Investigation of metal complex coordination structure using collision-induced dissociation and ion–molecule reactions in a quadrupole ion trap mass spectrometer

Richard W. Vachet[a,1], JudithAnn R. Hartman[b], Jeffrey W. Gertner[b], John H. Callahan[a,*]

[a]Code 6115, Chemistry Division, Naval Research Laboratory, Washington, DC 20375, USA
[b]Department. of Chemistry, United States Naval Academy, Annapolis, MD 21402, USA

Abstract

A technique described previously using ion–molecule (I–M) reactions to determine metal complex coordination number has been combined with collision-induced dissociation (CID) in a quadrupole ion trap mass spectrometer. Nitrogen-containing tripodal ligands complexed with some first-row transition metals [i.e. Mn(II), Co(II), Fe(II), and Ni(II)] are generated by electrospray ionization, and the coordination number of these metal complex ions are determined in the gas phase using I–M reactions. Information about the coordination sphere structure (i.e. metal–ligand connectivity) is then gathered by dissociating the metal complexes, reacting their product ions, and comparing the coordination number of these product ions with their parent ions. Results suggest that a change in the coordination number upon dissociation allows identification of the functionality involved in coordination to the metal. Conversely, no change in the coordination number indicates either the lost functional group is not involved in the coordination or the ligand rearranges to fill a coordination site vacated by the dissociated group. Distinguishing between the two processes (i.e. when coordination number does not change) was very difficult in these studies, but results suggest that, in some cases, ligand rearrangement does not occur. Also, some product ions did not provide any useful coordination information upon reaction. In these cases the product ions either did not contain the metal or contained the metal in a reduced oxidation state. The preliminary results presented here are promising and suggest that with further investigation of the dynamics of metal complex I–M reactions, a combination of CID and I–M reaction may allow metal-complex coordination structure to be determined using mass spectrometry. (Int J Mass Spectrom 204 (2001) 101–112) © 2001 Elsevier Science B.V.

Keywords: Metal ion–ligand complex; Collision-induced dissociation; Ion–molecule reactions; Electrospray; Coordination number

1. Introduction

The development of electrospray ionization (ESI) [1,2] has dramatically expanded the areas to which mass spectrometric analysis can be applied. The ability to gently transfer nonvolatile analytes from the solution phase to the gas phase has enabled a variety of compounds to be studied by mass spectrometry. ESI combined with the speed and sensitivity of mass spectrometry and tandem mass spectrometry (MS/MS) has enabled the characterization of trace levels of biological molecules. The advances in biochemical

* Corresponding author. E-mail: callahan@ccf.nrl.navy.mil
[1]National Research Council/Naval Research Laboratory Postdoctoral Associate. Current address: Department of Chemistry, University of Massachusetts-Amherst.

structural analysis by mass spectrometry have been most evident in peptide/protein sequencing [3–6], but advances have also been made in the structural studies of oligonucleotides [7,8] and carbohydrates [9,10]. The impact of mass spectrometry on inorganic and organometallic chemistry has not been as substantial, but significant progress has been made in recent years [11]. Although a vast array of work has studied the gas-phase reactions of metal ions with small neutrals [12,13], structural studies of inorganic and organometallic complexes using mass spectrometry have not been as extensively pursued.

Like mass spectrometry studies of peptides/proteins, structural analysis of inorganic complexes has typically been carried out using collision-induced dissociation (CID) to generate a series of product ions from which ion structure can be deduced. Yet unlike CID of peptides/proteins in which the dissociation chemistry is well understood, the structural analysis of inorganic complexes by CID is not as straightforward. Rearrangement reactions involving the loss of small neutrals from large ligands or the loss of intact ligands from multiligated metal ions are both commonplace, but neither is necessarily very structurally informative [14,15]. Further, the dissociation chemistry has been shown to depend upon the metal in the complex [16,17]. The chemistry prompted by the energetics of CID can complicate identification of the structures and, particularly, the connectivities of metal–ligand complexes. Consequently, another mass spectrometric means of studying the ligation of metal complexes would be useful.

Ion–molecule (I-M) reactions have been used effectively to provide structural information often not directly accessible by CID. For example, hydrogen/deuterium (H/D) exchange reactions have been used to count the number of hydrogen atoms bonded to oxygen, nitrogen, and sulfur heteroatoms [18], and they have also been very effective in providing insight into the secondary structure of peptides and proteins [19–22]. Other examples of the use of I-M reactions as diagnostic tools include the differentiation of isomers [23–28], structural analysis of peptides by endothermic reactions [29–31], and the enumeration of the basic sites in peptides and proteins [32]. I-M

