Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Mass Spectrometry 227 (2003) 97–109
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Probing the interactions of oxidized insulin chain A and metal ions using electrospray ionization mass spectrometry

Yen-Peng Ho*, Hsin-Piao Li, Li-Chuan Lu

Department of Chemistry, National Dong Hwa University, Hualien, Taiwan, ROC

Received 15 September 2002; accepted 13 November 2002

Dedicated to Professor Robert C. Dunbar on the occasion of his 60th birthday.

Abstract

This work examines the interactions between a large polypeptide, oxidized insulin chain A (ICA), and several metal ions (Na^+, K^+, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Pd^{2+}) by using tandem mass spectrometry. Fragmentation spectra of the anionic complexes show that each metal ion complex undergoes specific types of interactions, suggesting that different conformations are favored for each complex. The overall results are consistent with the data obtained from ion mobility measurements. Thus, the tandem mass spectrometry approach represents a complementary method for probing the conformations of metal–peptide complexes. The fragmentation spectra obtained from the complexes of the enzyme-digested ICA fragments with metal ions also reflect specific metal–peptide interactions parallel with those for corresponding metal–ICA complexes.

Keywords: Insulin chain A; Tandem mass spectrometry; Metalated complexes; Metal–peptide interactions; Enzymatic digestion

1. Introduction

The interactions between metal ions and proteins are related to many important biological processes [1–3]. The coordination sites in proteins are specific for particular metal ions and the metal ions induce conformational changes to optimize the metal ion–ligand interactions [2–5]. Each metal ion prefers certain types of ligand interactions. Some prefer to form loose complexes and some to form tight complexes. The differences in preferred binding interactions may explain why a protein can be either activated or inhibited by coordination with certain metal ions [6]. Generally, peptides with known or designed sequences are studied before proteins to understand specific interactions between the metal ions and proteins. According to the previous studies, electrospray ionization mass spectrometry (ESI-MS) can elucidate protein/peptide–metal interactions in the gas phase [7–20], which are directly related to those in solution. Recent reports of using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry have also revealed that the interactions of metal ions with peptides can be studied [21–29]. Collisionally activated dissociation (CAD) has been used more widely than several other mass spectrometric techniques to clarify specific interactions of metal ions and peptides. In general, fragmentation patterns obtained using

* Corresponding author. Tel.: +886-3-8662500x21223; fax: +886-3-8661487.
E-mail address: ypho@mail.ndhu.edu.tw (Y.-P. Ho).

1387-3806/03/$ – see front matter
doi:10.1016/S1387-3806(03)00049-6

CAD depend on the coordination chemistries of metal ions with peptides. Several studies of protein fragmentation have established that the conformation of the protein also influence fragmentation spectra [30].

In addition to CAD studies, several methods including ion mobility [31–34], H/D exchange [35–37], collision cross section [38], and kinetic energy release measurements [39] can be used to investigate the conformation of polypeptides. In particular, high-resolution ion mobility measurements done by Clemmer et al. have shown that many conformations exist for the oxidized insulin chain A (ICA) anions when they are complexed to metal ions. Multiple configurations for each transition metal–peptide ion are observed due to specific metal–peptide interactions. The authors also reported that the cross sections derived from ion mobility experiments for non-metalated ICAs increased with increasing charge states from −2 to −6. The −2 and −3 charge states have small cross sections corresponding to highly compact (roughly globular) structures. The −5 and −6 charge states have cross sections corresponding to open (linear) structures, which are induced by repulsive Coulombic forces. The cross sections of the −4 charge state, where repulsive Coulombic forces are roughly balanced with attractive forces, has a value between those for the highly compact structures and those for the linear structures. The authors defined six major conformer types for the non-metalated and metalated ICA ions. The conformation I–VI have increasing cross sections with a value from the smallest (410–430 $Å^2$) to the largest (>495 $Å^2$). For instance, conformation V is favored for the non-metalated ICA with a −4 charge state.

Our long-term goal is to elucidate the intrinsic interactions of metals and real biomolecules in the gas phase using mass spectrometry. To this end, we first considered a relatively large, readily available system, ICA (with 21 amino acid residues). Furthermore, ion mobility data should offer a good "reference point" for the results. Electrospray ionization-tandem mass spectrometry was used in the negative ion mode to investigate the interactions between ICA and various metal ions (Na^+, K^+, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pd^{2+}). The results obtained for such sizable polypeptide systems provide evidence for the formation of metal ion-dependent conformers and complement the data obtained by measuring ion mobilities.

2. Experimental

2.1. Materials and sample preparation

Insulin chain A (oxidized) and pepsin were purchased from Sigma (St. Louis, MO, USA). ICA is a 21-residue peptide that is part of the insulin hormone. The peptide has four cysteine residues that are oxidized to -SO_3H. HPLC grade methanol was purchased from Mallinckrodt Baker Inc. (Paris, KY, USA). Potassium bromide, and iron(II) chloride were from Acros (Geel, Belgium). Manganese(II) chloride, nickel(II) acetate tetrahydrate, cobalt(II) chloride hexahydrate, copper(II) acetate, zinc(II) acetate tetrahydrate, and palladium(II) chloride were purchased from Aldrich (Milwaukee, WI, USA). Sodium chloride was from Wako (Japan). The non-metalated ICA solution was prepared in 1:1 methanol/H_2O (v/v) at a concentration of 1×10^{-5} M. To form metal ion complexes of ICA, a 1×10^{-5} M ICA solution in 1:1 methanol/H_2O was mixed with the same volume of a 1×10^{-5} M solution of metal ion salt in 1:1 methanol/H_2O.

2.2. Digestion of ICA

Pepsin was dissolved in 2% acetic acid. The ICA sample in 2% acetic acid at a concentration of 5×10^{-5} M was mixed with the enzyme solution at an enzyme to substrate ratio of 1:100 (mol/mol) and incubated for 1.5 h at 37 °C. The digest solution was dried in a vacuum centrifuge equipped with a liquid nitrogen trap. The dried samples were dissolved in a methanol/H_2O (1:1 v/v) solution of metal ion salts before MS analysis.

2.3. Mass spectrometry

All experiments were performed using a Finnigan LCQ Duo ion trap mass spectrometer (San Jose, CA,

USA) equipped with an electrospray ionization source. The sample solution was infused at a flow rate of 2.5 μl/min. The autogain control (AGC) was maintained at 3×10^7 for MS scans, and 2×10^7 for MS/MS target, with microscan count of 3 and maximum injection time of 100 ms. The spray voltage was maintained at −3 kV. The capillary voltage and temperature were kept at 31 V and 100 °C, respectively. Collisionally activated dissociation (CAD) was performed in the ion trap region. Effective isolation-width for CAD experiments was set at 1.5 Da. The collision energy was set at 30% of the maximum instrument setting, which had an activation value of $q = 0.25$. Helium was used as collision and buffer gas at a pressure of approximately 1×10^{-3} mbar.

