the results from the CID spectra are presented in Tables 2 and 3. We postulated previously that the presence of cross-ring cleavage ions observed in the CID spectra depend on the accessible conformations of the carbohydrate and the binding interaction of the sugar to the metal [10,17,19]. Because the Co-coordinated monosaccharides are not covalently bound to the auxiliary ligands, changing the size of the ligand should have no effect on the possible binding sites of the sugar to the metal and very little effect on the accessible conformations of the carbohydrate. Therefore, the fact that changing the auxiliary ligand from DAP to EN had little effect on the cross-ring cleavage ions in the product ion spectra further supports our initial hypothesis that carbohydrate-metal binding interaction dictates the dissociation pathways [10,17,19].

3.3. Comparing the complexes with different coordination numbers

Although changing the ligand size, from DAP to EN, does not substantially affect the types of product ions observed for the complexes studied, changing the number of coordinating nitrogens dramatically changes the dissociations observed in the CID spectra. This can be demonstrated by comparing the CID spectra of DAP_2/$GlcNH_2$/4/6, DIEN/$GlcNH_2$/3/5, and DAP/$GlcNH_2$/2/4 [Figs. 6(a)–6(c)]. As the number of coordinating nitrogens on the auxiliary ligand decreases (from 4 to 3 to 2 for a–c, respectively) the number and type of product ions produced changes. For example, loss of ligand is prominent in Fig. 6(a), but not Fig. 6(b) or Fig. 6(c). Likewise, the $C_3H_7NO_2$ loss becomes more abundant as the number of ligands decreases, and the C_2H_4NO loss becomes less abun-