reactions have also been used in conjunction with CID to provide more selective structural information. For instance, CID followed by I-M reactions was used to selectively target a specific class of antibiotics [33]. More recently, we demonstrated that I-M reactions in a quadrupole ion trap can be used to determine the coordination number of metal complexes [34]. This method was based on the idea that coordinately unsaturated metal complexes could be "titrated" in the gas phase by reagent ligands to determine their coordination number. Other recent studies have also observed the reactivity of coordinately unsaturated metal complexes [35–40] and doubly charged transition metal complexes [41,42]. In one study, the reactivity of O_2 with coordinately unsaturated complexes of Cr(II), Ru(II), and Os(II) revealed the reversible adduction of O_2 to the Cr(II) and Ru(II) complexes but the oxidation of the Os(II) complex [36]. Another study used CID to prepare coordinately unsaturated Pt complexes that were subsequently reacted with small neutrals [37]. Coordinately unsaturated organometallic complexes of Ir [38,39] and Rh [40] have also been produced by CID of electrosprayed ions and reacted with neutrals to study the mechanisms of C-H bond activation [38,39] and homogeneous catalytic hydrogenation [40]. We present here the initial studies of I-M reactions combined with CID to provide the coordination sphere structure of a limited series of nitrogen-containing tripodal ligands with Mn(II), Fe(II), and Ni(II). These studies are meant to assess to potential of combining I-M reactions and CID to provide coordination structure and are not meant to be definitive. Coordination structure analysis is usually relegated to spectroscopic methods, but a structural analysis technique based on mass spectrometry would be potentially powerful, allowing the coordination structure to be determined for metal complexes that are only present at trace levels (e.g. metalloproteins) or are difficult to study by other techniques.

2. Experimental

Experiments were done on a Finnigan-Thermoquest LCQ quadrupole ion trap that has been modified

as described previously to allow the introduction of reagent gases into the analyzer region [34]. In addition to the modifications described in our previous work, other adjustments have been made to the instrument to facilitate reagent gas introduction and pressure measurement. The helium inlet system on the LCQ was replaced by a leak valve that allowed the variable addition of helium to the vacuum system. Also, notches were drilled into the quartz spacers between the ring electrode and the endcap electrodes. These notches allowed the free flow of the introduced gas throughout the vacuum system. This modification allowed the reagent gas pressure to be measured more accurately since the instrument's ion gauge was now more exposed to the gas load. A calibration of the pressure in the vacuum system was achieved by observing the reaction rate of the +11 charge state of ubiquitin with various pressures of NH_3 [28]. It should be noted that drilling the notches in the quartz spacers required the repositioning of the tuning screw associated with the rf coil to maintain the optimum rf drive frequency of 760 kHz.

Ions were generated by electrospraying 50:50 water:methanol solutions containing the metal complex of interest at a concentration of 100 μM. In a few cases metal complex ions that were difficult to synthesize and isolate were produced in the gas phase by spraying solutions that contained a mixture of a transition metal salt and the ligand of interest in a 50:50 mixture of water and methanol. The ligands and metal complexes used in these studies were synthesized as described elsewhere [34,43]. The metal salts $MnCl_2 \cdot 4H_2O$ and $NiCl_2 \cdot 6H_2O$ were obtained from Sigma Chemical Company (St. Louis, MO). The metal salts $Co(NO_3)_2$ and $FeCl_2 \cdot 6H_2O$ were obtained from Aldrich Chemical Co. (Milwaukee, WI).

The I-M reactions were carried out using the LCQ software to isolate the ions corresponding to the *m/z* of interest. Once isolated, the ions were allowed to react with a static pressure of the desired reagent gas for a chosen amount of time. An advanced scan definition editor was obtained from Finnigan-Thermoquest in order to vary the reaction time. Using the technique described recently [34], the coordination number of the metal complexes was determined from

the resultant reactivity. Four different reagent gases were used to assess the coordination chemistry of the metal complexes: pyridine, water, methanol, and ammonia. The following trend allowed the coordination number of the metal complexes to be determined: six-coordinate complexes are unreactive, five-coordinate complexes are only reactive with pyridine (adding only one pyridine molecule), four-coordinate complexes are reactive with ammonia (adding just one ammonia molecule) and pyridine (adding two pyridine molecules), and three-coordinate complexes are reactive with all the reagents including water and methanol (adding two pyridine, one ammonia or one water/methanol molecule). The standard protocol for determining the coordination number consisted of reacting the complex with more reactive ligands until a reaction was observed, and then comparing the result to the aforementioned qualitative trend. In general, a reaction was considered to have occurred if the product ion had a relative intensity greater than 20% after a 100 ms reaction time. These criteria are somewhat arbitrary, but based on previous results [34] 100 ms is sufficient time for most of these reactions to reach an equilibrium. The establishment of equilibrium was tested by allowing a reaction to proceed for the specified reaction period to establish the apparent product ion/parent ion equilibrium ratio. The product ion was then isolated and allowed to "react" for the same period. In such cases, the product ion/parent ion ratio was reestablished by dissociation of the product ion, indicating the parent and product ions are in equilibrium. In most cases, the product ion/parent ion ratios were greater than 10 (and many often greater than 100), far in excess of the 20% criterion indicated above. Typical reagent gas pressures for pyridine and ammonia were between 1×10^{-6} and 5×10^{-6} Torr (uncorrected for the reagent gas polarizability). Water and methanol from the electrospray solvent flow (2 μL/min) accumulated in the vacuum system at pressures sufficient enough to preclude the need to introduce these reagents separately. The helium pressure was usually maintained around 1.5×10^{-4} Torr (uncorrected). To gather information about coordination structure, as outlined below, the product ions of metal complex ions were reacted to determine their coordi-

nation number. In these experiments, the parent ion *m/z* was isolated and then made to undergo CID by the application of a resonant excitation signal for 30 ms at q_z=0.25. The resonance excitation voltage used in each case was the lowest amplitude needed to fully attenuate the parent ion's intensity. The resulting product ions were then isolated and allowed to react with the static pressures of the reagent gases, and their coordination numbers were determined.