3. Results and discussion

Non-metalated ICA systems were examined first, in order to understand the effects of metal ions on the structures of metal ion-bound ICA complexes. The spectrum of ICA anions reveals predominant charge states of −4 and −3, with −2 and −5 being minor states (Fig. 1a). The ICA peptide has six acidic residues, including four sulfated cysteine (Cox) and two glutamic acid (E) residues and the C-terminus (N) as potential deprotonation sites. The $[ICA\text{-}4H]^{4-}$ ions were chosen for CAD studies. The CAD spectrum of $[ICA\text{-}4H]^{4-}$, as shown in Fig. 1b, contains mainly c type ions and the ions corresponding to the losses of neutrals from the precursor ions. The other types of ions, including a, b, y, z and internal ions, were also observed. The nomenclature used in this work is generally based on that of Biemann and coworkers [40]. The symbol $(b_m y_n)_o$ refers to an internal fragment of the 21-residue peptide, which contains o residues between the m and $22 - n$ amino acid residues. Most ions formed were accompanied by neutral losses. The loss of mass 18 represents the elimination of H_2O and may be indicative of glutamic acid (E) residues [41]. The mass of 17, 44, 56, 61, 88 and 106 presumably corresponds to the neutrals: NH_3, CO_2, 2 CO, NH_3 plus CO_2, 2 CO_2 and the tyrosine side chain. Loss of tyrosine side chain has been reported in the negative ion mode [42–44]. Loss of 17 is indicative of glutamine (Q) or asparagine (N) residues [41]. Loss of CO_2 has been reported to be associated with the C-terminal residue of anionic peptides [43,45–47]. In the dissociation of the ICA complex, loss of CO_2 is also indicative of the glutamic acid residues (E).

Fig. 2 displays CAD spectra of sodium and potassium–ICA complexes ($[ICA + Na]^{4-}$ and $[ICA + K]^{4-}$). For simplicity, the formula is not balanced with respect to hydrogen in the employed notations. The spectra are dominated by the ions that are associated with losses of neutrals from the precursor complexes and the loss of the C-terminus ($[c_{20}+Na]^{4-}$ and $[c_{20}+K]^{4-}$). The neutral loss of mass 30 matches a loss of CH_2O from serine [17,28,45]. Neutral losses of mass 48 and 60 correspond to the elimination of CH_2O plus H_2O and two CH_2O neutrals, respectively. The $[c_{20} + Na]^{4-}$ and $[c_{20} + K]^{4-}$ ions correspond to the loss of the C-terminal amino acid. An asterisk indicates metalated ions. For instance, $[c_{20} + Na]^{4-}$ is denoted as $[c^*_{20}]^{-4}$. The fragments from the two complexes are similar except in that more fragment ions were generated by the losses of neutrals from the potassium complex. The two CAD spectra and that of the non-metalated ICA all show prominent ions ($[c_{20}]^{4-}$ or $[c^*_{20}]^{-4}$) corresponding to the loss of the C-terminal amino acid. However, $[ICA\text{-}4H]^{4-}$ produced a significant amount of the $[y_{19} - 44]^{3-}$ and $[z_{19}]^{3-}$ ions, which were not observed from alkali metal ion complexes. Further, more internal fragments were observed from the $[ICA\text{-}4H]^{4-}$ ion.

In the ion mobility experiment at 390 K, the cross sections of both the sodium complex and the $[ICA\text{-}4H]^{4-}$ ions did not change substantially and the complexes were assigned to the type V conformers. The common fragmentation pathways (losses of the C-terminal amino acid, tyrosine side chain and H_2O) revealed in the spectra of Figs. 1b and 2a may indicate the presence of similar conformations. However, the bond cleavages from the non-metalated ICA leading to the formation of other major fragment ions seem to occur at many amino acid residues. This suggests that the non-metalated ICA may also assume another

