J. Am. Soc. Mass Spectrom. (2011) 22:233–244
DOI: 10.1007/s13361-010-0035-2

RESEARCH ARTICLE

Formation of Peptide Radical Cations ($M^{+\bullet}$) in Electron Capture Dissociation of Peptides Adducted with Group IIB Metal Ions

Xiangfeng Chen, Wai Yi Kelly Chan, Pui Shuen Wong, Hoi Sze Yeung, Tak Wah Dominic Chan

Department of Chemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, SAR, China

Abstract

Peptides adducted with different divalent Group IIB metal ions (Zn^{2+}, Cd^{2+}, and Hg^{2+}) were found to give very different ECD mass spectra. ECD of Zn^{2+} adducted peptides gave series of *c*-/*z*-type fragment ions with and without metal ions. ECD of Cd^{2+} and Hg^{2+} adducted model peptides gave mostly *a*-type fragment ions with $M^{+\bullet}$ and fragment ions corresponding to losses of neutral side chain from $M^{+\bullet}$. No detectable *a*-ions could be observed in ECD spectra of Zn^{2+} adducted peptides. We rationalized the present findings by invoking both proton-electron recombination and metal-ion reduction processes. As previously postulated, divalent metal-ions adducted peptides could adopt several forms, including (a) $[M + Cat]^{2+}$, (b) $[(M + Cat - H) + H]^{2+}$, and (c) $[(M + Cat - 2H) + 2H]^{2+}$. The relative population of these precursor ions depends largely on the acidity of the metal–ion peptide complexes. Peptides adducted with divalent metal-ions of small ionic radii (i.e., Zn^{2+}) would form predominantly species (b) and (c); whereas peptides adducted with metal ions of larger ionic radii (i.e., Hg^{2+}) would adopt predominantly species (a). Species (b) and (c) are believed to be essential for proton-electron recombination process to give c-/z-type fragments via the labile ketylamino radical intermediates. Species (c) is particularly important for the formation of non-metalated c-/z-type fragments. Without any mobile protons, species (a) are believed to undergo metal ion reduction and subsequently induce spontaneous electron transfer from the peptide moiety to the charge-reduced metal ions. Depending on the exothermicity of the electron transfer reaction, the peptide radical cations might be formed with substantial internal energy and might undergo further dissociation to give structural related fragment ions.

Key words: Electron capture dissociation, Peptides, Group IIB transition metal ions, Electron transfer

Introduction

Electron capture dissociation (ECD) is an important and useful technology for sequencing of peptides/proteins [1, 2]. The key characteristics of ECD include preferential cleavages of disulfide bond and backbone N–C_α linkages. These properties have provided complimentary structural information in comparison with the more conventional "slow heating" ion dissociation methods, such as infrared multi-photon dissociation [3–5] and collision induced dissociation [6, 7]. In addition, ECD of peptides/proteins generates more sequence specific cleavages and exhibits less dependence on the amino acid residues [8–10]. Regarding the mechanistic aspects, there are two prevailing models for ECD of protonated peptides/proteins. The "hot hydrogen" model postulates that the incoming electron neutralizes the solvated

Electronic supplementary material The online version of this article (doi:10.1007/s13361-010-0035-2) contains supplementary material, which is available to authorized users.

Correspondence to: T.W.D. Chan; *e-mail:* twdchan@cuhk.edu.hk

Received: 3 September 2010
Revised: 9 November 2010
Accepted: 11 November 2010
Published online: 1 February 2011

proton and generates a hydrogen radical, $H^{\bullet}$. The transfer and relocation of $H^{\bullet}$ to backbone carbonyl group leads to the formation of labile ketylamino radical and induces the dissociation of the adjacent N–C_α bond at the C-terminal side of the carbonyl group [1, 2, 11, 12]. Alternatively, the "superbase" model involves initial capture of electron by the π^* orbital of the charge-perturbed carbonyl group. The anionic radical formed at the carbonyl group is even more basic than the side chain of the arginine residue and would induce abstraction of the nearby proton. The ketylamino radical formed would undergo cleavage along the N–C_α linkage to form the usual c-/z-ions [13–19].

To better understand the dissociation process(es) and to further improve the performance of the electron capture dissociation method, many researchers have studied the influence of various experimental parameters on the dissociation behavior of peptides/protein ions. An important experimental parameter is the nature of the charge carriers. Several researchers have studied the impact of replacing the mobile protons with fixed charges using functionalities such as quaternary amino groups [20–23]. Our group and other researchers have focused on the use of divalent and trivalent metal ions as charge carriers [24–38]. Among different charge carriers, metal ions are of particular interest. First, metal ions as charge carriers might provide a tunable parameter for internal energy deposition during the electron capture event. Second, the diverse reactivities of metal ions in gas phase might provide additional structural information. ECD with metal ions as charge carriers have been used to characterize O-sulfated tyrosine [24], petrobacin [25], metabolites [26], oligosaccharides [27], phosphocholines [28], supramolecular complexes [29], and dendrimers [30].

Despite the many applications of metal ions in ECD, the involvement of metal ions in the ECD process is not well-understood. Williams and coworkers studied the ECD behavior of model peptide cationized with two different alkaline metal ions (Li^+ and Cs^+). It was concluded that the incoming electron tends to neutralize the cation of higher recombination energy [31]. Our group studied the ECD fragmentation of model peptides adducted with alkaline earth metal ions. The incoming electron was believed to be captured by the mobile proton, rather than metal ions [32]. The metal ions did not seem to play any significant roles in the dissociation process. For transition metal ions, Heck and coworkers postulated that the electron was initially captured by metal ions and was transferred to the peptide to form both typical and atypical ECD fragments [33]. Håkansson and coworkers studied the ECD behavior of Substance P adducted with both a metal ion and proton [34]. The authors suggested that the electron transfer from metal ions to peptide could be correlated to the second ionization energies of metals. More recently, Yuri et al. attributed the different cleavage patterns obtained from ECD of peptides adducted with different metal ions to the conformational changes of the peptide models [35].

This paper aims to study the effect of Group IIB metal ions (Zn^{2+}, Cd^{2+}, and Hg^{2+}) adduction on the ECD fragmentation of model peptides. Table 1 summarized the ionization energies and coordination chemistries of the three metal ions. Model peptides with a general framework of ZGGGXGGGZ, where X is either V or W; and Z is either R, K, H, were used in this study. The use of these model peptides, as opposed to the use of bioactive peptides, was to include residues with mechanistic illustration and to reduce the complexity of the spectra. Basic amino acids, R, K, and H were included in the model peptide to facilitate direct comparison of the ECD spectra of metalated peptides with the protonated analogues of different proton carriers. Glycine spacers were used to separate amino acid residues and to provide a molecular framework of high flexibility. Valine and tryptophan were included to alter the ionization energy of the peptides. The main goal of this paper is to compare the ECD behavior of peptides cationized with transition metal ions having fully filled d-shell.

Experimental

Sample Preparation

All materials were obtained commercially and were used without further purification. Zinc (II) acetate was obtained from Riedel-de Haën (Seelze, Germany), Cd (II) nitrate and Hg(II) nitrate were obtained from Sigma and Aldrich (St. Louis, MO, USA). Model peptides were custom-synthesized by Peptron Inc., (Daejeon, South Korea). The samples were prepared at concentrations of 1×10^{-4}–2.5×10^{-4} M in 1:1 water:methanol (Labscan Ltd., Bangkok, Thailand). The concentrations of the metal salts were 5 mM in peptide solutions.