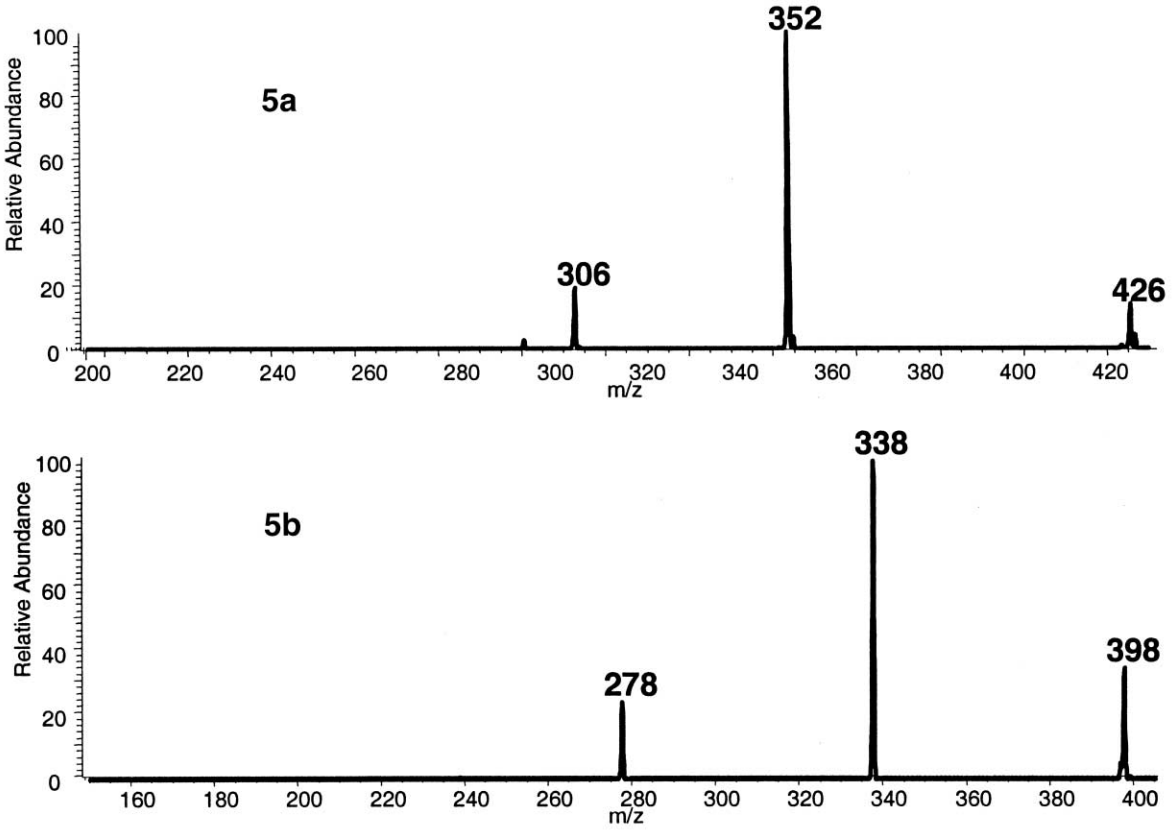

Fig. 5. CID spectra of DAP_2/ManNAc/4/6 (a) and EN_2ManNAc/4/6 (b). The product ions observed correspond to a loss of DAP or EN (*m/z* 310 and 296) and $C_4H_8O_4$ (*m/z* 306 and 278) for (a) and (b), respectively.

dant (relative to other cross-ring cleavages). By changing the number of coordinating nitrogens, both the electronics of the metal and the sterics of the metal-ligand system are different. These differences account for the change in ion intensity observed in Fig. 6.

For the case of the C_2H_4NO neutral loss, this product ion is the largest cross-ring cleavage observed in the CID spectrum of DAP_2/$GlcNH_2$/4/6, whereas all other cross ring cleavages are less than 5% relative abundance. The C_2H_4NO loss is the most abundant ion in the CID spectrum of DIEN/$GlcNH_2$/3/5; however, other cross-ring cleavage ions appear in substantial abundance. Finally, in the CID spectrum of DAP/$GlcNH_2$/2/4 the C_2H_4NO loss is completely absent. These differences can be explained based on the electronics of the metal. The mechanism for this dissociation has been recently studied in-depth and is depicted in Fig. 7 [17]. In the first step, the metal is reduced to a +1 oxidation state. The Co, with 18 *d* electrons, then acts as a nucleophile to displace the leaving group. This reaction is similar to previously observed one-electron oxidative addition reactions, and these reactions proceed more readily for electron-rich systems [20]. As the number of coordinating nitrogen ligands decreases from 4 to 3 to 2, the number of *d* electrons for Co is reduced from 18 to 16 to 14. Thus, it is not surprising that when the Co has only 14 *d* electrons, in the case of the DAP/$GlcNH_2$/2/4 complex, it does not appear to act as a nucleophile. The C_2H_4NO loss is not observed in Fig. 6(c). In this case, changing the number of coordinating