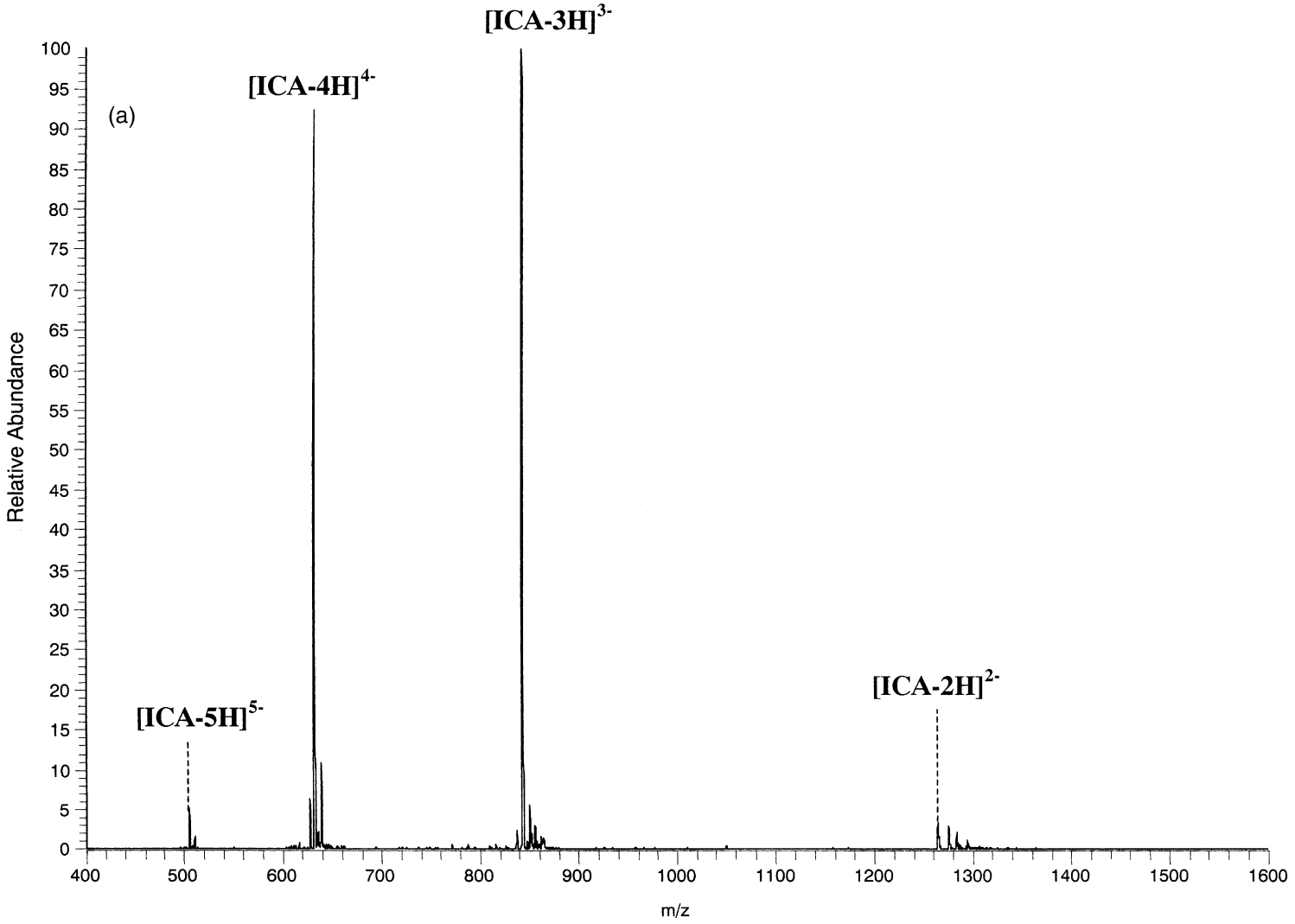

Fig. 1. (a) Spectrum of ICA anions. (b) The CAD spectrum of $[ICA\text{-}4H]^{4-}$. Potential deprotonation sites in the sequence are in boldface. Cox in the sequence stands for sulfated cysteine.

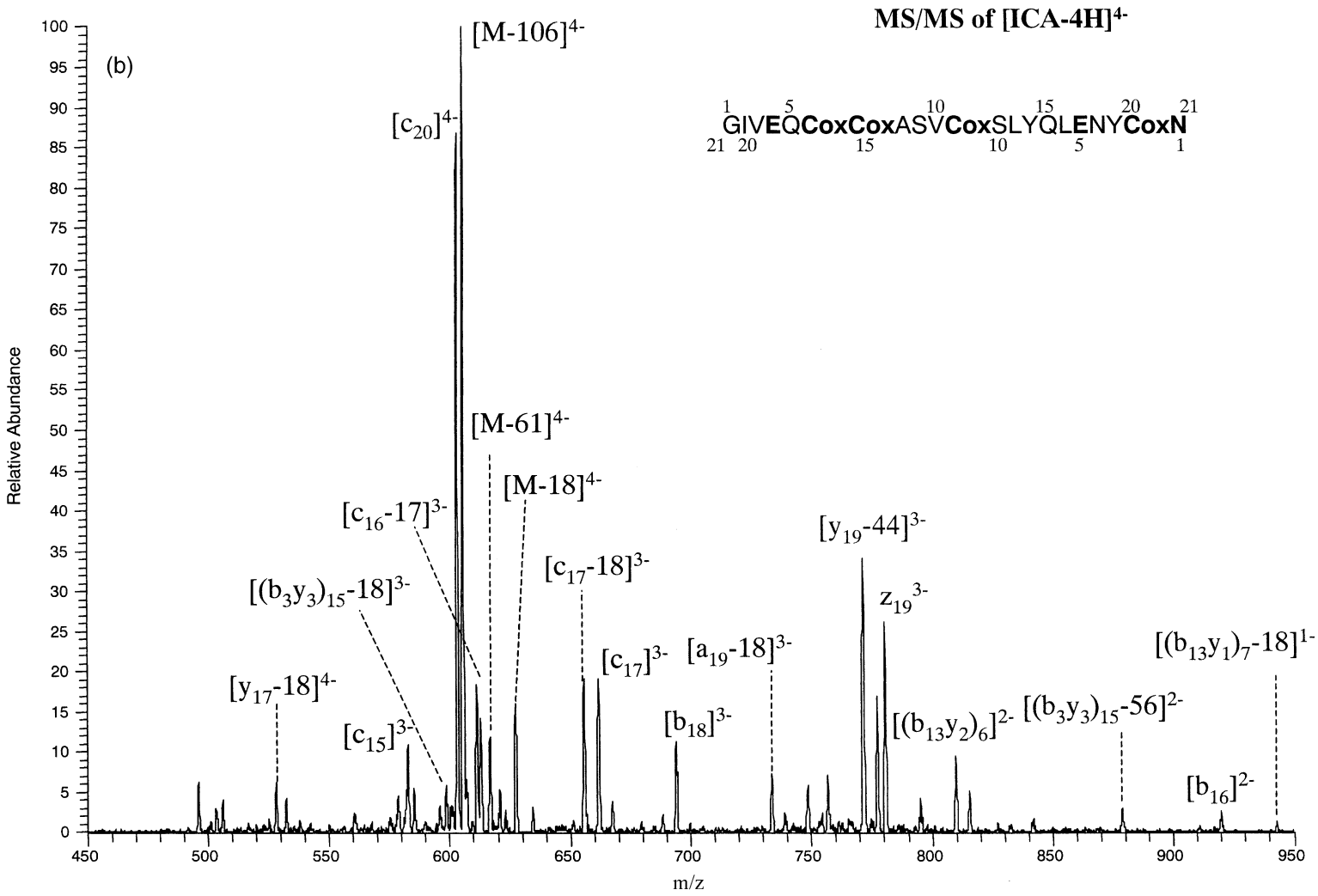

Fig. 1. (*Continued*).