Instrumentation

All experiments were performed by using a 4.7 Tesla FTMS system (APEX III, Bruker Instrument Inc., Boston, MA, USA). This instrument was equipped with standard, commercially available external electrospray ion source (Analytica, Branford, CT, USA) [41]. ESI ion source was modified to adopt a homemade nanospray assembly [42]. Eight to 10 μL of sample solution was loaded into a tapered capillary tip and was electrically grounded using a 15 μm gold-plated tungsten wire. Intact molecular ions were produced. Ion transmission from the external source into the trapped ion cell was achieved by using the standard electrostatic lens system. Sidekick ion accumulation method was used to facilitate the ion trapping process. Ten cycles of multiple ion filling (MIF) were used to enhance the intensity of precursor ions [43]. In all experiments, static trapping potentials of 1.0 V were used. Initial calibration of the instrument was achieved by using a peptide mixture containing LGF, angiotensin II, and melittin. A standard electrically heated

Table 1. A summary of the physical and chemical information of Group IIB metal ions

Element	Symbol	1st IP[a]	2nd IP[a]	Electronic configuration		Pauling radius (Å)[a]		Ionic radius (Å)[b]		
		eV	eV	M^{2+}	M^{+}	M^{2+}	M^{+}	4-Coordinated	6-Coordinated	8-Coordinated
Hydrogen	H	13.60	-	-	-	-	-	-	-	-
Zinc	Zn	9.39	17.96	$3d^{10}$	$3d^{10}4s^{1}$	0.74	0.88	0.74	0.88	1.04
Cadmium	Cd	8.99	16.91	$4d^{10}$	$4d^{10}5s^{1}$	0.97	1.14	0.92	1.09	1.24
Mercury	Hg	10.44	18.80	$5d^{10}$	$5d^{10}6s^{1}$	1.10	1.25	1.10	1.16	1.28

[a]Data obtained from reference [39].
[b]For doubly charged species; data obtained from reference [40].

filament source was used to produce pulses of electron beam. The filament was made of rhenium ribbon and was fixed at a distance of 108 mm from the rear end of the Infinity cell. Details of the geometry of the filament source have previously been described [44]. Typical experimental conditions were 3.3 A filament heating current, 3.5–3.8 V average filament bias voltage, and 300~500 MS electron irradiation time. All ECD mass spectra were acquired in broadband mode using 256 kbyte dataset. Thirty to fifty scans were normally summed to improve the signal-to-noise ratio. The time-domain signals were zero-filled once before Fourier transformation.

Calculations

All calculations were performed by using Gaussian 03 package [45]. Equilibrium geometries were determined by full optimization followed by harmonic frequency calculations to confirm the nature of minima and transition states. All the stationary points were optimized using the Becke three parameters hybrid (B3LYP) exchange-correlation functional in the framework of the Kohn-Sham density functional theory (DFT) [46–48]. The standard split-valence basis set 6-31++G(d,p) was used for H, C, N, and O atoms. All the metal cations were described by employing the Hay-Wadt effective core potential (ECP) with LANL2DZ basis set [49–51]. Single point energy calculation were calculated at the B3LYP level in conjunction with LANL2DZ+6-311++G(3df,2p) basis set. The calculated total energies for all species are available as supporting information, which can be found in the electronic version of this article.

Results and Discussion

Spectral Features

Figure 1a–c show the ECD spectra of RGGGWGGGR adducted with Zn^{2+}, Cd^{2+}, and Hg^{2+}, respectively. The mass assignments of the corresponding Hg^{2+} adducted peptide are given in Table 2. Product ions are labeled according to our previously proposed nomenclature, in which $\bar{c}-$ and $\bar{z}-$ denotes the corresponding N- and C-terminal fragments originated from the homolytic cleavage of the $N–C_{\alpha}$ linkages, respectively. Any surplus of proton/hydrogen atom or metal ion/atom is indicated in the label [32]. Interestingly, peptides adducted with Group IIB metal ions generated different ECD tandem mass spectra. Among the three metal ions studied, the ECD fragmentation of Zn^{2+} adducted RGGGWGGGR is similar to that of alkaline earth metal ions. Typical ECD fragment ions, i.e., c-/z-ions, with and without metal ions were generated. Even and odd electron side chain losses from the metalated z-ions could also be observed. The ECD spectrum of Cd^{2+} adducted model peptides is substantially different from that of Zn^{2+}. Only minor c-/z-ions with and without metal ions were also observed. Abundant signals corresponding to the peptide radical cation ($M^{+\bullet}$) and its neutral loss fragment ions were observed in the ECD spectrum of Cd^{2+} adducted peptides. For Hg^{2+} adducted RGGGWGGGR, only one non-metalated c-ions was observed. The spectrum was dominated by fragment ions derived from neutral losses of $M^{+\bullet}$. A relatively weak signal corresponding to the peptide radical cation was observed. Some non-metalated a-/z-type fragment ions were generated in the ECD spectrum of Hg^{2+} adducted peptide. The ECD spectral information for metal ions adducted ZGGGWGGGZ (Z = K or H) were found to be similar to that of RGGGWGGGR and were summarized in Table 3.

CID of Hg^{2+} and Cd^{2+} Adducted Peptides

The formation of peptide radical cations ($M^{+\bullet}$) is of both fundamental and analytical importance. These ions have never been reported in the literature relevant to ECD of metal ions adducted peptides binary complex. A conceivable explanation for the formation of $M^{+\bullet}$ might involve an electron transfer (ET) reaction between the peptide and the divalent metal cation. This electron transfer process has previously been found to occur in the collision induced dissociation (CID) of metal-ligand-peptide ternary complexes [52–55], such as copper(II) terpyridine peptide complexes. In order to determine the role of the electron capture process in the formation of the peptide radical cations, CID of Cd^{2+} and Hg^{2+} adducted peptides were conducted using argon as collision gas. Figure 2 shows the CID tandem mass spectra of Cd^{2+} and Hg^{2+} adducted peptides. CID of Cd^{2+} adducted peptide generated primarily non-metalated *b*-ions; whereas CID of Hg^{2+} adducted

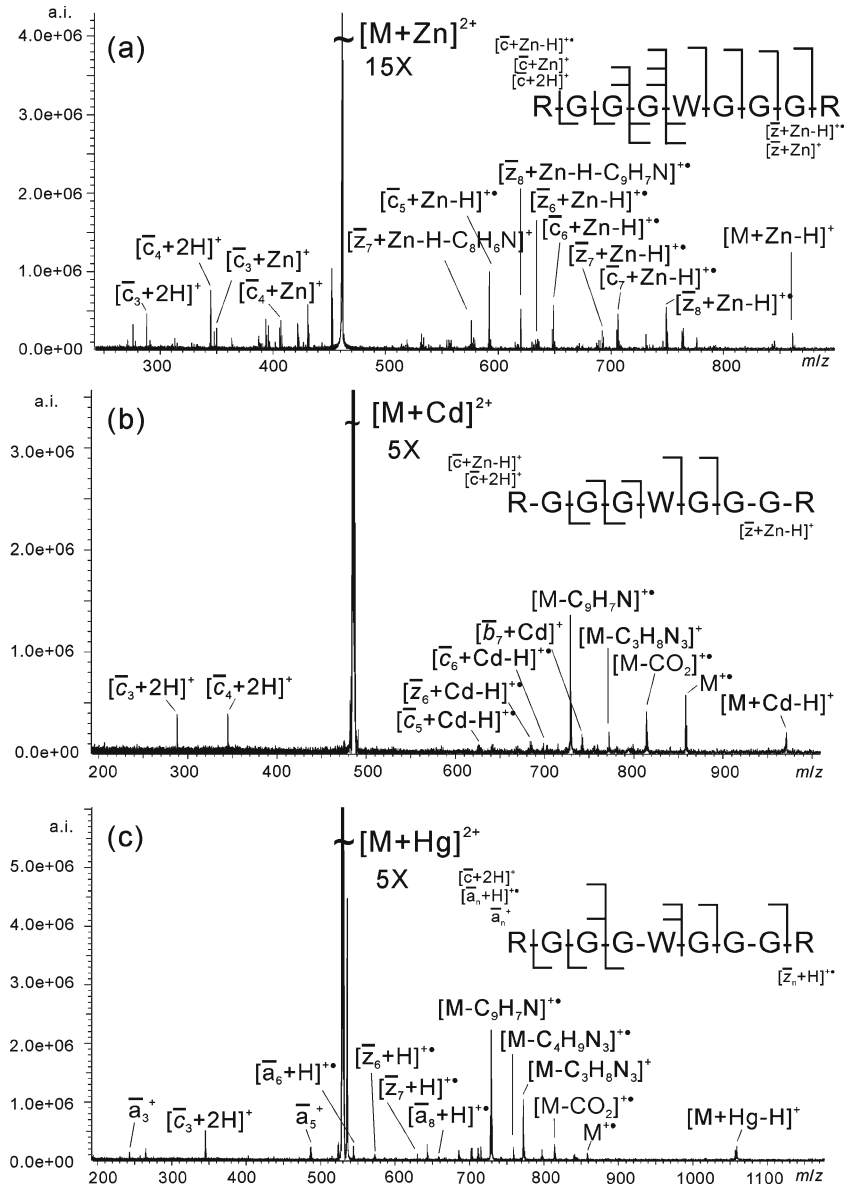


Figure 1. Typical ECD mass spectra of RGGGWGGGR adducted with (**a**) Zn^{2+}, (**b**) Cd^{2+}, (**c**) Hg^{2+}