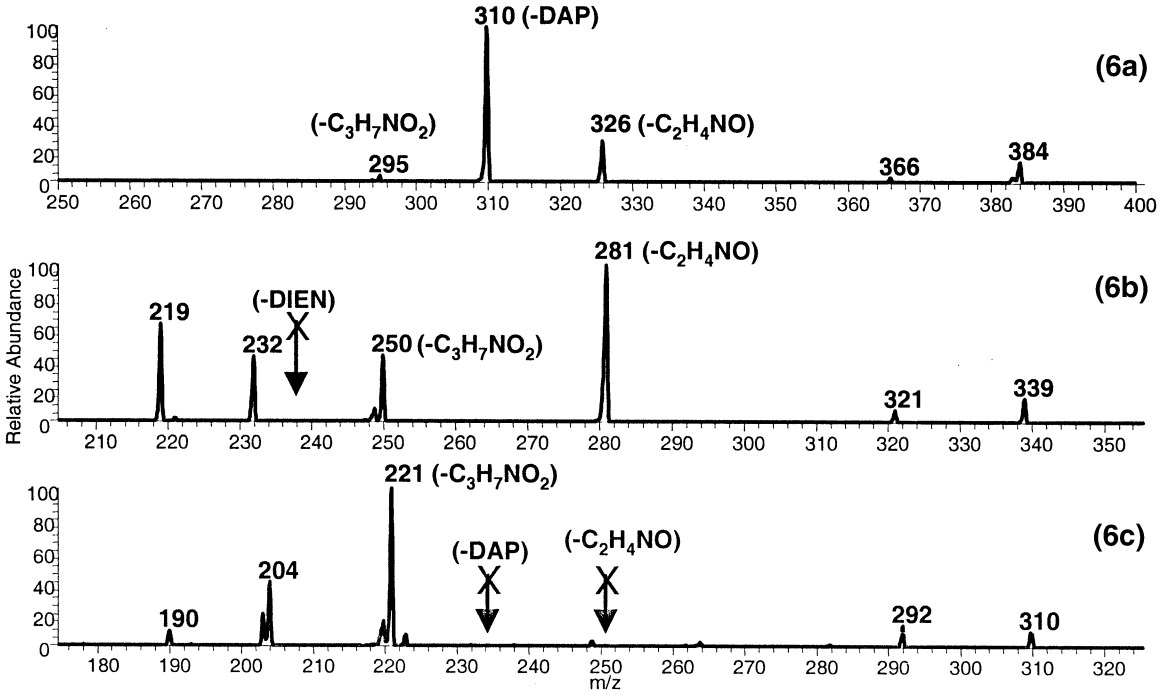

Fig. 6. CID spectra of $DAP_2/GlcNH_2/4/6$ (a), $DIEN/GlcNH_2/3/5$ (b), and $DAP/GlcNH_2/2/4$ (c).

nitrogens, and changing the metal's electronic affinity, directly affects the neutral losses.

The importance of the auxiliary ligands as a source of electronic stability for cobalt can also be demonstrated in another example. In the case where cobalt has four coordinating nitrogens, ligand dissociation (loss of DAP or EN) is always the base peak in the spectrum. For example, see Figs. 3, 4, and 5. For these dissociations, the metal undergoes a transition from a completely filled *d* shell, 18 electrons, to a *d* shell with 14 electrons. Yet, for the complexes with 3 or 2 coordinating nitrogens, loss of the auxiliary ligand is not observed. [See Tables 2 and 3 and Figs. 6(b) and 6(c)]. In these cases, loss of ligand would require the metal center to be depleted to only 10 *d* electrons; thus more favorable dissociations, like those that reduce the oxidation state of Co, will be more prominent.

The favored geometry and reactivity of the transition metal complex is not only governed by electronics, but by sterics as well [21–23]. These steric effects can influence the dissociation pathways of metallated complexes undergoing CID. To demonstrate this point, one can compare the relative abundance of the product ion corresponding to the loss of $C_3H_7NO_2$ from the metallated hexosamine complexes. The relative abundance of this product ion increases as the number of coordinating nitrogens decrease (Fig. 6). In this case, the ligands will affect the transition state energy of the reaction by presenting a steric barrier. The dissociation mechanism has been studied in depth, and the mechanism and transition state for the dissociation is depicted in Fig. 8 [17]. By comparing the proposed transition state structures for $DAP_2/GlcNH_2/4/6$, $DIEN/GlcNH_2/3/5$, and $DAP/GlcNH_2/2/4$, Fig. 9, it is clear that as the number of coordinating nitrogens decreases, the transition state structure of the monosaccharide becomes less congested stericly. Therefore, the dissociation will occur more readily because of its lower energy barrier. Accordingly, the relative abundance of the product ion representing this loss ($C_3H_7NO_2$) increases, from 4%, to 45%, to 100% for $DAP_2/GlcNH_2/4/6$, $DIEN/GlcNH_2/3/5$, and $DAP/GlcNH_2/2/4$, respectively (Fig. 6).