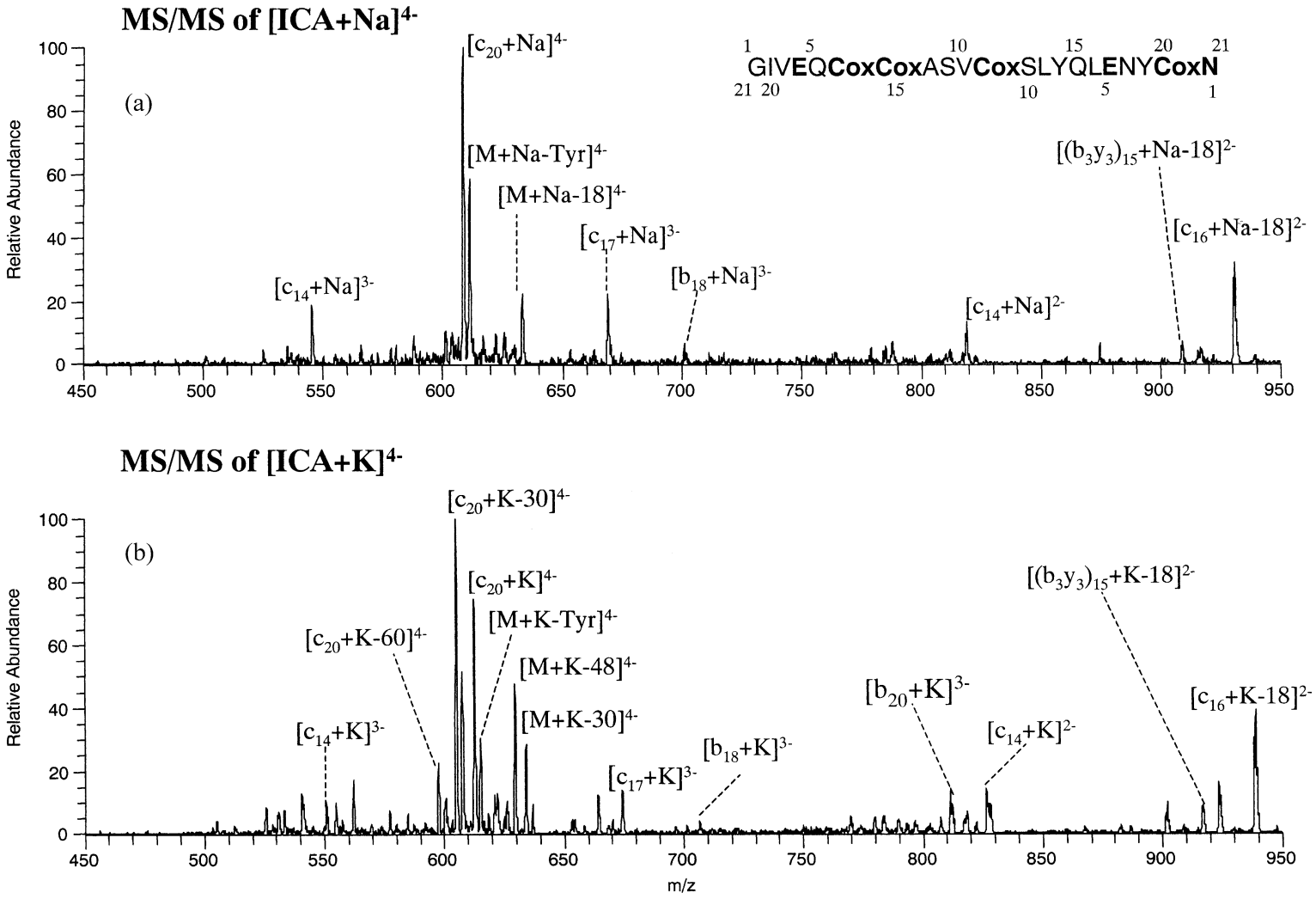

Fig. 2. CAD spectra of (a) sodium ($[ICA + Na]^{4-}$) and (b) potassium ($[ICA + K]^{4-}$) ICA complexes.

conformation. Notably, although the ions were electrosprayed into a heated capillary at a temperature (373 K) close to that in Clemmer's experiments, the instrumental setups for these two experiments definitely differ. Therefore, our CAD results do not necessarily reflect the exact conformations derived from the ion mobility data. Furthermore, the tandem mass spectrometric method would be unlikely to solve multiple conformations independently and should be considered complementary to the ion mobility method.

CAD of $[ICA + Mn]^{4-}$ (Fig. 3) resulted in major ion fragments formed by the losses of the C-terminal amino acid (N) and neutrals. The dominance of the neutral-loss fragments is very obvious. In addition to the neutral-loss fragments as observed from the potassium complex, the neutral loss of mass 78 was observed, indicative of the combined losses of two CH_2O neutrals and H_2O. The loss of CH_2O from the serine residues implies interactions between the metal ion and the serine side chains [17] in addition to the most probable interaction sites including SO_3^- and COO^- [31]. The reason for this interpretation is that the losses of CH_2O did not occur in the non-metalated system. Interestingly, less abundant ions associated with the loss of CH_2O were also obtained from the potassium complex but not from the sodium complex. For these two complexes, the larger radius of the potassium ion might make the metal–serine interaction more accessible and induce such neutral loss. The overall fragmentation chemistries for the Mn^{2+} complex indicate that Mn^{2+} interacts with ICA to form a stable complex such that the fragmentation only occurs at side chains and the C-terminus. It may be argued that the lack of extensive fragmentation is due to the large size of ICA. However, the non-metalated ICA ions did yield some fragments other than those arising from the losses of neutrals and the C-terminus (Fig. 1b). At 390 K, the cross sections (~450–470 Å) of the Mn^{2+}–ICA complex determined by ion mobility experiments are smaller than those (~485 Å) of non-metalated- and sodium–ICA complexes. The structure was assigned as conformation III. The authors believed that divalent ions stabilized compact conformers to yield relatively small cross sections. This is consistent with our CAD results. The fragmentation patterns of Fe^{2+}, Co^{2+} and Zn^{2+}–ICA complexes are similar to that of the Mn^{2+}–ICA complex (data not shown).

The CAD spectra of Ni^{2+} and Pd^{2+}–ICA complexes (Fig. 4) reveal a striking change in the fragmentation patterns. The spectra are dominated by $[y_{17}]^{3-}$ and $[b_4 + Ni]^-$ or $[b_4 + Pd]^-$. The two complementary products are formed by cleavage of the Glu–Gln (E–Q) amide bond, with metal ions remaining attached to the b_4 fragment. The results suggest that both Ni^{2+} and Pd^{2+} (d^8 configuration) preferentially bind with the four N-terminal amino acids (i.e. the GIVE segment). Although, a favorable attachment of sodium ion to a specific sequence such as the PPGF segment has been reported [28], it would be expected that the larger (y_{17}) of the two fragments would more strongly bind the metal ion [48] due to enhanced coordination with SO_3^- and COO^-. Further, it seems peculiar to have ions existing as $[b_4 + Ni^{2+}]^-$ or $[b_4 + Pd^{2+}]^-$ although the observed masses were correct. Unfortunately, CAD of b_4^{*-} only yielded fragment ions corresponding to the losses of H_2O and CO_2. To resolve this problem may require some theoretical calculations. The results seem to indicate that the two metal ion complexes form very loose conformers. Interestingly, the ion mobility results show that the nickel system effectively selects for a new type of conformation VI with cross section > 495 Å. Our findings may explain the existence of this loose conformation.

Fig. 5 shows the CAD spectrum of $[ICA + Cu]^{4-}$. As well as the fragments corresponding to the losses of neutrals from the precursor ions and the loss of the C-terminus ($[[C_{20}^*]^{4-}$), prominent $[y_{17}]^{3-}$ ions characteristic of the dissociation of the Ni^{2+}–ICA complex were observed. The losses of mass 44 and 88 represent the losses of one and two CO_2 molecules. The fragmentation results suggest that the Cu^{2+}–ICA complex includes at least two conformers, one similar to that of Ni^{2+}–ICA and the other similar to those of Mn^{2+}–ICA. Interestingly, CAD of $[ICA + Cu]^{5-}$ yielded exclusively $[y_{17}]^{4-}$ and $[y_{15}]^{4-}$ ions and the corresponding H_2O loss fragments (data not shown). Ions associated with the losses of neutrals from the precursor ions and the loss of the C-terminal residues