peptide gave both metalated and non-metalated *b*-/*y*-ions. The absence of any *y*-ions and the formation of series of non-metalated *b*-ions in the CID spectrum of Cd^{2+} adducted peptide imply that Cd^{2+} binds preferentially to the C-terminal side of the peptide. The formation of $[M + Cd - 60]^{2+}$ also supports this postulation. Loss of *m/z* 60 from the precursor ions of peptides containing C-terminal arginine residue has previously been investigated [56] and was assigned to be the concomitant loss of a H_2O and HN=C=NH moieties from the C-terminal carboxylic acid and the arginine side chain, respectively. Nevertheless, no evidence for the formation of peptide radical cation $M^{+\bullet}$ and related species was found in both CID spectra. The absence of $M^{+\bullet}$ species in these CID spectra indicates that the electron capture is a pre-requisite process for the electron transfer between peptides and metal ions.

Formation of Peptide Radical Ion $M^{+\bullet}$ Under ECD Conditions

Since electron capture is an essential process for the formation of peptide radical cation ($M^{+\bullet}$) and its related species, it is logical to examine the feasibility of the electron transfer process between the monovalent Group IIB metal ions and the peptide molecules. Theoretically, the energetic of the electron transfer reaction is governed by:

$$\Delta H_{et} = \mathrm{R.E.(Cat^+)} - \mathrm{I.E.(M)} \qquad (1)$$

where R.E.(Cat^+) is the recombination energy of the monovalent metal ion with electron and I.E.(M) is the ionization energy of the peptide. Ignoring the effect of coordination environment of metal ion, the R.E.(Cat^+) is equal to the first ionization energy of the metal atom. Table 1

Table 2. Assignment of peaks in the ECD spectrum of RGGGWGGGR adducted with Hg^{2+}

	Theoretical mass	Experimental Mass	S/N	Error in ppm
$[M+Hg-H]^+$	1059.3843	1059.3806	11.6	–3.5
$M^{+\bullet}$	858.4209	858.4258	6.3	5.8
$[M-CO_2]^{+\bullet}$	814.4310	814.4433	13.2	15.1
$[M-C_3H_8N_3^{\bullet}]^+$	772.3485	772.3455	48.7	–3.8
$[M-C_4H_9N_3]^{+\bullet}$	759.3407	759.3361	10.7	–6.0
$[M-C_9H_7N]^{+\bullet}$	729.3624	729.3636	103.6	1.7
$[M-CO_2-C_3H_8N_3^{\bullet}]^+$	728.3587	728.3547	35.9	–5.5
$[M-CO_2-C_4H_9N_3]^{+\bullet}$	715.3508	715.3458	11.0	–7.1
$[M-C_9H_7N-H_2O]^{+\bullet}$	711.3518	711.3414	10.0	–14.7
$[\bar{y}_8+2H]^+$	703.3270	703.3310	9.9	5.6
$[\bar{z}_8+H]^{+\bullet}$	687.3084	687.3129	2.7	6.5
$[M-CO_2-C_9H_7N]^{+\bullet}$	685.3726	685.3727	8.0	0.2
$[\bar{a}_8+H]^{+\bullet}$	658.3294	658.3311	3.4	2.7
$[\bar{z}_7+H]^{+\bullet}$	630.2868	630.2786	5.5	–13.1
$[\bar{z}_6+H]^{+\bullet}$	573.2654	573.2599	5.3	–9.6
$[\bar{a}_6+H]^{+\bullet}$	544.2864	544.2815	11.6	–9.1
$[M+Hg]^{2+}$	530.1958	530.1967	1121.4	1.6
$[\bar{a}_5+H]^{+\bullet}$	487.2650	487.2664	11.0	2.9
$\bar{a}_5^+$	486.2572	486.2586	10.5	2.9
$[\bar{c}_3+2H]^+$	345.1993	345.1990	23.8	–0.9
$\bar{a}_3^+$	243.1564	243.1573	6.9	3.6

summarizes some related physical properties of Group IIB elements. The first ionization energies for Hg, Cd, and Zn are 10.44, 8.99, and 9.39 eV, respectively. Compared with that of the amino acid residue having the lowest I.E., i.e., tryptophan (I.E. = 7.24 eV) [57], it is obvious that electron transfer from the peptide moiety to any of the monovalent Group IIB metal ions is energetically feasible.

In the literature, the phenomenon of electron transfer from gas phase organic molecules to monovalent metal ions has been examined extensively [58–61]. Bohme and cow-

Table 3. Summary of product ions abundance of ECD of ZGGGWGGGZ (Z = R, K, H) adducted with Group IIB metal ions

		Zn^{2+}			Cd^{2+}			Hg^{2+}		
	n	R	K	H	R	K	H	R	K	H
$[M+Cat - H]^+$		4.5	2.9	7.9	3.7	7.5	6.0	3.1	2.0	7.2
$[\bar{c}_n+Cat/-H]^+$	8	-	8.2	11.4	-	7.5	52.1	-	-	4.1
	7	5.8	5.1	1.8	-	4.5	3.4	-	3.6	-
	6	7.2	7.6	4.1	2.5	2.9	5.1	-	2.1	-
	5	13.3	11.5	12.5	2.3	3.8	7.6	-	-	-
	4	11.6	22.5	17.9	-	4.1	8.1	-	-	-
	3	5.5	9.9	18.4	-	-	1.3	-	-	-
$[\bar{z}_n+Cat/-H]^+$	8	8.0	-	3.3	-	-	-	-	-	-
	7	2.5	-	-	3.8	2.1	-	-	-	-
	6	1.9	5.7	7.2	2.9	2.0	3.4	-	-	-
	5	2.15	4.2	2.3	-	3.0	2.6	-	-	-
$M^{+\bullet}$		-	-	-	13.8	10.2	1.2	1.7	1.0	-
$[M-CO_2]^{+\bullet}$		-	-	-	9.9	39.6	2.8	3.5	2.4	-
$[M-W_{Sc}]^{+\bullet}$		-	-	-	32.7	3.7	2.1	27.4	5.1	13.0
$[M-W_{Sc}-CO_2]^{+\bullet}$		-	-	-	-	-	-	2.1	-	5.6
$[M-Z_{Sc}^{\bullet}]^+$		-	-	-	18.3	-	-	15.6	8.5	7.8
$[M-Z_{Sc}^{\bullet}-CO_2]^+$		-	-	-	-	6.5	-	12.4	19.0	6.9
$[\bar{a}_n/+H]^{+/\bullet}$	8	-	-	-	-	-	-	0.9	-	-
	6	-	-	-	-	-	-	3.1	-	-
	5	-	-	-	-	-	-	6.8	15.4	17.6
	3	-	-	-	-	-	-	1.8	-	-
$[\bar{c}_n+2H]^+$	7	-	-	-	-	-	-	-	2.2	-
	4	12.1	-	-	9.3	2.6	-	-	-	4.7
	3	7.8	-	-	9.3	-	-	6.3	-	-
$[\bar{z}_n+H]^{+\bullet}$	8	-	-	-	-	-	-	0.7	-	-
	7	-	-	-	-	-	-	1.5	-	-
	6	-	-	-	-	-	2.1	1.4	-	-
	5	-	-	-	-	-	-	-	2.7	-
$[\bar{y}_n+2H\ /-CO_2]^+$	8	-	-	-	-	-	-	3.3	6.3	9.7
	7	-	-	-	-	-	-	1.2	3.8	16.1
	6	-	-	-	-	-	-	-	-	2.9
	5	-	-	-	-	-	-	1.4	-	-