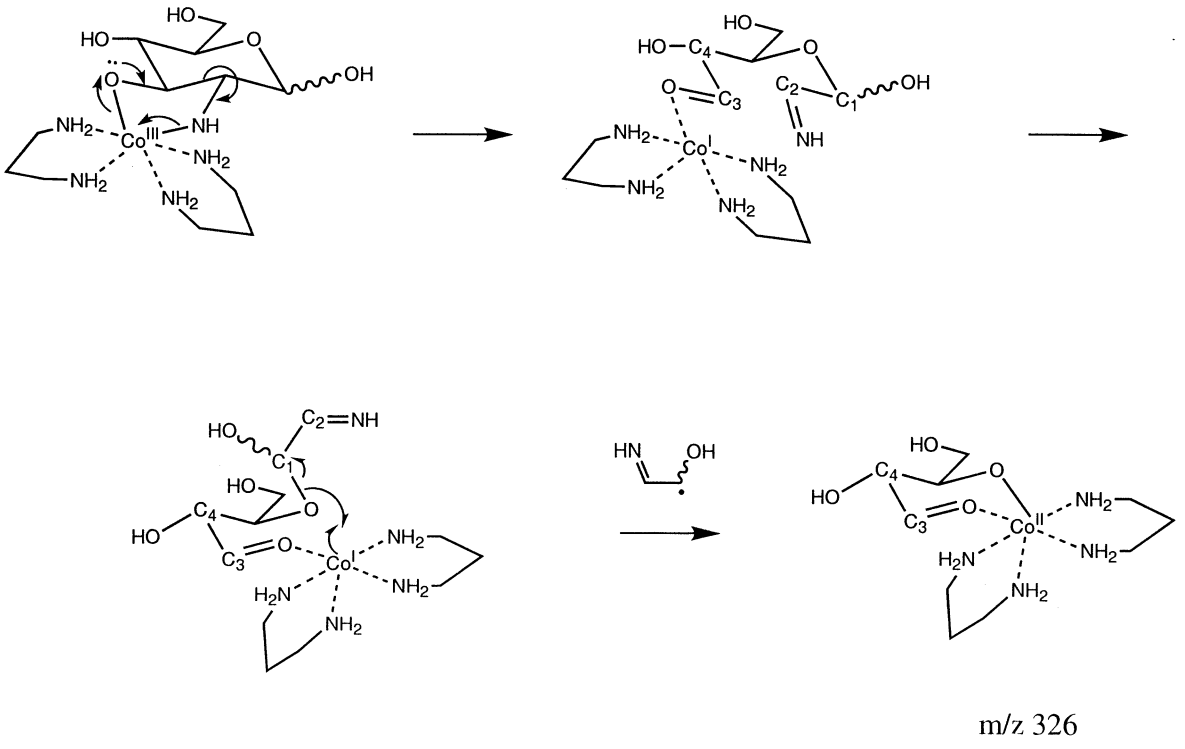

Fig. 7. Dissociation mechanism for loss of C_2H_4NO from the complex DAP_2/$GlcNH_2$/4/6. Reprinted from [17] with permission of Elsevier Science.

In summary, changing the number of ligands may have at least two effects on the observed dissociations. First, by reducing the number of ligands about the metal, steric interaction between the ligands and the monosaccharide is reduced. Therefore, some dissociations, like the loss of $C_3H_7NO_2$, become more favored. The number of ligands present also dictates the number of electrons around the metal. So by changing the number of ligands, different dissociations may become more or less favorable, depending on their electronic requirements. This is the case for the neutral losses of C_2H_4NO and DAP (or EN). These steric and electronic effects have been explored in depth for solution-phase chemistry [21–23].

3.4. Correlating trends in collision induced dissociation spectra to dissociation mechanisms

Comparing CID spectra with different numbers of auxiliary ligands can be helpful in assessing the validity of different dissociation mechanisms. For example, it was postulated previously that the loss of formaldehyde from the complex DAP/GalNAc/2/4 occurred as described in Fig. 10 [12]. In the first two steps of the dissociation, the negative charge moves away from the metal center: first to the ring oxygen, then to the C6 oxygen. This process will be low in energy for a metal with a filled d shell, and higher in energy for a metal with fewer d electrons. Therefore, to test the validity of the mechanism, the DAP/GalNAc/2/4 CID spectrum is compared with that of DIEN/GalNAc/3/5 and DAP_2/GalNAc4/6 [Figs. 11(a)–11(c), respectively]. In these three complexes, the number of auxiliary nitrogens increases from 2, to 3, to 4 respectively. Likewise, the number of *d* electrons for cobalt increases from 14 to 16 to 18. If the postulated mechanism for loss of CH_2O is accurate, the abundance of the ion corresponding to loss of CH_2O should increase with increasing *d* electrons