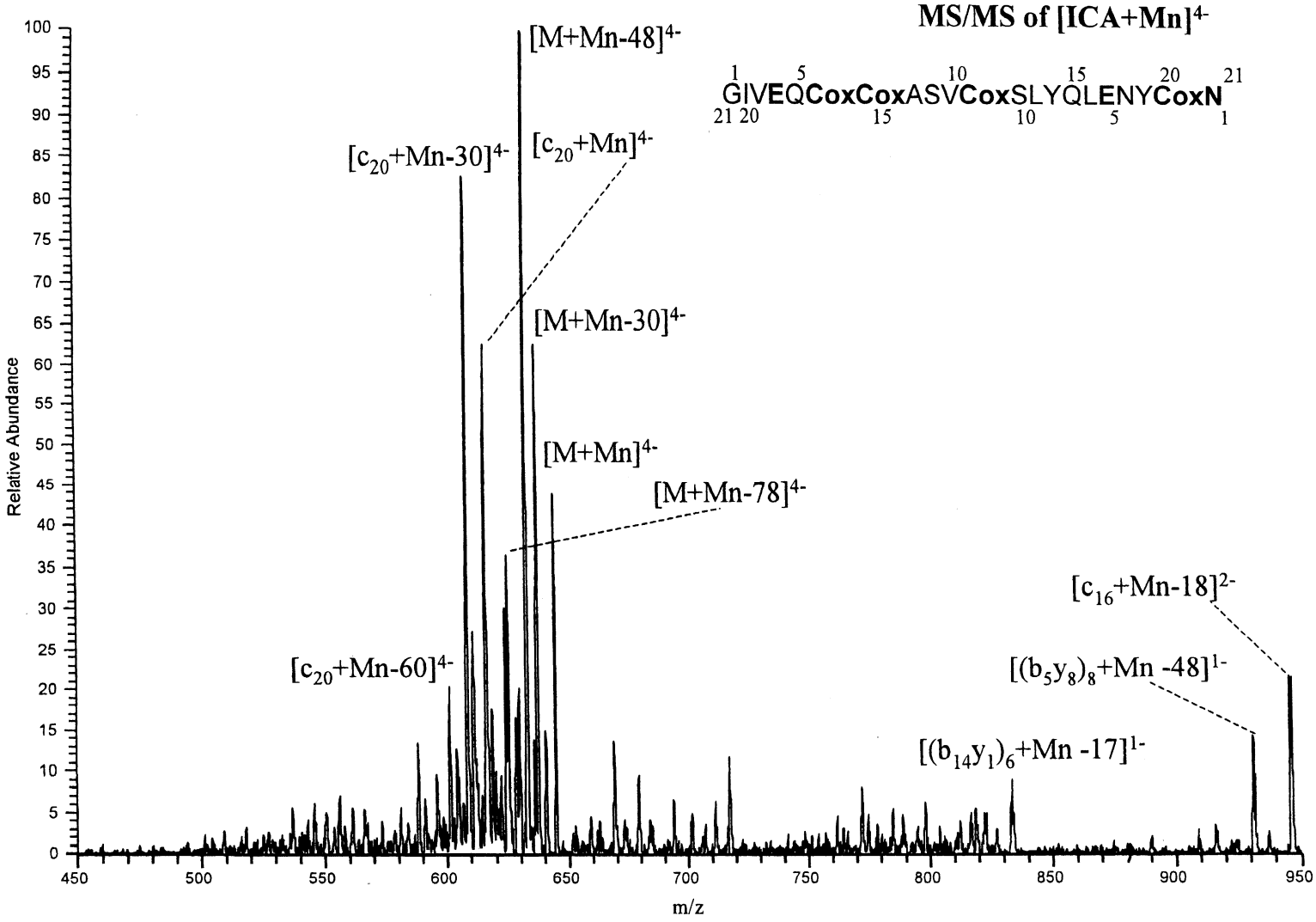

Fig. 3. CAD spectrum of $[ICA + Mn]^{4-}$.

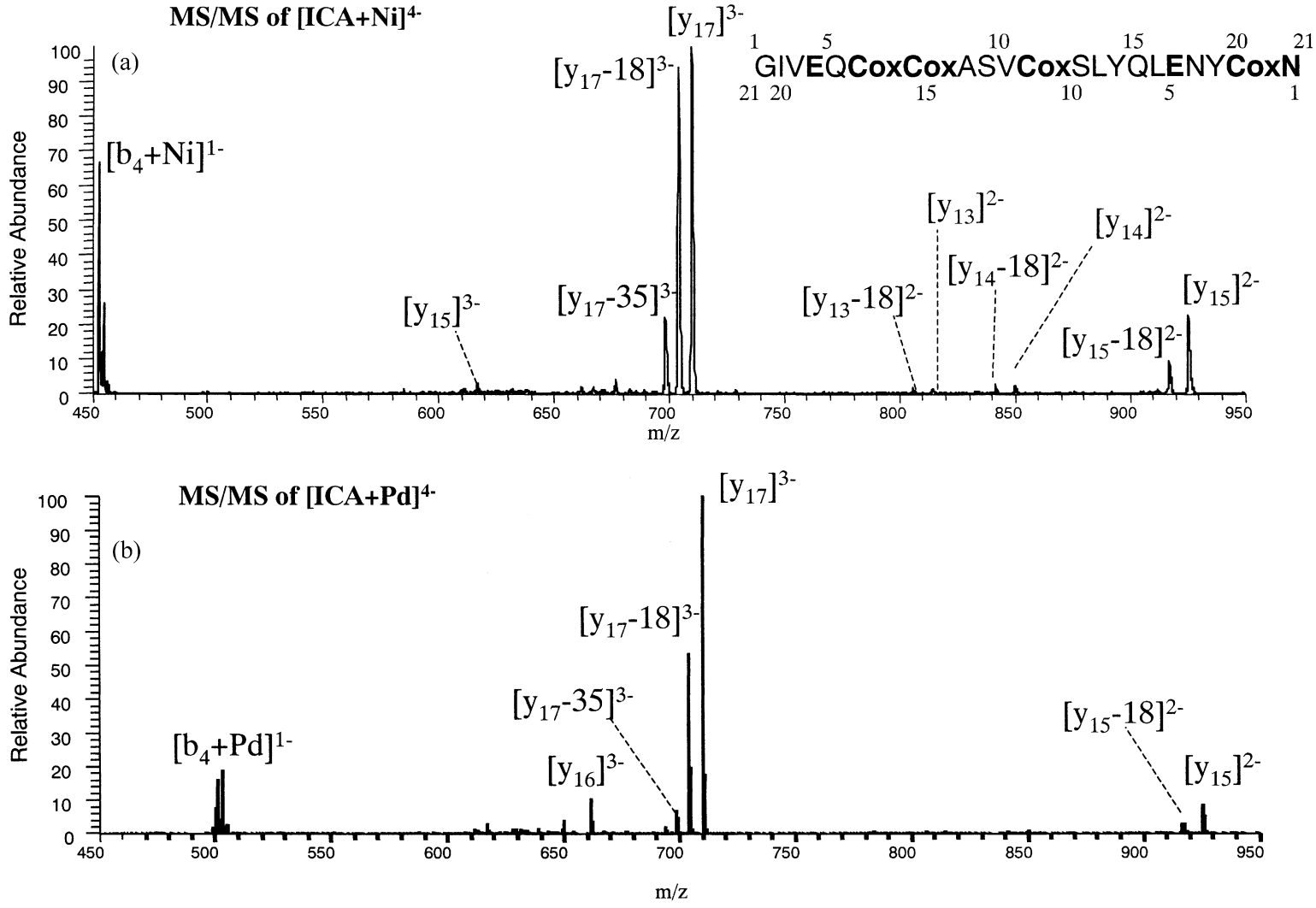

Fig. 4. CAD spectra of (a) Ni^{2+} and (b) Pd^{2+}–ICA complexes.