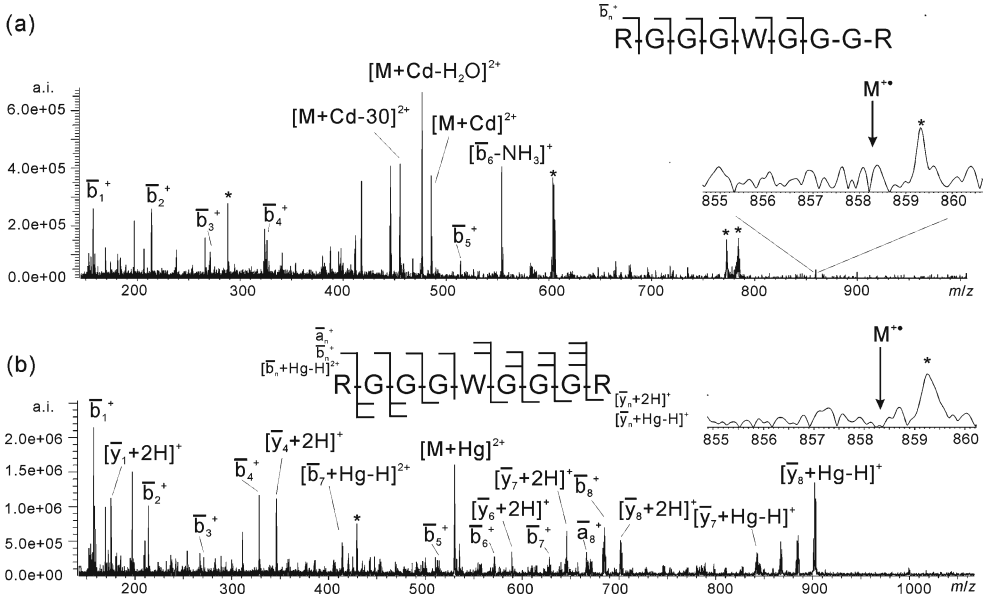


Figure 2. CID mass spectra of RGGGWGGGR adducted with (**a**) Cd^{2+}, (**b**) Hg^{2+}

orkers [60] found that Hg^{+} and Zn^{+} can undergo charge transfer reaction with benzene (I.E=9.24 eV) in gas phase leading to the formation of $[C_6H_6]^{+\bullet}$ species. In a comprehensive study of the reactivity of Hg^{+}, Bohme and coworkers [61] concluded that Hg^{+} can ionize organic molecules with ionization energy lower than 10.1 eV.

Herein, we proposed that the peptide radical cation $M^{+\bullet}$ was generated through an "electron capture induced spontaneous electron transfer" model. Reduction of the metal ion by the incoming electron generates the monovalent metal ions peptide complex, $[M + Cat(I)]^{+}$. If the recombination energy of monovalent metal ion is larger than the ionization energy of peptides, the system is likely to relax spontaneously and access the $[M^{+\bullet} + Cat(0)]^{+}$ state via electron transfer from peptide to metal monocation. Without any charges in the metal centre, the metal-peptide complex would dissociate to give the observed $M^{+\bullet}$.

Influences of Amino Acid Residues in the Peptides

To get more information relating to the charge transfer behavior of Group IIB metal ions under electron capture dissociation conditions, another model peptide with similar peptide framework, i.e., RGGGVGGGR, was examined. By replacing the tryptophan residue with valine residue, the ionization energy of the peptide should be increased to a value close to the ionization energy of arginine residue (i.e., I.E=9.20 eV) [62]. Figure 3a–c shows typical ECD spectra of divalent Group IIB metal ion adducted RGGGVGGGR. With adduction of Zn^{2+} and Hg^{2+} species, the spectral behavior of RGGGVGGGR shows high similarities with respect to that of RGGGWGGGR under ECD conditions. ECD of Zn^{2+} adducted RGGGVGGGR gave exclusive c-/z-type fragment ions, whereas ECD of Hg^{2+} adducted RGGGVGGGR gave mainly $M^{+\bullet}$ and related species. An interesting feature of the ECD spectrum of Hg^{2+} adducted RGGGVGGGR is the fewer number of fragment ions arising from the backbone cleavages. Consistent with the expected lowering of the energy release through the charge transfer reaction between the peptide moiety and the Hg^{+} ion, i.e., R. E. (Hg^{+}) – I.E. (RGGGVGGGR), the extent of backbone cleavage was substantially reduced. From the ECD spectrum of the Cd^{2+} adducted RGGGVGGGR, it is interesting to find that no peptide radical ions ($M^{+\bullet}$) and related species were generated. The ECD spectrum is dominated with non-metalated and metalated c-/z-type fragment ions. Cross comparing the ECD spectra of RGGGVGGGR and RGGGWGGGR obtained with Cd^{2+} adduction (Figures 1b and 3b), it is intriguing to note that the initial ionization site of the peptide can lead to the remote loss of radical fragments. Since there is no apparent charge transfer reaction between the RGGGVGGGR moiety and the charge-reduced Cd^{+}, it is therefore logical to postulate that ionization of RGGGWGGGR by the charge-reduced Cd^{+} proceeds primarily through the transfer of an electron from the tryptophan side chain to the Cd^{+}. Losses of odd-electron arginine side chains (see Figure 1b) from the RGGGW $^{+\bullet}$GGGR must therefore proceed via a rearrangement reaction involving the migration of the radical centre from the tryptophan side chain to the arginine residue. Scheme **1** shows a possible reaction mechanism for side chain(s) loss in the ECD of RGGGWGGGR adducted with Cd^{2+}. Similar to the reaction process involving the loss of arginine side chain from the z-ions, the indole radical cation might abstract the C_α hydrogen in the arginine residue to form a distonic ion, i.e., the charge resides on the tryptophan side chain and the radical is located at the C_α of the arginine residue. The radical site would induce the formation of C_α=C_β double bond with