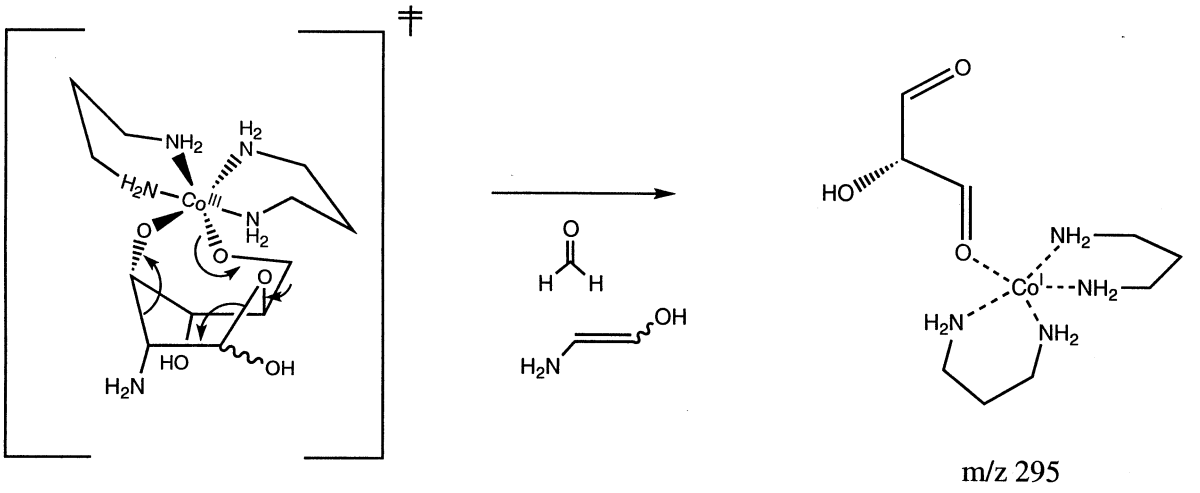

Fig. 8. Dissociation mechanism for loss of $C_3H_7NO_2$ from the complex DAP_2/$GlcNH_2$/4/6. Reprinted from [17] with permission of Elsevier Science.

around cobalt. The opposite trend is observed. The DIEN/GalNAc/3/5 CID spectrum [Fig. 11(b)] has a very small formaldehyde loss, and the DAP_2/GalNAc/4/6 spectrum [Fig. 11(c)] has no formaldehyde loss. Thus the proposed mechanism is likely inaccurate.

Using the information that the CH_2O loss increases with decreasing auxiliary ligands present, a new mechanism, which remains consistent with results of previous isotopic labeling studies [12], is postulated. This mechanism, Fig. 12, can be used to explain why the loss becomes more prevalent as the metal becomes more electron-poor; during the course of the mechanism, the metal is reduced to a +1 oxidation state, stabilizing an electron-poor metal center. In addition, the Co may be further stabilized by the fact that the formaldehyde remains bound to the metal until another portion of the carbohydrate chelates to the metal. Not only is the oxidation state of the metal reduced but the number of electrons about the metal has a net increase from 14 to 16 electrons. In conclusion, the mechanism in Fig. 12 is more consistent with the electronic requirements of the dissociation, as inferred from comparing the CID spectra of DAP/GalNAc/2/4, DIEN/GalNAc/3/5, and DAP_2/

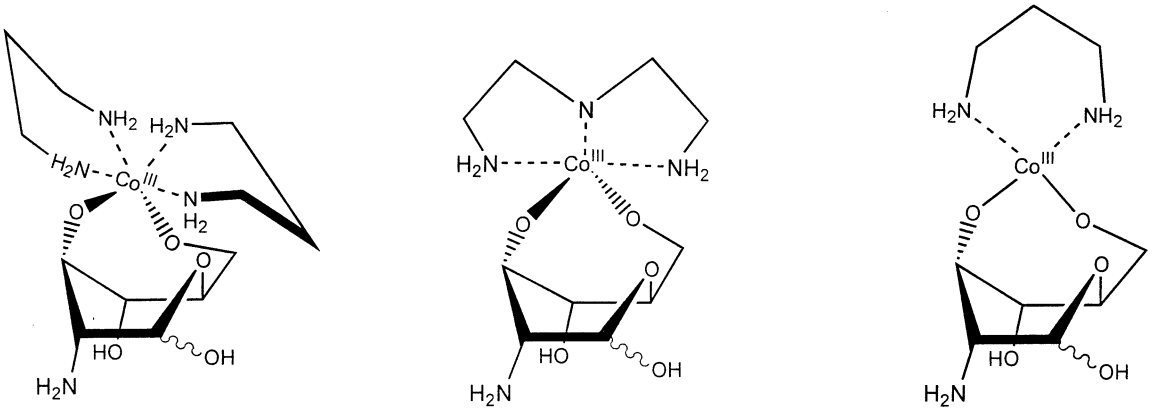

Fig. 9. Transition states for the loss of $C_3H_7NO_2$ for the complexes DAP_2/$GlcNH_2$/4/6, DIEN/$GlcNH_2$/3/5, and DAP/$GlcNH_2$/2/4.