Semiempirical calculations were carried out on one of the product ions of Mn(TREN-pyr)$^{2+}$ using Hyperchem (Hypercube, Inc., Waterloo, Ontario) to assess the relative stabilities of a series of isomers. Each isomer of the four-coordinate product ion of interest was first constructed as a tetrahedral complex (or as close as possible) before geometry optimization was done using Zerner Intermediate Neglect of Differential Overlap (ZINDO). Energy minimization was continued until there was minimal change (0.5 kcal/mol) in the ion's energy.

3. Results and discussion

The general concept for coordination structure determination is to compare the coordination number of a parent ion with its product ions. Two assumptions are made: (1) a change in the coordination number of the product ion relative to its parent ion suggests that the functionality lost was initially involved in the coordination to the metal and (2) the absence of any change in the coordination number upon formation of a product ion suggests that either the functionality lost is not involved in the coordination or a rearrangement occurs to fill a coordination site vacated by the dissociated functional group. The second possibility in assumption two would add significant ambiguity to coordination structure determination. The work presented here has focused on the CID and reactivities of metal complexes in order to test these assumptions. The elucidation of metal complex coordination structure in these studies consists of three basic steps. First, the coordination number of a parent ion is obtained using the I-M reaction protocol developed previously [34] and described previously. Second, the ion is

dissociated using CID, and a spectrum of product ions is collected. Finally, these product ions are subjected to I-M reactions to determine their coordination number. In effect, the I-M reactions guide the elucidation of the functionality involved in coordination using the data provided by CID.

The aforementioned method was tested using complexes of the ligands shown in Fig. 1 and some of the first-row transition metals [Mn(II), Fe(II), Co(II), and Ni(II)]. The following describes representative examples of these metal complexes. To demonstrate the feasibility of the I-M reaction/CID approach, 2:1 complexes of AM-pyr and the metals, Mn(II) and Ni(II), were generated in the gas phase and subjected to I-M reactions and CID. The mass spectra in each case (data not shown) confirm that two AM-pyr ligands are bound to the metals. The resultant ions, M(AM-pyr)$_2^{2+}$, are six coordinate, as revealed by their inability to react with any added reagent gas. Fig. 2 shows an example of the CID MS/MS spectrum of Mn(AM-pyr)$_2^{2+}$ (*m/z* 226.5). The MS/MS spectra of the Ni(II) complex with AM-pyr is similar (see Table 1). Four product ions from this spectrum are indicated with their appropriate neutral loss, and these four product ions were subjected to I-M reactions to determine their coordination number. Product ions at *m/z* 187 and 180 correspond to the loss of pyridine and 2-methylpyridine, respectively. (A description of the mechanisms involved in these and other dissociation reactions are beyond the scope of this article and therefore will not be addressed here. The proposed molecular formulas for the fragment losses, however, are listed in Table 1.) In both cases one of the four pyridines is lost. This should lower the coordination number of the resulting complex to five, and, in fact, the I-M reactions show that these product ions are five coordinate (the isolated CID product ion reacts with a single gas-phase pyridine molecule). Because the four pyridine moieties are chemically identical, the loss of one pyridine ring and the resulting coordination number determination (i.e. five) indicate that the nitrogen atoms from all four pyridine moieties are involved in the coordination. The product ion at *m/z* 166.5 is formed by the loss of a pyridine along with a secondary amine, which should be expected to lower
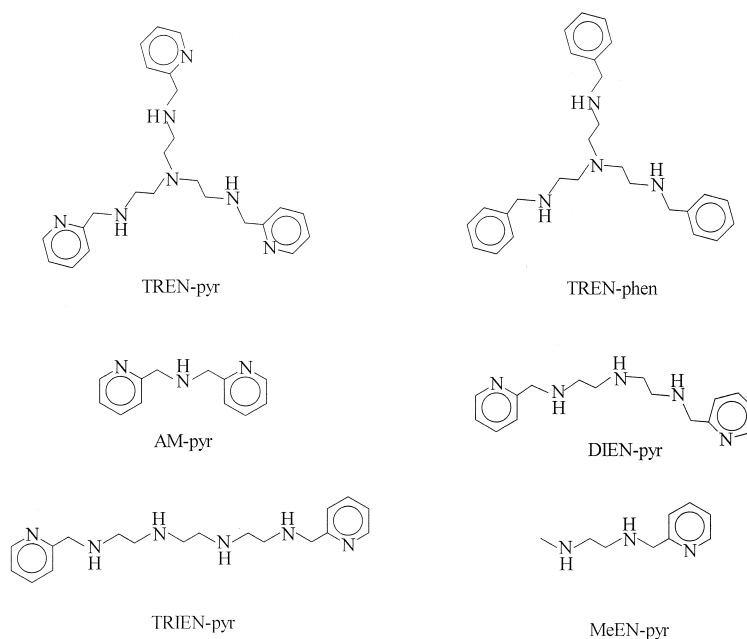
Fig. 1. Structures of the ligands used in this study.