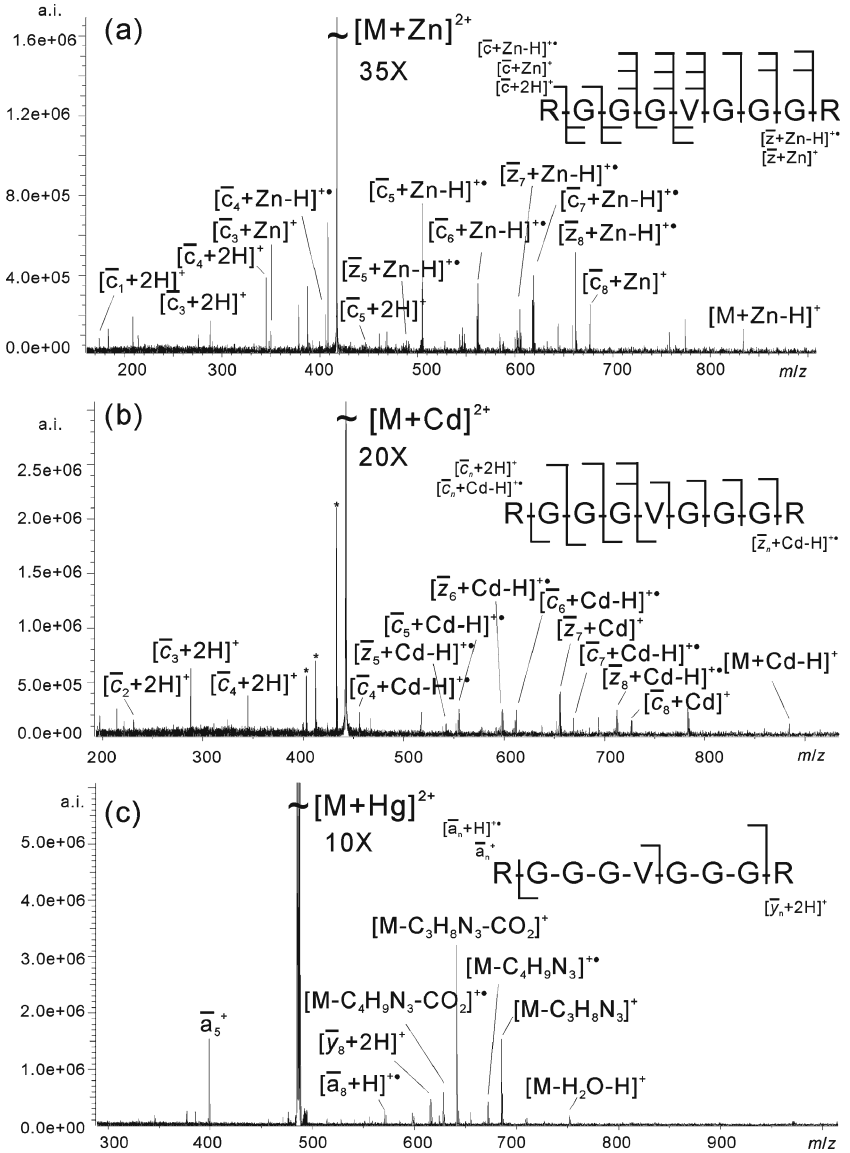
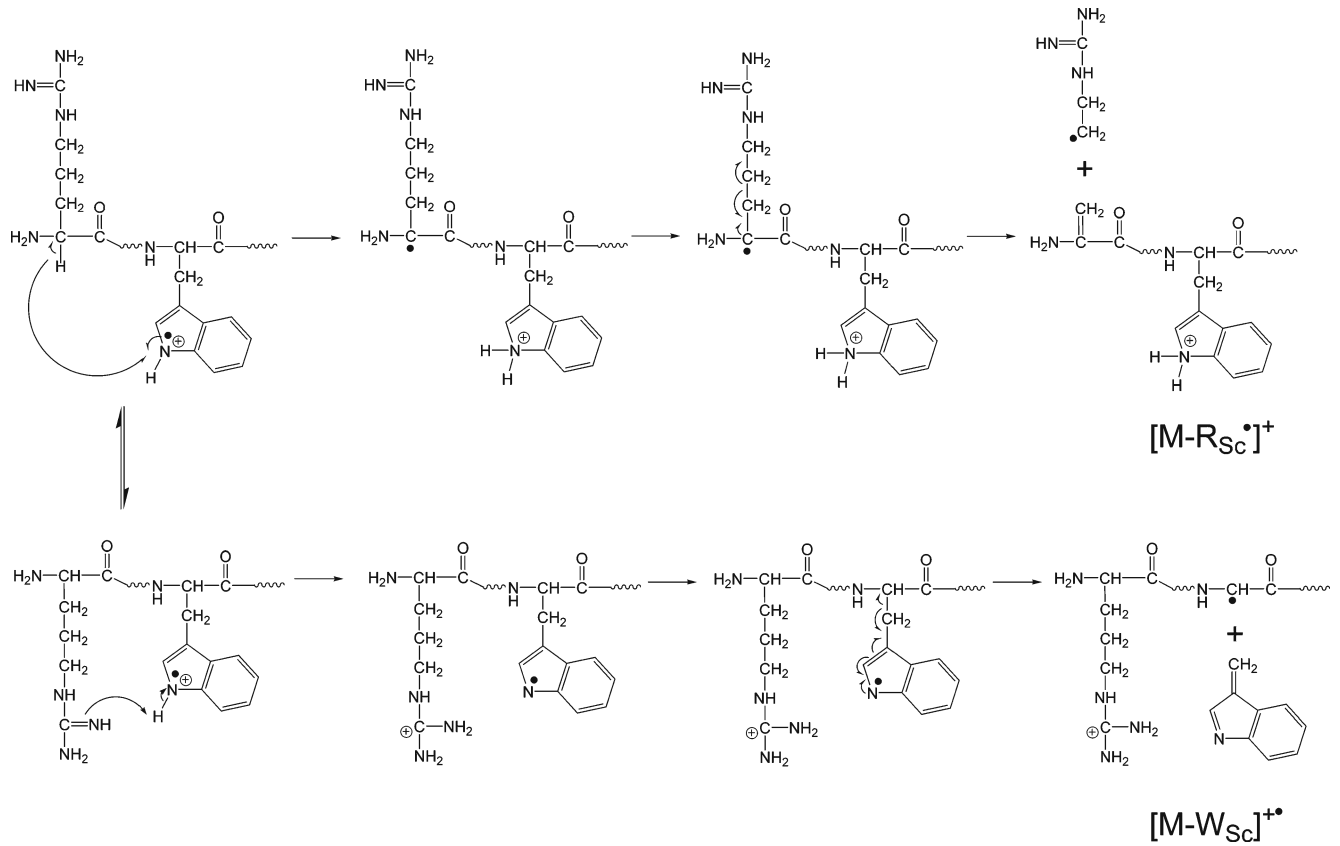


Figure 3. Typical ECD mass spectra of RGGGVGGGR adducted with (**a**) Zn^{2+}, (**b**) Cd^{2+}, (**c**) Hg^{2+}

concomitant loss of the $[C_3H_8N_3]^{\bullet}$ through the cleavage of the C_β–C_γ linkage. For tryptophan side chain loss, proton abstraction from NH of tryptophan side chain by arginine separates the charge and radical, then homolytic cleavage C_α–C_β bond can lead to the loss of $[C_9H_7N]$.

ECD of Zn^{2+} Adducted Peptides

A major discrepancy arising from the current experimental results and the theoretical prediction is related to the ECD behavior of Zn^{2+} adducted peptides. From Table 1, the first ionization energy of Zn is ~0.4 eV higher than Cd. If the driving force of the electron transfer reaction between the peptide moiety and the monovalent metal ions is solely related to their I.E. and R.E. respectively, Zn^{2+} adducted peptides should show a spectral behavior similar to that of the Cd^{2+} adducted peptides or even approaching to that of the Hg^{2+} adducted peptides, i.e., at least the ECD spectrum of Zn^{2+} adducted RGGGWGGGR should contain the peptide radical cation. In the study of the gas-phase reactions between the monovalent transition metal ions and benzene, Bohme and co-workers demonstrated [60] that electron transfer reaction can actually occur in Hg^{+}/benzene and Zn^{+}/benzene systems but not the Cd^{+}/benzene system. The latter could presumably be explained on the basis of the higher ionization energy of benzene (versus tryptophan residue). However, both our experimental results and literature reports demonstrate that ECD of Zn^{2+} adducted peptides gives only typical metalated and non-metalated c-/z-type fragments. Regardless of the ionization energy of the peptide moiety, no peptide radical cation or its related species could be observed.