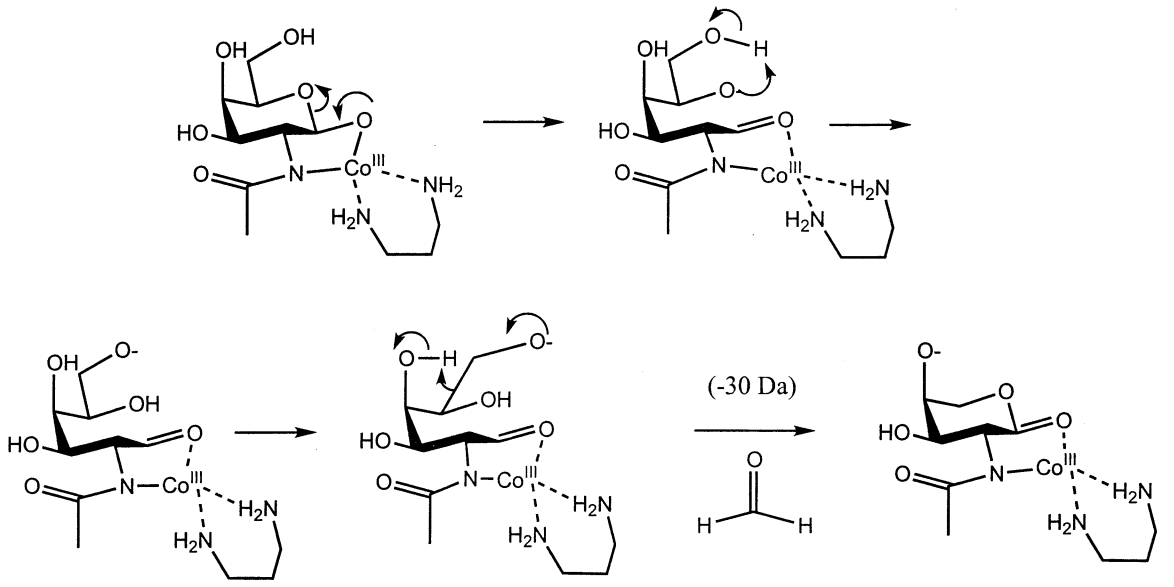

Fig. 10. Previously proposed mechanism for the loss of CH_2O from the complex DAP/GalNAc/2/4. Reprinted in part from [12] with permission of the American Chemical Society.