the coordination number from six to four. I-M reactions confirm this assumption (the isolated CID product ion reacts with two gas-phase pyridine molecules, or one ammonia molecule). Finally, the product ion at m/z 253 corresponds to a loss of an entire AM-pyr ligand as a protonated species. The resulting product

Fig. 2. CID MS/MS spectrum of Mn(AM-pyr)$_2^{2+}$ (m/z 226.5). The structures correspond to the neutral fragment lost in the formation of the indicated product ion. Ion–molecule reactions were used to determine the coordination number (x) of each product ion, and this value is shown next to the corresponding product ion ($N=x$).

ion should be three coordinate because only one tridentate AM-pyr ligand remains coordinated to Mn(II). Using I-M reactions, the coordination number of this product ion is, indeed, determined to be three (the isolated CID product ion reacts with water and methanol, as well as ammonia and pyridine).

Not all the product ions in Fig. 2 provide information about the coordination structure of Mn(AM-pyr)$_2^{2+}$. The product ion at m/z 200 corresponds to the protonated ligand (AM-pyr+H)$^+$ and is the complementary ion to m/z 253. Because this product ion does not contain a metal, it is not expected to react with the reagent ligands, and indeed it does not. The same can be said for the ions at m/z 80, 94, and 109. The peaks at m/z 271 and 285 in Fig. 2 are not formed directly from the parent complex. These ions are the water and methanol adducts of m/z 253, respectively. The ability to react with water and methanol (electrospray solvents) is the test for a three-coordinate complex [34]. The presence of m/z 271 and 285 in the MS/MS spectrum of Mn(AM-pyr)$_2^{2+}$ confirms the result that m/z 253 is three-coordinate (as shown previously, where m/z 253 was isolated and found to react with
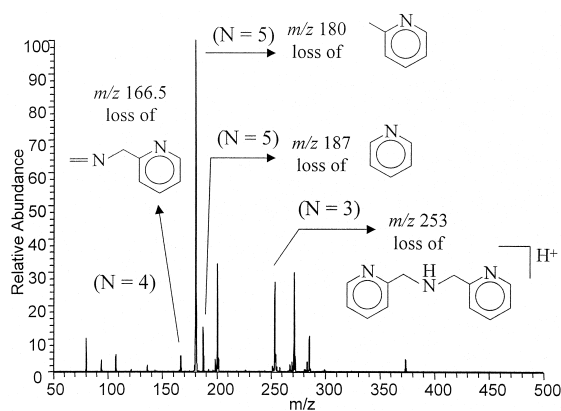
Table 1
Identity, *m/z*, and coordination number of the product ions formed during CID MS/MS of selected metal complexes; note: product ions that do not contain the metal or contain the metal in a reduced oxidation state are not listed

Complex (*m/z*, coord. no.)	Product ion *m/z*	Fragment loss(es)	Product ion coord. no.
Mn(AM-pyr)$_2^{2+}$ (226.5, 6)	253	$[C_5H_4B–CH_2–NH–CH_2–C_5H_4N]^{H+}$	3
	187.5	C_5H_5N	5
	180	$C_5H_4N–CH_3$	5
	166.5	$C_5H_4N–CH_2N=CH_2$	4
Ni(AM-pyr)$_2^{2+}$ (226.5, 6)	156	$[C_5H_4N–CH_2NH–CH_2–C_5H_4N]^{H+}$	3
	254	H_2 and $[C_5H_4N–CH_2–NH–CH_2–C_5H_4N]^{H+}$	3
	168	$C_5H_4N–CH_2N=CH_2$	4
Mn(TREN-pyr)$^{2+}$ (237, 6)	197.5	C_5H_5N	5
	183	$C_5H_4N–CH_2–NH_2$	4
	170	$C_5H_4N–CH_2–NH–CH–CH_2$	4
	168	H_2 and $C_5H_4N–CH_2–NH–CH_2–CH_3$	4
Fe(TREN-pyr)$^{2+}$ (237.5, 6)	198	C_5H_5N	5
	191	$C_5H_4N–CH_3$	5
	183.5	$C_5H_4N–CH_2–NH_2$	4
	176.5	$C_5H_4N–CH_2NH–CH_3$	4
	169.5	$C_5H_4N–CH_2–NH–CH_2–CH_3$	4
Co(TREN-pyr)$^{2+}$ (239, 6)	219	$C_5H_4N–CH_2–NH–CH_2–CH_3$ and $[C_5H_4N–H_2–N=CH_2]^{H+})$	3
	199.5	C_5H_5N	5
	185	$C_5H_4N–CH_2–NH_2$	4
	184	H_2 and $C_5H_4N–CH_2–NH_2$	4
	170	$C_5H_4N–CH_2–NH–CH_2–CH_3$	4
Ni(TREN-pyr)$^{2+}$ (238.5, 6)	199	C_5H_5N	5
	191	H_2 and $C_5H_4N–CH_3$	5
	184.5	$C_5H_4N–CH_2–NH_2$	4
	169.5	H_2 and $C_5H_4N–CH_2–NH–CH_2–CH_3$	4