Scheme **1.** A proposed mechanism for side chain(s) loss in the ECD of RGGGWGGGR adducted with Cd^{2+}

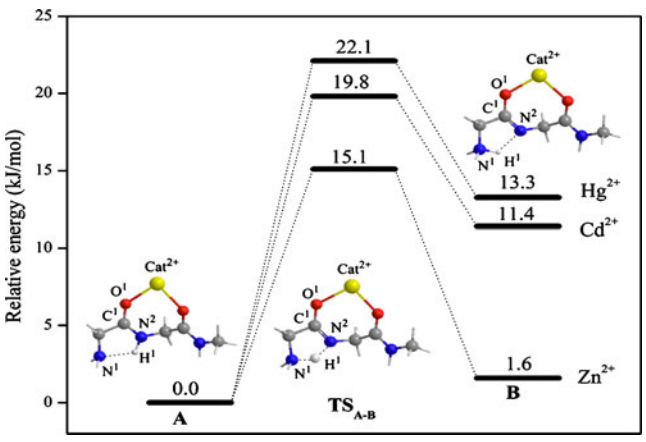
One way to explain the observed discrepancies is related to the solvation modulation of the recombination energy of the transition metal ions. In the metal ion adducted peptide systems, the transition metal ions should be coordinated by the polar functionalities of the peptide moiety, such as the carbonyl oxygen and arginine side chains. Reduction of the metal ions from their monovalent states to the neutral states would drastically alter the ion solvation. Part of the ion-electron recombination energy would therefore be used to compensate the change in the solvation energy. The equation governing the electron transfer should therefore be modified as:

$$\Delta H_{et} = \text{R.E.}(Cat^{+}) - \text{I.E.}(M) - \text{solvation energy} \quad (2)$$

The order of solvation energies for monovalent Group IIB metal ions should be inversely proportional to the size of the metal ions (see Table 1). It is therefore possible that the inclusion of the solvation energy term might toggle the energetics of the charge transfer reaction from spontaneous to non-spontaneous for Zn-system but not for the other group IIB metal systems. A better understanding on the solvation of monovalent metal ions by the peptide moiety is needed to evaluate this hypothesis.

The Role of Precursor Ion Heterogeneity

On top of the perturbation of solvation energy on the electron transfer reaction, the heterogeneity of the precursor ions might also play an important role in governing the resulting spectral features of these Group IIB metal ion



Scheme **2.** Potential energy surfaces associated with the deprotonation reactions of Zn^{2+}, Cd^{2+}, and Hg^{2+} adducted N-methyl glycyl-glycinamide. Single point energy calculated at the B3LYP/LANL2DZ+6-311++G(3df,2p) based on the structures optimized at B3LYP/LANL2DZ+6-31++G(d,p) level. The scale factor for ZPE is 0.961

Table 4. Optimized bond distances (Å) for the reactants, transition states and products involved in the deprotonation reaction at the B3LYP/LANL2DZ+6-31 ++G(d,p) level

	Zn^{2+}			Cd^{2+}			Hg^{2+}		
	A	TS_{A-B}	B	A	TS_{A-B}	B	A	TS_{A-B}	B
$R(H^1\text{-}N^1)$	1.938	1.318	1.050	1.954	1.292	1.054	2.003	1.292	1.053
$R(H^1\text{-}N^2)$	1.041	1.288	1.912	1.038	1.310	1.868	1.033	1.309	1.874
$R(C^1\text{-}O^1)$	1.286	1.297	1.320	1.278	1.290	1.310	1.268	1.287	1.309
$R(C^1\text{-}N^2)$	1.323	1.303	1.286	1.326	1.305	1.290	1.328	1.305	1.289
$R(Cat^1\text{-}O^1)$	1.901	1.894	1.883	2.113	2.107	2.092	2.253	2.219	2.198

adducted peptides. Previous experimental and theoretical studies of the metal ions peptide complexes have shown that the metal ions can induce deprotonation of the amide functions of peptides [63–66] and generate different forms of zwitterions. To get additional insights into the effect of Group IIB metal ions coordination on the deprotonation reaction of amide group, theoretical calculation using N-methyl glycyl-glycinamide as a truncated peptide model was investigated.

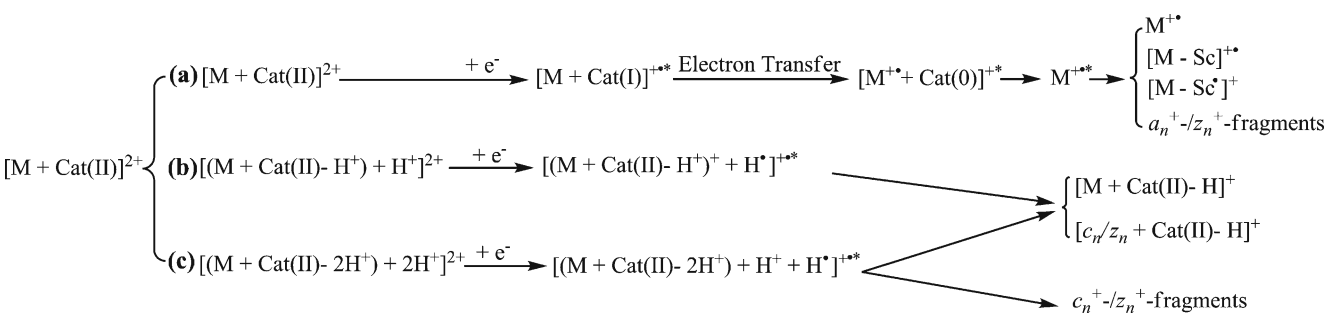
Scheme **2** shows the potential energy curves of deprotonation reaction mediated by Zn^{2+}, Cd^{2+}, and Hg^{2+}, respectively. The optimized structural parameters for the reactants, transition states and products are tabulated in Table 4. The stationary points (A, TS_{A-B}, and B) adopt a conformation in which the metal ion (Cat^{2+}) was bi-coordinated by the carbonyl oxygen atoms. As shown in Scheme **2**, the Zn^{2+} mediated intramolecular proton transfer from the amide to the N-terminal amino group is almost thermo-neutral (ΔH = +1.6 kJ mol^{-1}) and has a fairly low activation barrier (E_{TS} = +15.1 kJ mol^{-1}). In the presence of high proton affinity functionalities, the gas phase Zn^{2+} coordinated moieties should, therefore, adopt a zwitterionic form in which the backbone amide group(s) and /or the C-terminal carboxylic acid group are deprotonated, and the basic functionalities are protonated. Three conformers, with a general formula $[(\mathrm{M} + \mathrm{Cat}^{2+} - n\mathrm{H}^{+})^{(2-\mathrm{n})+} + n\mathrm{H}^{+}]^{2+} (n = 0, 1, \mathrm{and} 2)$, should exist in the precursor ions.

After established the existence of mobile proton(s) in the precursor ions, it is time to reconsider the recombination processes. Beside the metal ion-electron recombination, the mobile proton-electron recombination might constitute the second neutralization channel for the incoming electron. Along this channel, the hydrogen radical would be formed, and induce typical ECD fragmentation, leading to the formation of c-/z-ions and $[M + Cat - H]^+$. Because of the lower energy needed for Zn^{2+} mediated deprotonation reaction, it is believed that the proton-electron recombination prevails over the metal ions-electron recombination. Fragment ion species derived from this reaction channel should resemble to those derived from typical ECD of protonated peptides, i.e. mostly c-/z-type fragments.

For Cd^{2+}/peptide system, the energy barrier of deprotonation was found to be higher than that of Zn^{2+} (E_{TS} = +15.1 kJ mol^{-1}). The reactions are calculated to be more endothermic, ΔH = +11.4 kJ mol^{-1}. The population of zwitterionic conformers in precursor ions should then be much lower than that of Zn^{2+} containing systems. For ECD of Cd^{2+} adducted peptide, it is believed that the metal ion reduction should be more competitive with respect to the proton-electron recombination. The simultaneous occurrence of c-/z-type fragment ions and $M^{+\bullet}$ related species in the ECD spectra of Cd^{2+}/peptide systems supports a notion that metal ion reduction and proton-electron recombination are competitive processes. For the Hg^{2+} adducted model, the deprotonation is calculated to be even more unfavorable than for the Cd^{2+} adducted case. Considering the highest electron transfer reactivity of mercury mono-cation, it is believed that the metal ion-electron recombination is dominant in the electron recombination process. This is inconsistent with the experimental observations.