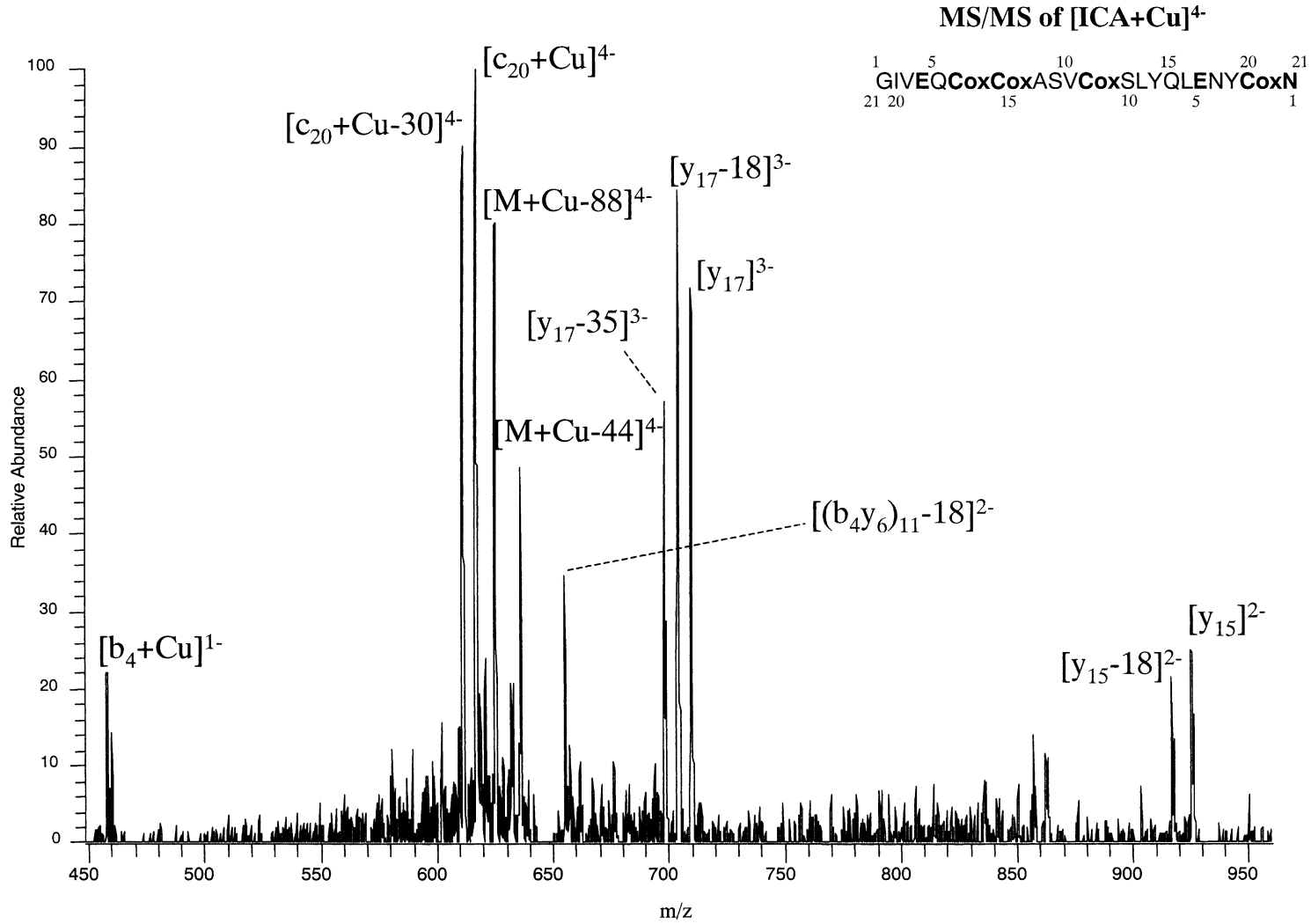

Fig. 5. CAD spectrum of $[ICA + Cu]^{4-}$.

were not observed, indicating that the −5 complex has mainly a conformation analogous to that of Ni^{2+}–ICA. When the negative charge state of the Cu^{2+} complex is changed from −4 to −5, the repulsive Coulombic force is increased and a highly extended conformation should be favored. This argument is consistent with the assumption that the Ni^{2+}–ICA complex has a loose conformation. The transition from compact to elongated structures with increasing charge state has been reported for the non-metalated–ICA complex [31].

The proteolytic solutions of ICA were mixed with metal ion salts and the metalated peptides were selected and subjected to CAD, to further understand the interactions between the metal ions and ICA. Mass spectrometric analysis of the proteolytic peptide fragments obtained by pepsin digestion revealed two complementary peptides comprising residues 1–13 ($ICA_{1–13}$) and 14–21 ($ICA_{14–21}$) among several other fragments. Fig. 6 presents the CAD spectra of the Mn^{2+}, Cu^{2+} and Ni^{2+}–$ICA_{1–13}$ ions that carry $a-1$ charge. All three spectra show prominent $[y^*_{11}]^-$ ions. The CAD spectrum of the Mn^{2+}–$ICA_{1–13}$ ion also reveals a significant amount of $[b^*_{12}]^-$ ions and ions corresponding to the losses of neutrals from the precursor (Fig. 6a). The loss of mass 82 is presumably indicative of a combined elimination of HCO_2H and two H_2O molecules [17]. The fragmentation patterns of the Cu^{2+} and Ni^{2+}–$ICA_{1–13}$ complexes (Fig. 6b and c) are virtually identical and dominated by the $[y^*_{11}]^-$ ions. Hence, the coordination sites of $ICA_{1–13}$ for Ni^{2+} and Cu^{2+} must be comparable. For the Ni^{2+}–ICA complex, a $[b^*_4]^-$ ion was observed, indicating a strong interaction between the metal ion and the four N-terminal amino acids. The lack of this ion