water). The preceding example demonstrates that simple losses that are expected to lower the coordination number do produce the expected effect. Table 1 shows the data from a similar treatment of the Ni(AM-pyr)$_2^{2+}$ complex.

The preceding example shows that CID with I-M reactions can be used to characterize structure in a trivial system. A more complicated case is represented by the ligand, TREN-pyr (Fig. 1), which contains seven nitrogen atoms, of which only six are likely to be involved in a 1:1 complex with first-row transition metals. I-M reactions of Ni(TREN-pyr)$^{2+}$ (*m/z* 238.5) reveal that it is six coordinate, and the CID MS/MS spectrum is shown in Fig. 3. The four product ions labeled in Fig. 3 are useful for determin-
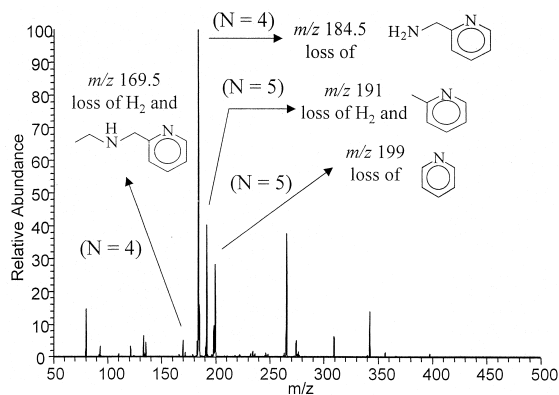

Fig. 3. CID MS/MS spectrum of Ni(TREN-pyr)$^{2+}$ (*m/z* 238.5). The structures correspond to the neutral fragment lost in the formation of the indicated product ion. Ion–molecule reactions were used to determine the coordination number (*x*) of each product ion, and this value is shown next to the corresponding product ion (*N* = *x*).

ing the coordination structure of Ni(TREN-pyr)$^{2+}$. The product ion at m/z 199 corresponds to a loss of a single pyridine group, and I-M reactions reveal that this product ion is five coordinate, suggesting that each of the chemically identical pyridine moieties is involved in the coordination to Ni(II). The product ion at m/z 184.5 corresponds to the loss of 2-aminomethyl pyridine. This product ion was determined by I-M reactions to be four-coordinate, which indicates that the two nitrogen atoms lost in its formation are involved in the coordination to Ni(II). Because each of the aminomethyl pyridine functionalities are chemically identical, each of these pairs of nitrogen atoms is likely involved in the coordination of Ni(II). The remaining tertiary amine in the center of the ligand is therefore not involved in the coordination of Ni(II). This result is confirmed by the product ion at 169.5, which corresponds to the loss of one entire arm of the TREN-pyr ligand (and H$_2$), yet it is found to still be only four coordinate (not five coordinate) by I-M reactions. The product ion at m/z 191 also provides useful information regarding the coordination of Ni(II) by TREN-pyr. A loss of 2-methylpyridine and H$_2$ leads to this product ion, and the resulting coordination number of the product ion is five, which is reasonable considering only a single coordinating pyridine moiety is lost.

As was observed in the MS/MS spectrum of Mn(AM-pyr)$_2^{2+}$ (Fig. 2), a few product ions formed from Ni(TREN-pyr)$^{2+}$ do not provide any coordination information. Peaks at m/z 80, 133, and 135 correspond to product ions that do not contain a metal and therefore are not expected to react. The product ions at m/z 265, 309, and 342, however, are uninformative for other reasons. None of these product ions was found to be reactive, but at least two of these product ions (i.e. m/z 265 and 342) contain Ni; the identity of the product ion at m/z 309 was undetermined. Based upon the masses of the fragments lost, the product ions at m/z 265 and 342 are probably both formed with the concomitant loss of radical cations. The product ion at m/z 342 corresponds to a complex in which Ni(II) has been reduced to Ni(I) and the ligand has lost one of its three arms. The product ion at m/z 135 is the complementary ion to m/z 342, and

it is a radical cation with the following formula: C$_5$H$_4$N-CH$_2$-NH-CH$_2$-CH$_2^+$. Radical cation formation suggests that the Ni has been reduced. The ion at m/z 265 mostly likely corresponds to the loss of one arm of the ligand and an additional pyridine ring. Because these two losses occur separately, a complementary ion at m/z 212 is not expected, but a radical cation ion at m/z 133 along with a neutral pyridine explains this dissociation. Again, the presence of a radical cation product suggests that Ni has been reduced in the metal complex at m/z 265. Previous studies revealed that metals in an oxidation state of 1 were much less reactive than their +2 counterparts and did not follow the same reactivity trend as metals in an oxidation state of 2 [34]. Consequently, the reduction of the metal in the formation of a particular product ion yields an unhelpful result under the current reaction scheme. The results of the complexes of TREN-pyr with Fe(II), Co(II), and Mn(II) provide similar results and are summarized in Table 1.