Dissociation Model of ECD of Peptides Adducted with Group IIB Metal Ions

Scheme **3** summarizes the reactions in the ECD of Group IIB metal ion adducted model peptides. It is believed that



Scheme **3**. A summary of the proposed reactions in the ECD of model peptides adducted with Group IIB metal ions

there are two reaction channels within the metalated peptide ions upon the capture of a low-energy electron, i.e., metal ion reduction and electron-proton recombination. The choice of the reaction channel is determined by the deprotonation reactivity of metal ion upon complexation with the peptide moiety. If the metal ion has low deprotonation ability and the solvation modulated recombination energy of the monovalent metal ion is larger than the ionization energy of the peptide, the reaction would take place along the upper branch. Electron capture-induced spontaneous electron transfer would occur. The $M^{+\bullet}$ and related fragment ions would be preferentially formed. This situation is best illustrated in the ECD of Hg^{2+}/peptide system. In contrast, if the metal ion has high deprotonation ability and/or the solvation modulated recombination energy of the monovalent metal ion is lower than the ionization energy of the peptide, the reaction would take place along the lower branch to form the usual labile ketylamino radical. Subsequent decomposition of this intermediate would lead to the metalated and/or non-metalated c-/z-type fragment ions. This situation is best illustrated in the ECD of Zn^{2+}/peptide system. If the metal ion has medium deprotonation ability, and the solvation modulated recombination energy of the monovalent metal ion is higher than the ionization energy of the peptide moiety, both reaction channels would be operative and generate an ECD spectrum containing both c-/z-type fragment ions and $M^{+\bullet}$ (and related fragment ions), i.e., ECD of Cd^{2+}/RGGGWGGGR system. Selecting a peptide moiety with higher ionization energy, i.e., RGGGVGGGR, the charge-transfer reaction between the peptide moiety and the monovalent metal ion might become energetically unfavorable. The corresponding ECD spectrum would then be dominated by the classical c-/z-fragment ions.

Conclusions

Electron capture dissociation of model peptides with Group IIB metal ions as charge carriers reveals interesting fragmentation chemistry. Typical ECD fragment ions were observed in the ECD of peptides adducted with Zn^{2+}. Peptide radical cations $M^{+\bullet}$ and related fragment species were observed in the ECD of model peptides adducted with Cd^{2+} or Hg^{2+}. Based on these distinctive reaction products, it is postulated that both electron-proton recombination and metal-ion reduction processes could be operated. The relative proportion of these processes depends on the kinetic factors (i.e., the number density of the zwitterionic form of the precursor ions) and/or the thermodynamic factors (i.e., the solvation-modulated electron-metal ion recombination energy). Regardless of the controlling factor, the reduction of divalent metal ions by the electron capture event could induce spontaneous electron transfer from the peptide moiety to the monovalent metal centre and generate hydrogen-deficient $M^{+\bullet}$ species. Depending on the overall reaction exothermicity, the $M^{+\bullet}$ species might be formed with substantial internal energy and undergo further decomposition to give structural specific information.

Acknowledgments

The authors acknowledge that the work described in this paper was fully supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China (project no. 400209). The calculations were carried out in part using the ORION calculation workstation in the Information Technology Service Center (ITSC) at the Chinese University of Hong Kong. The authors thank the ITSC for generous allocation of computing time and space of the system.

References

1. Zubarev, R.A., Kelleher, N.L., McLafferty, F.W.: Electron capture dissociation of multiply charged protein cations. A nonergodic process. *J. Am. Chem. Soc.***120**, 3265–3266 (1998)
2. Zubarev, R.A., Horn, D.M., Fridriksson, E.K., Kelleher, N.L., Kruger, N.A., Lewis, M.A., Carpenter, B.K., McLafferty, F.W.: Electron capture dissociation for structural characterization of multiply charged protein cations. *Anal. Chem.***72**, 563–573 (2000)
3. Adamson, J.T., Håkansson, K.: Infrared multiphoton dissociation and electron capture dissociation of high-mannose type glycopeptides. *J. Proteome Res.***5**, 493–501 (2006)
4. Woodlin, R.L., Bomse, D.S., Beauchamp, J.L.: Multiphoton dissociation of molecules with low power continuous wave infrared laser radiation. *J. Am. Chem. Soc.***100**, 3248–3250 (1978)
5. Kjeldsen, F., Haselmann, K.F., Budnik, B.A., Sorensen, E.S., Zubarev, R.A.: Complete characterization of post-translational modification sites in the bovine milk protein PP3 by tandem mass spectrometry with electron capture dissociation as the last stage. *Anal. Chem.***75**, 2355–2361 (2003)
6. Gauthier, J., Trautman, T.R., Jacobson, D.B.: Sustained off-resonance irradiation for collision-activated dissociation involving fourier transform mass spectrometry. collision-activated dissociation technique that emulates infrared multiphoton dissociation. *Anal. Chim. Acta***246**, 211–225 (1991)
7. Jennings, K.R.: The changing impact of the collision-induced decomposition of ions on mass spectrometry. *Int. J. Mass Spectrom.***200**, 479–493 (2000)
8. Shi, S.D.H., Hemling, M.E., Carr, S.A., Horn, D.M., Lindh, I., McLafferty, F.W.: Phosphopeptide/phosphoprotein mapping by electron capture dissociation mass spectrometry. *Anal. Chem.***73**, 19–22 (2001)
9. Kelleher, R.L., Zubarev, R.A., Bush, K., Furie, B., Furie, B.C., McLafferty, F.W., Walsh, C.T.: Localization of labile post-translational modifications by electron capture dissociation: The case of o-carboxyglutamic acid. *Anal. Chem.***71**, 4250–4253 (1999)
10. Hakansson, K., Cooper, H.J., Emmett, M.R., Costello, C.E., Marshall, A.G., Nilsson, C.L.: Electron capture dissociation and infrared multiphoton dissociation MS/MS of an N-glycosylated tryptic peptide to yield complementary sequence information. *Anal. Chem.***73**, 4530–4536 (2001)
11. Zubarev, R.A., Kruger, N.A., Fridriksson, E.K., Lewis, M.A., Horn, D.M., Carpenter, B.K., McLafferty, F.W.: Electron capture dissociation of gaseous multiply-charged proteins is favored at disulfide bonds and other sites of high hydrogen atom affinity. *J. Am. Chem. Soc.***121**, 2857–2862 (1999)
12. McLafferty, F.W., Horn, D.M., Breuker, K., Ge, Y., Lewis, M.A., Cerda, B., Zubarev, R.A., Carpenter, B.K.: Electron capture dissociation of gaseous multiply charged ions by fourier-transform ion cyclotron resonance. *J. Am. Soc. Mass Spectrom.***12**, 245–249 (2001)
13. Sawicka, A., Skurski, P., Hudgins, R.R., Simons, J.: Model calculations relevant to disulfide bond cleavage via electron capture influenced by positively charged groups. *J. Phys. Chem. B***107**, 13505–13511 (2003)
14. Sobczyk, M., Anusiewicz, W., Berdys-Kochanska, J., Sawicka, A., Skurski, P., Simons, J.: Coulomb-assisted dissociative electron attach-