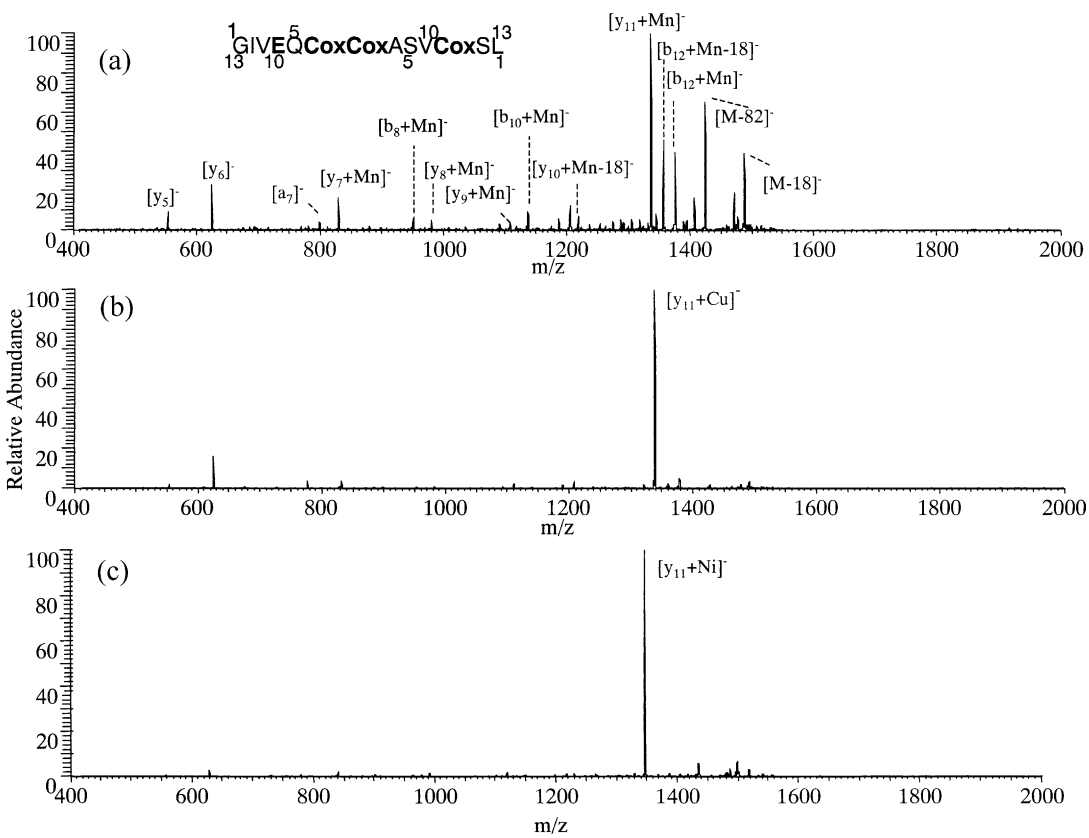

Fig. 6. CAD spectra of (a) Mn^{2+}, (b) Cu^{2+} and (c) Ni^{2+}–$ICA_{1–13}$ anions (−1 charge state).

in the fragmentation of Ni^{2+}–$ICA_{1–13}$ suggests that the strong interaction between the Ni^{2+} ion and the four N-terminal amino acids must be induced by the multiple negative charges on ICA. Notably, comparing the fragmentation spectra of the complementary $ICA_{14–21}$–Ni^{2+} and Cu^{2+} complexes shows an analogous fragment $[y_5^*]^-$ observed to have been formed from both complexes and some ions corresponding to neutral losses only observed to have been formed from the Cu^{2+}–$ICA_{14–21}$ complex (data not shown). Recall that an analogous $[y_{17}]^{3-}$ ion was generated from both the Ni^{2+}–ICA and Cu^{2+}–ICA complexes and some product ions corresponding to the neutral losses and the loss of the C-terminus ($[C_{20}^*]^{4-}$) were only produced from the Cu^{2+}–ICA complex. Although different charge states (−4 vs. −1) and different sequence lengths cause different interactions between the metal ions and the peptides, the analogy in these CAD experiments explains the similarities and dissimilarities in binding chemistries of Ni^{2+} and Cu^{2+} with the peptides examined. The different coordination chemistries of $ICA_{14–21}$ with Ni^{2+} and Cu^{2+} may be attributable to the different interactions between the two aromatic side chains (one from each of the two tyrosines in the sequence) and Ni^{2+} or Cu^{2+}.The Mn^{2+} ion binds with $ICA_{1–13}$ in a way such that the fragmentation patterns (Fig. 6a) differ from those of the Ni^{2+} and Cu^{2+} complexes. Detailed examination of the spectrum of Mn^{2+}–$ICA_{1–13}$ reveals several $[[y_n^*]^-$ and $[b_n^*]^-$ ions in relatively low abundance. The residue 7 (Cox) among the potential binding ones (E, three Cox and C-terminus) is common to those $[y_n^*]^-$ and $[b_n^*]^-$ fragments, suggesting an interaction between residue 7 and Mn^{2+}. Notably, many coordination sites may be involved in the binding between $ICA_{1–13}$ and Mn^{2+}, this fact is confirmed by the presence of the major fragment $[y_{11}^*]^-$. The residue 7 (Cox) is likely a binding site required to form a stable complex.

4. Conclusions

This work examined the interactions between a large polypeptide (ICA) and metal ions by using tandem mass spectrometry. Many product ions corresponding to neutral losses (e.g. NH_3, H_2O, CO, and CO_2) were observed to have come from the −4 precursor anions and the fragment ions. Such neutral losses are indicative of specific amino acid residues. Although the predominant neutral losses revealed by the CAD spectra of the precursors may suggest a stable complex conformation, various types of neutral losses from each metal–ICA complex indicate different intrinsic interactions between the metal ions and ICA. Alkali metal ions, Mn^{2+}, Fe^{2+}, Co^{2+} and Zn^{2+} bind with ICA in a specific configuration such that dissociation occurs only at some side chains and the C-terminus. The CAD spectra of Ni^{2+} and Pd^{2+}–ICA complexes show a dramatic change in the fragmentation patterns and suggest that the Ni^{2+} and Pd^{2+} ions (d^8 configuration) are bound to the four N-terminal amino acid residues of ICA. The fragmentation patterns of the Cu^{2+}–ICA complex reveal the existence of at least two conformations, one similar to Ni^{2+}–ICA and the other similar to Mn^{2+}–ICA. The repulsive Coulombic force arising from the negative charges on $[ICA + Cu]^{5-}$ drives the complex to assume a highly extended conformation, in contrast to the −4 complex that has a compact and an extended conformation. Enzyme-digested ICA fragments that contain complementary peptide residues 1–13 ($ICA_{1–13}$) and 14–21 ($ICA_{14–21}$) were subjected to CAD after they were combined with metal ions including Mn^{2+}, Ni^{2+}, and Cu^{2+}. The data show similar and dissimilar metal–peptide interactions parallel to those for corresponding metal–ICA complexes. Most results of the metal–ICA experiments agree with the data from ion mobility measurements. CAD thus represents a complementary method for probing the metal-induced peptide ion conformations.