A possible problem with the approach described in this work is that dissociation of metal complexes could lead to the formation of product ions that, when reacted, do not definitively identify the metal coordination sites. A lack of product ion channels or an abundance of product ions that do not contain the metal or contain the reduced metal would limit the coordination information available by this method. Fig. 4 demonstrates this possibility with the MS/MS spectrum of Co(TREN-phen)$^{2+}$ (m/z 237.5). I-M reactions reveal that this complex is four-coordinate, but only one of the product ions is directly useful for providing coordination information. The product ion at m/z 191.5 corresponds to a loss of toluene, and the resulting coordination number of this product ion is determined to be four. Toluene loss in this case is significant because it shows that the loss of a group not involved in coordination results in no coordination number change. Unfortunately, no other product ions in the MS/MS spectrum (Fig. 4) provide information about the coordination of Co(II) in this complex. The product ion at m/z 91 corresponds to the benzyl (or tolyl or tropylium) cation and therefore is not helpful. The product ions at m/z 234.5, 235, and 235.5 correspond to product ions formed by multiple dehy-
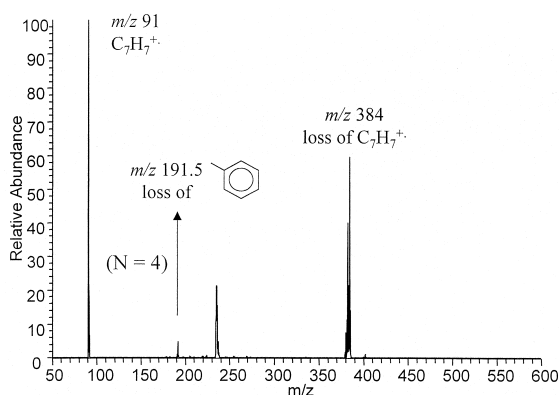
Fig. 4. CID MS/MS spectrum of Co(TREN-phen)$^{2+}$ (m/z 237.5). The structure corresponds to the neutral fragment lost in the formation of m/z 191.5. Ion–molecule reactions were used to determine the coordination number (x) of this product ion, and its value is shown next to the product ion ($N=x$).

rogenations and/or hydrogen losses. The mechanisms for the formation of these products ions is not completely understood at this point, but none of these product ions is valuable for providing coordination information. The remaining product ions at m/z 384 and 382 correspond to species in which Co(II) has been reduced to Co(I) and therefore do not provide any additional insight. Although Co(TREN-phen)$^{2+}$ represents a trivial case in which a coordination number of four strongly implicates all four nitrogen atoms as the coordinating atoms, no product ions directly identify these nitrogen atoms. Unfortunately, mass spectrometry experiments in which the product ions of m/z 191.5 are observed do not reveal any ions that correspond to a loss of a nitrogen functionality. From these studies, metal complexes [including Mn(II), Fe(II), and Ni(II)] with this ligand represent uncommon cases in which the CID MS/MS spectra contain product ions that do not provide coordination structure information upon reaction with the reagent ligands. Very likely the presence of the benzene functionality and its ability to provide a very facile dissociation pathway (i.e. benzyl cation formation) prevents the formation of product ions that would otherwise provide more direct coordination information.

An assumption fundamental to the CID I-M reac-

tion approach is that initially non-coordinating groups do not rearrange to fill coordinating positions. Loss of functionality involved in coordination and the subsequent occupation of a coordination site by an initially uncoordinated group would introduce ambiguity into the structural analysis. In the previous examples and in most complexes studied thus far, this potential hindrance does not appear to occur. For example, when M(TREN-pyr)$^{2+}$ complexes fragment to lose an entire arm, the resulting ligand product is similar in structure to DIEN-pyr, which has been shown in previous studies to be a pentacoordinate ligand. However, the product ion that results from CID of M(TREN-pyr)$^{2+}$ has a coordination number of four, not five. A coordination number of five would be expected if the complex rearranged to allow the (formerly) tertiary nitrogen to interact with the metal. An explanation for the absence of rearrangement is that the coordination interactions between the other two arms and the metal ion are not disrupted during the loss of the third arm. In fact, the loss of the electron donating properties of the third arm would tend to strengthen the remaining interactions. Given that the other two arms of the ligand remain strongly coordinated to the metal, the tertiary nitrogen may not be able to move into a coordinating position.