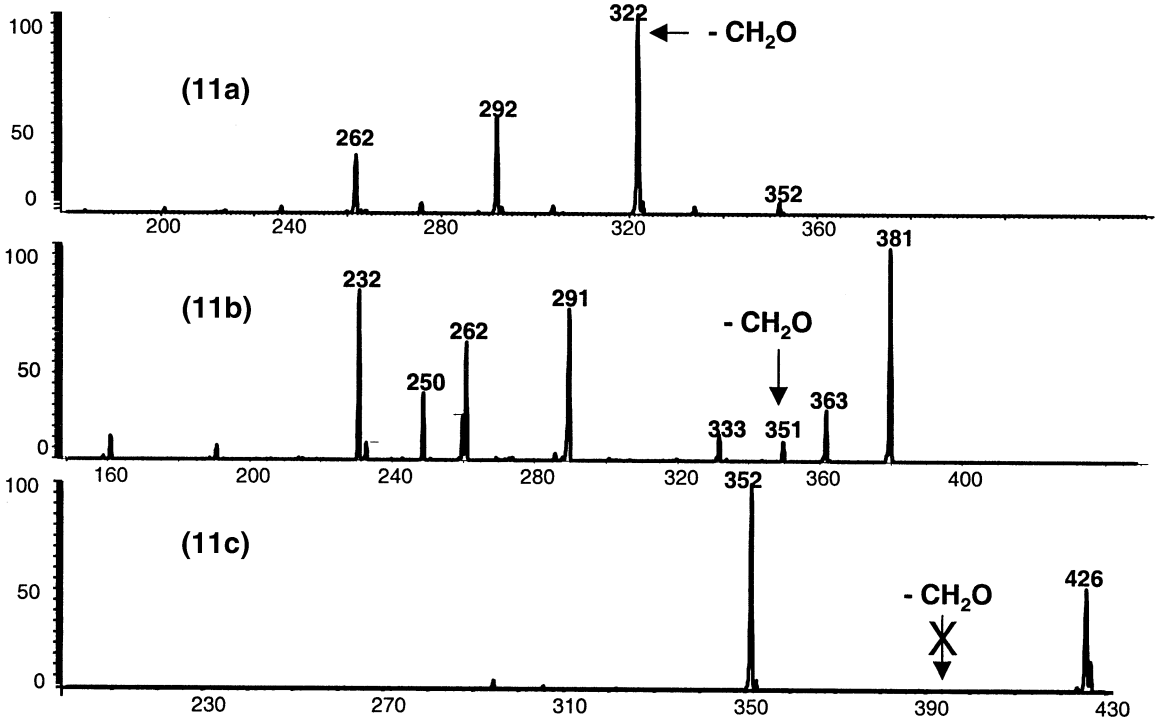

Fig. 11. CID spectra for the complexes DAP/GalNAc/2/4 (a), DIEN/GalNAc/3/5 (b), and DAP_2/GalNAc/4/6 (c).

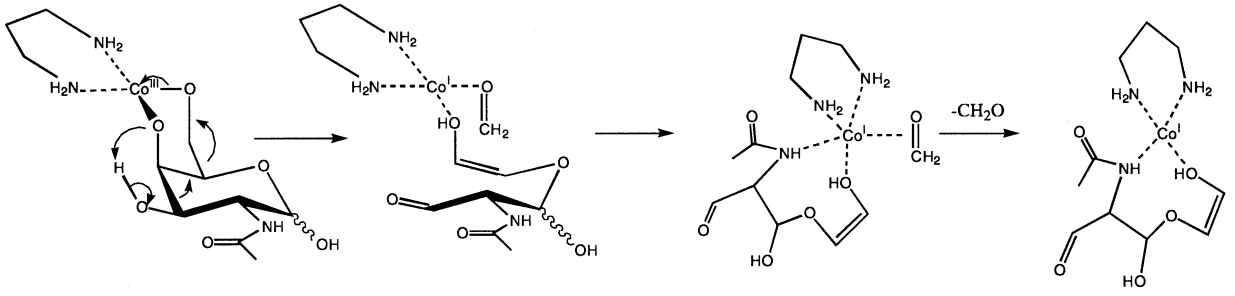

Fig. 12. New mechanism for the loss of CH_2O from the complex DAP/GalNAc/2/4.

GalNAc/4/6 [Figs. 11(A)– 11(c), respectively]. The number of electrons around the metal increases, from 14 to 16 to 18 for Figs. 11(a)–11(C), and correspondingly, the loss of CH_2O (which stabilizes an electron-poor cobalt) decreases.

4. Conclusions

By comparing CID spectra for various metal-ligated monosaccharide complexes, several conclusions can be drawn. First several different types of complexes provided spectra that could be used to differentiate the diastereomers. These complexes included the DIEN/Hex/3/5 and DAP_2/Hex/4/6 complexes **(1A and 1C)** for both the hexosamines and the *N*-acetylhexosamines, and the EN_2HexNAc/4/6 complexes **(1B)**. Second, for all of the coordination complexes studied, changing the size of the ligand (from DAP to EN) did not substantially affect the CID spectra. Finally, changing the number of coordinating nitrogens did effect product ions detected in the CID spectra. Using dissociation mechanisms that were studied in-depth previously, it can be demonstrated that, as in solution phase chemistry, both steric and electronic effects are important. These effects can be used to explain trends in reactivity for the different metal-ligated complexes. By considering the steric and electronic requirements of the metal-ligand systems, the accuracy of a proposed dissociation mechanism was probed, and a new mechanism, which is consistent with the expected trends in ion abundance for the different coordination complexes, is postulated. Thus, by conducting these studies we have not only probed the role of the auxiliary ligands in the Co-coordinated monosaccharide complexes, we have also developed an aid for assessing the validity of mechanisms proposed for various metal-coordinated complexes undergoing CID.

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