Acknowledgements

The authors would like to thank the National Science Council of the Republic of China for financially supporting this research.

References

[1] M.J. Kendrick, M.T. May, M.J. Plishka, K.D. Robinson, Metals in Biological Systems, Ellis Harwood, Chichester, UK, 1992.
[2] R.E. Hay, Bio-inorganic Chemistry, Ellis Horwood, Chichester, UK, 1984, p. 69.
[3] J.P. Gluker, in: C.B. Anfinsen, J.T. Edsall, D.S. Eisenber, F.M. Richards (Eds.), Advances in Protein Chemistry, vol. 42, Academic Press, San Diego, CA, 1991.
[4] D.W. Deerfield, D.L. Olson, P. Berkositz, P.A. Byrd, K.A. Koehler, L.G. Pederson, R.G. Hiskey, J. Biol. Chem. 262 (1987) 4017–4023.
[5] R.B. Martin, in: H. Sigel (Ed.), Metal Ions in Biological Systems, vol. 17, Marcel Dekker, New York, 1984, p. 1.
[6] T.G. Spiro, in: G.L. Eichhorn (Ed.), Inorganic Biochemistry, Elsevier, Amsterdam, The Netherlands, 1973, p. 549.
[7] G. Pappalardo, G. Impellizzeri, R.P. Bonomo, T. Campagna, G. Grasso, M.G. Saita, New J. Chem. 26 (2002) 593.
[8] J.A. Loo, Mass Spectrom. Rev. 16 (1997) 1.
[9] G. Duan, Y. Wang, M. Aviram, M. Swaroop, J.A. Loo, J. Bian, Y. Tian, C.L. Mueller, Y. Sun, Mol. Cell. Biol. 19 (1999) 3145.
[10] P. Hu, Q.-Z. Ye, J.A. Loo, Anal. Chem. 66 (1994) 4190.
[11] T.D. Veenstra, K.L. Johnson, A.J. Tomlinson, T.A. Craig, R. Kumar, S. Naylor, J. Am. Soc. Mass Spectrom. 9 (1998) 8.
[12] Q.P. Lei, X. Cui, D.M. Kurtz Jr., I.J. Amster, I.V. Chernushevich, K.G. Standing, Anal. Chem. 70 (1998) 1838.
[13] J.A. Loo, Int. J. Mass Spectrom. 204 (2001) 113.
[14] P. Hu, J.A. Loo, J. Am. Chem. Soc. 117 (1995) 11314.
[15] J.A. Loo, P. Hu, R.D. Smith, J. Am. Soc. Mass Spectrom. 5 (1994) 959.
[16] A. Reiter, J. Adams, H. Zhao, J. Am. Chem. Soc. 116 (1994) 7827.
[17] P. Hu, M.L. Gross, J. Am. Chem. Soc. 114 (1992) 9153.
[18] O.V. Nemirovskiy, M.L. Gross, J. Am. Soc. Mass Spectrom. 9 (1998) 1020.
[19] L.M. Teesch, J. Adams, J. Am. Chem. Soc. 112 (1990) 4110.
[20] J. Volt, F.U. Bosch, M. Wunderkind, M. Schumacher, K. Melcher's, K. Bench, W. Steinhilber, K.P. Schafer, G. Toth, B. Penke, M. Przybylski, J. Chromatogr. A. 800 (1998) 29.
[21] R.W. Nelson, T.W. Hutchens, Rapid Commun. Mass Spectrom. 6 (1992) 4.
[22] S.T. Fountain, H. Lee, D.M. Lubman, Rapid Commun. Mass Spectrom. 8 (1994) 407.
[23] P.-C. Liao, J. Allison J. Mass Spectrom. 30 (1995) 408.
[24] I.A. Kaltashov, R.J. Cotter, W.H. Feinstone, G.W. Ketner, A.S. Woods, J. Am. Soc. Mass Spectrom. 8 (1997) 1070.
[25] T. Wyttenbach, G.V. Helden, M.T. Bowers, J. Am. Chem. Soc. 118 (1996) 8355.
[26] A. Wattenberg, H.-D. Barth, B. Brutschy, J. Mass Spectrom. 32 (1997) 1350.
[27] B. Salih, C. Masselson, R. Zenobi, J. Mass Spectrom. 33 (1998) 994.
[28] B.A. Cerda, L. Cornett, C. Wesdemiotis, Int. J. Mass Spectrom. 193 (1999) 205.
[29] D. Jayawardene, C. Dass, J. Mass Spectrom. 37 (2002) 389.
[30] J.A. Loo, C.G. Edmonds, R.D. Smith, Science 248 (1990) 201.
[31] J.A. Taraszka, J. Li, D.E. Clemmer, J. Phys. Chem. B 104 (2000) 4545.
[32] T. Wyttenbach, J.E. Bushnell, M.T. Bowers, J. Am. Chem. Soc. 120 (1998) 5098.
[33] M.F. Jarrold, Acc. Chem. Res. 32 (1999) 360.
[34] C.S. HaoglundkHyzer, A.E. Counterman, D.E. Clemmer, Chem. Rev. 99 (1999) 3037.
[35] C.J. Cassady, S.R. Carr, J. Mass Spectrom. 31 (1996) 247.
[36] D. Suckau, Y. Shi, S.C. Beu, M.W. Senko, J.P. Quinn, F.M. Wampler, F.W. McLafferty, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 790.
[37] M. Freitas, A.G. Marshall, Int. J. Mass Spectrom. 182 (1999) 221.
[38] Y.L. Chen, B.A. Collings, D.J. Douglas, J. Am. Soc. Mass Spectrom. 8 (1997) 681.
[39] I.A. Kaltashov, C. Fenselau, Proteins 27 (1997) 165.
[40] K. Biemann, S.A. Martin, Mass Spectrom. Rev. 6 (1987) 1.
[41] A.M. Bradford, R.J. Waugh, J.H. Bowie, Rapid Commun. Mass Spectrom. 9 (1995) 677.
[42] N.P. Ewing, C.J. Cassady, J. Am. Soc. Mass Spectrom. 12 (2000) 105.
[43] R.J. Waugh, J.H. Bowie, R.N. Hayes, Int. J. Mass Spectrom. Ion Processes 107 (1991) 333.
[44] S.T. Steinbrenner, J.H. Bowie, Rapid Commun. Mass Spectrom. 11 (1997) 253.
[45] R.J. Waugh, M. Eckersley, J.H. Bowie, R.N. Hayes, Int. J. Mass Spectrom. Ion Processes 98 (1990) 134.
[46] M. Eckersley, J.H. Bowie, R.N. Hayes, Int. J. Mass Spectrom. Ion Processes 93 (1989) 199.
[47] R.J. Waugh, J.H. Bowie, Rapid Commun. Mass Spectrom. 8 (1994) 169.
[48] S.W. Lee, H.S. Kim, J.L. Beauchamp, J. Am. Chem. Soc. 120 (1998) 3188.