Although the above observation supports the argument that initial strong coordination interactions are not disrupted (other than those involving the moiety that is lost), it is not the only possible explanation for the absence of rearrangement following CID of M(TREN-pyr)$^{2+}$ complexes. When one arm of the ligand is lost (with transfer of hydrogen to the neutral), a π bond must be formed between the (formerly) tertiary nitrogen and an adjacent carbon, thus the resulting ligand does not have a structure identical to DIEN-pyr. This would force some rigidity on the ligand structure, and it can be argued that this may also inhibit rearrangement. A more stringent test of the possible effect of rearrangement is found in the dissociation of the Mn(TREN-pyr)$^{2+}$ complex, wherein fragmentation produces a product ion with a structure nominally identical to Mn(DIEN-pyr)$^{2+}$. Under these circumstances, the product ion has an even better chance of undergoing rearrangement. The

CID MS/MS spectra of Mn(TREN-pyr)$^{2+}$ (data not shown) and Ni(TREN-pyr)$^{2+}$ (Fig. 3) are very similar. However, one low intensity product ion (*m/z* 170) is observed in the CID MS/MS spectrum of Mn-(TREN-pyr)$^{2+}$ that is not seen in the CID MS/MS spectrum of Ni(TREN-pyr)$^{2+}$. This product ion corresponds to the loss of an entire arm of the TREN-pyr ligand with the subsequent transfer of H from the departing neutral, presumably, to the tertiary nitrogen. This product ion should be identical to a 1:1 complex of DIEN-pyr (Fig. 1) with Mn(II). I-M reactions of Mn(DIEN-pyr)$^{2+}$ reveal that Mn(DIEN-pyr)$^{2+}$ is five coordinate, indicating that all five nitrogen atoms are involved in the coordination of Mn(II). I-M reactions of *m/z* 170 from CID MS/MS of Mn(TREN-pyr)$^{2+}$, however, reveal that this product ion is more reactive than Mn(DIEN-pyr)$^{2+}$. This product ion reacts like a four-coordinate complex, although the extent of reaction is near the threshold chosen for a positive reaction, suggesting that some fraction of the ions could be five coordinate. Thus, some ions may have undergone rearrangement to fill a fifth site, a significant portion have a four-coordinate structure. Alternatively, the fraction of ions that are seemingly five coordinate may instead represent a case where the noncoordinating secondary amine simply hinders the reaction of a truly four-coordinate system [44]. The overall result suggests that the tertiary amine was probably not initially coordinated to Mn(II) in Mn-(TREN-pyr)$^{2+}$ and cannot, in every case, position itself to fill one of the coordination sites left vacant by the departing neutral fragment even though this amine has been converted to a secondary amine (Fig. 5, structure **I**).

The above mentioned argument assumes that when one arm of the TREN-pyr ligand is lost, a hydrogen is transferred to the former tertiary amine, effectively producing DIEN-pyr. The apparently four-coordinate product ion, however, could be the result of a completely different isomer. Another possible scenario involves the transfer of the H from the departing neutral to a nitrogen other than the tertiary nitrogen. The net outcome of such a process is a change in the nitrogen atoms that are involved in coordination. The resulting structure (Fig. 5, structure **II** or **III**) would
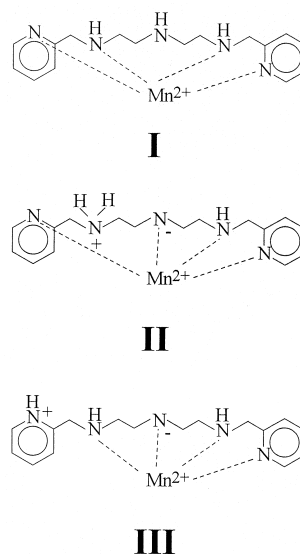

Fig. 5. Three possible structures for the product ion of Mn(TREN-pyr)$^{2+}$ at *m/z* 170. Semiempirical calculations suggest that structure **I** is the most stable (see text).

involve the interaction of Mn with the now negatively charged tertiary nitrogen and the protonation of one of the other nitrogen atoms. In structures **II** and **III**, one of the nitrogen atoms that was coordinated to Mn in the parent ion is no longer coordinated. Although structures **II** and **III** cannot be refuted by the data at hand, rearrangement to such structures is unlikely. Semiempirical calculations show that structure **I** is ~18 kcal/mol lower in energy than structure **II** and ~69 kcal/mol lower in energy than structure **III**. These calculations and the aforementioned CID/I-M reaction results suggest that the product ion at *m/z* 170 is similar in structure to Mn(DIEN-pyr)$^{2+}$ but with only four of the nitrogen atoms interacting with the metal instead of five. The absence of ligand rearrangement cannot be definitively proven with the data at hand, thus further studies into the likelihood of ligand rearrangement and ways to identify this process are needed. Consequently, at this point, in cases where ligand rearrangement could likely occur, very limited information should be inferred from dissociations that result with no change in coordination number unless the dissociation patterns of the complex are very well understood.

An interesting point to note is that I-M reactions reveal that $Mn(TREN-pyr)^{2+}$ is six coordinate, whereas recent crystallographic studies of this complex [45] suggest that the complex is seven coordinate (with the tertiary nitrogen coordinated to the metal). Our reaction scheme cannot distinguish between a six- and seven-coordinate species, but CID results argue against an interaction between the tertiary amine and the metal. In particular, if the tertiary nitrogen is involved in coordination, loss of a pyridine moiety should not change the reactivity of the product ion because it should still be six coordinate. The observed change in reactivity argues that the gas-phase structure of $Mn(TREN-pyr)^{2+}$ is described by a six-coordinate complex with the loss of the pyridine generating a five-coordinate product ion. Loss of a pyridine group is very unlikely to change the tripodal structure of this ligand, so the orientation and thus coordination (or lack thereof) of the tertiary nitrogen should not change very much. An explanation for the discrepancy between the gas-phase and crystallographic results is not available at this point, but future studies will address such issues as the relationship between solid-state and gas-phase structures in metal complexes.

Another issue that can be raised regarding the gas-phase reaction of metal complex ions with reagent ligands is the possibility of the reagent ligand displacing a weakly coordinating site. Such an event would introduce significant ambiguity into the method. In none of the cases described previously or studied previously [34] did ligand displacement appear to occur. The reactions of the complex $Ni(TRIEN-pyr)^{2+}$ with pyridine, however, may represent a case in which a weakly coordinating atom is displaced by pyridine. The solid-state structure of this complex has been reported previously in the literature [46]. The complex was found to be a distorted octahedron with all six nitrogen atoms coordinated to give five chelate bond angles averaging 81.7° (as compared to the ideal bond angle of 90°). In contrast, I-M reactions suggest that this complex is five coordinate (i.e. reacts with one pyridine only) in the gas phase. One explanation for the discrepancy is that the TRIEN-pyr is under significant steric strain to maintain the octahedral geometry observed in the crystal structure. This suggestion is supported by the observed distortions to the octahedral coordination sphere. Quite possibly in the gas phase this complex relaxes to give a less sterically strained five-coordinate geometry. Another explanation assumes the complex retains its six-coordinate geometry in the gas phase, but the distorted octahedron causes one of the nitrogen atoms to be weakly coordinating. Upon reaction with pyridine this weakly coordinated nitrogen is then displaced by a pyridine. Unfortunately, our gas-phase results cannot currently distinguish between the two possibilities. Interestingly, gas-phase reactions of a 2:1 complex of MeEN-pyr (Fig. 1) with Ni show that $Ni(MeEN-pyr)_2^{2+}$ is six coordinate. MeEN-pyr is essentially half of the TRIEN-pyr ligand and thus consists of the same number and type of coordinating nitrogen atoms but in a more flexible arrangement. This result suggests that it is the strained nature of the TRIEN-pyr around Ni(II) that causes the observed reactivity of $Ni(TRIEN-pyr)^{2+}$ (i.e. five coordinate). Studies of the thermodynamics of the gas-phase reaction of $Ni(TRIEN-pyr)^{2+}$ with pyridine are currently underway with the hope of providing insight into the dynamics of this reaction. Ligand displacement by the reagent gas should be recognized as a significant drawback to the technique described previously, and studies to identify its likelihood will be crucial.

4. Conclusions

The initial studies of a method combining ion–molecule (I-M) reactions and CID that attempts to determine the coordination structure of metal complexes have been described. The technique relies on a comparison of the coordination number, as determined by I-M reactions, of a parent ion with its product ions to determine the functionality involved in metal binding. Results show that a decrease in the coordination number of a product ion relative to its parent ion identifies that the lost functionality is involved in the coordination to the metal. Conversely, no change in the coordination number of the product ion relative to its parent ion suggests one of two

possibilities. One, the lost functional group is not involved in the coordination, or two, ligand rearrangement occurs to fill a site vacated by the dissociating functional group. Distinguishing between the two possibilities is difficult at the present time, but in at least one instance $(Mn(TREN-pyr)^{2+})$ ligand rearrangement seems to be hindered to some extent. Future studies will attempt to distinguish between the two possibilities and investigate the likelihood of ligand rearrangement in other metal–ligand complexes.

In some cases the product ions formed during CID did not provide any information upon reaction with the reagent ligands. These uninformative product ions were ions that either did not contain the metal or contained the metal in a reduced oxidation state and thus did not react according to the previously described method [34]. Finally, only very limited evidence was found for the displacement of coordinating functionality by the reagent ligands (e.g. pyridine), which would add some ambiguity to the measurement. Studies are underway to assess the applicability of this technique for the coordination structure determination of metal–peptide and metal–oligosaccharide complexes and other complexes with oxygen and sulfur functionality. These types of complexes may be more likely to be affected by the two aforementioned concerns (i.e. ligand rearrangement and ligand displacement).

Acknowledgements

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