ment: Application to a model peptide. *J. Phys. Chem. A***109**, 250–258 (2005)
15. Sobczyk, M., Simons, J.: The role of excited rydberg states in electron transfer dissociation. *J. Phys. Chem. B***110**, 7519–7527 (2006)
16. Simons, J.: Mechanisms for S–S and N–Ca bond cleavage in peptide ECD and ETD mass spectrometry. *Chem. Phys. Lett.***484**, 81–95 (2010)
17. Syrstad, E.A., Turecek, F.: Towards a general mechanism of electron capture dissociation. *J. Am. Soc. Mass Spectrom.***16**, 208–224 (2005)
18. Jones, J.W., Sasaki, T., Goodlett, D.R., Turecek, F.: Electron capture in spin-trap capped peptides. An experimental example of ergodic dissociation in peptide cation-radicals. *J. Am. Soc. Mass Spectrom.***18**, 432–444 (2007)
19. Turecek, F., Chen, X.H., Hao, C.T.: Where does the electron go? Electron distribution and reactivity of peptide cation radicals formed by electron transfer in the gas phase. *J. Am. Chem. Soc.***130**, 8818–8833 (2008)
20. Li, X., Cournoyer, J.J., Lin, C., O'Connor, P.B.: The effect of fixed charge modifications on electron capture dissociation. *J. Am. Soc. Mass Spectrom.***19**, 1514–1526 (2008)
21. Chamot-Rooke, J., Rest, G., Dalleu, A., Bay, S., Lemoine, J.: The combination of electron capture dissociation and fixed charge derivatization increases sequence coverage for o-glycosylated and o-phosphorylated peptides. *J. Am. Soc. Mass Spectrom.***18**, 1405–1413 (2007)
22. Jensen, C.S., Holm, A.I.S., Zettergren, H., Overgaard, J.B., Hvelplund, P., Nielsen, S.B.: On the charge partitioning between *c* and *z* fragments formed after electron-capture induced dissociation of charge-tagged Lys-Lys and Ala-Lys dipeptide dications. *J. Am. Soc. Mass Spectrom.***20**, 1881–1889 (2009)
23. Chamot-Rooke, J., Malosse, C., Frison, G., Tureček, F.: Electron capture in charge-tagged peptides. Evidence for the role of excited electronic states. *J. Am. Soc. Mass Spectrom.***18**, 2146–2121 (2007)
24. Liu, H.C., Håkansson, K.: Electron capture dissociation of tyrosine o-sulfated peptides complexed with divalent metal cations. *Anal. Chem.***78**, 7570–7576 (2006)
25. Liu, H.C., Yoo, H.J., Håkansson, K., Lee, J.Y., Sherman, D.H.: Collision-activation dissociation, infrared multiphoton dissociation, and electron capture dissociation of the *Bacillus anthracis* siderophore petrobacin and its metal ion complexes. *J. Am. Soc. Mass Spectrom.***18**, 842–849 (2007)
26. Liu, H.C., Yoo, H.J., Håkansson, K.: Characterization of phosphate-containing metabolites by calcium adduction and electron capture dissociation. *J. Am. Soc. Mass Spectrom.***19**, 799–808 (2008)
27. Adamson, J.T., Håkansson, K.: Electron capture dissociation of oligosaccharides ionized with alkali, alkaline earth, and transition metals. *Anal. Chem.***79**, 2901–2910 (2006)
28. James, P.F., Perugini, M.A., O'Hair, R.A.J.: Electron capture dissociation of complexes of diacylglycerophosphocholine and divalent metal ions: competition between charge reduction and radical induced phospholipid fragmentation. *J. Am. Soc. Mass Spectrom.***19**, 978–986 (2008)
29. Kaczorowska, M.A., Hotze, A.C.G., Hannon, M.J., Cooper, H.J.: Electron capture dissociation mass spectrometry of metallo-supramolecular complexes. *J. Am. Soc. Mass Spectrom.***21**, 300–309 (2010)
30. Kaczorowska, M.A., Cooper, H.J.: Electron capture dissociation and collision-induced dissociation of metal ion [Ag(+), Cu(2+), Zn(2+), Fe (2+), and Fe(3+)] Complexes of polyamidoamine (PAMAM) dendrimers. *J. Am. Soc. Mass Spectrom.***20**, 674–681 (2009)
31. Iavarone, A.T., Paech, K., Williams, E.R.: Effects of charge state and cationizing agent on the electron capture dissociation of a peptide. *Anal. Chem.***76**, 2231–2238 (2004)
32. Fung, Y.M.E., Liu, H., Chan, T.W.D.: Electron capture dissociation of peptides metalated with alkaline-earth metal ions. *J. Am. Soc. Mass Spectrom.***17**, 757–771 (2006)
33. Kleinnijenhuis, A.J., Mihalca, R., Heeren, R.M.A., Heck, A.J.R.: Atypical behavior in the electron capture induced dissociation of biologically relevant transition metal ion complexes of the peptide hormone oxytocin. *Int. J. Mass Spectrom.***253**, 217–224 (2006)
34. Liu, H.C., Håkansson, K.: Divalent metal ion-peptide interactions probed by electron capture dissociation of trications. *J. Am. Soc. Mass Spectrom.***17**, 1731–1741 (2006)
35. Yuril, B.E.V., Palmblad, M., Dallebout, H., Heeren, R.M.A.: Electron capture dissociation of peptide hormone changes upon opening of the tocin ring and complexation with transition metal cations. *Rapid Commun. Mass Spectrom.***23**, 31–38 (2009)
36. Turecek, F., Jones, J.W., Holm, A.I.S., Panja, S., Nielsen, S.B., Hvelplund, P.: Transition metals as electron traps. I. Structures, energetics, electron capture, and electron-transfer-induced dissociations of ternary copper–peptide complexes in the gas phase. *J. Mass Spectrom.***44**, 707–725 (2009)
37. Turecek, F., Jones, J.W., Holm, A.I.S., Panja, S., Nielsen, S.B., Hvelplund, P.: Transition metals as electron traps. II. Structures, energetics, and electron transfer dissociations of ternary Co, Ni, and Zn–peptide complexes in the gas phase. *J. Mass Spectrom.***44**, 1518–1531 (2009)
38. Zubarev, R.A., Haselmann, K.F., Budnik, B., Kjeldsen, F., Jensen, F.: Towards an understanding of the mechanism of electron-capture dissociation: a historical perspective and modern ideas. *Eur. J. Mass Spectrom.***8**, 337–349 (2002)
39. Weast, R.C. (ed.). CRC handbook of chemistry and physics (67th ed.); Boca Raton, FL. CRC Press: E-76-77 (1987).
40. Shannon, R.D.: Revised effective ionic radii and systematic studies of interatomic distances in halides and chalcogenides. *Acta Crystallogr. A***32**, 751–767 (1976)
41. Lau, R.L.C., Jiang, J.Z., Ng, D.K.P., Chan, T.W.D.: Fourier transform ion cyclotron resonance studies of lanthanide(III) porphyrin-phthalocyanine heteroleptic sandwich complexes by using electrospray ionization. *J. Am. Soc. Mass Spectrom.***8**, 161–169 (1997)
42. Fong, K.W.Y., Chan, T.W.D.: A novel nonmetallized tip for electrospray mass spectrometry at nanoliter flow rate. *J. Am. Soc. Mass Spectrom.***10**, 72–75 (1999)
43. Chan, T.W.D., Duan, L., Sze, T.P.: Accurate mass measurements for peptide and protein mixtures by using matrix-assisted laser desorption/ionization fourier transform mass spectrometry. *Anal. Chem.***74**, 5282–5289 (2002)
44. Chan, T.W.D., Ip, W.H.H.: Optimization of experimental parameters for electron capture dissociation of peptides in a fourier transform mass spectrometer. *J. Am. Soc. Mass Spectrom.***13**, 1396–1406 (2002)
45. Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Montgomery, J.A., Vreven Jr., T., Kudin, K.N., Burant, J.C., Millam, J.M., Iyengar, S.S., Tomasi, J., Barone, V., Mennucci, B., Cossi, M., Scalmani, G., Rega, N., Petersson, G.A., Nakatsuji, H., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Klene, M., Li, X., Knox, J.E., Hratchian, H.P., Cross, J.B., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Ayala, P.Y., Morokuma, K., Voth, G.A., Salvador, P., Dannenberg, J.J., Zakrzewski, V.G., Dapprich, S., Daniels, A.D., Strain, M.C., Farkas, O., Malick, D.K., Rabuck, A.D., Raghavachari, K., Foresman, J.B., Ortiz, J.V., Cui, Q., Baboul, A.G., Clifford, S., Cioslowski, J., Stefanov, B.B., Liu, G., Liashenko, A., Piskorz, P., Komaromi, I., Martin, R.L., Fox, D.J., Keith, T., Al-Laham, M.A., Peng, C.Y., Nanayakkara, A., Challacombe, M., Gill, P.M.W., Johnson, B., Chen, W., Wong, M.W., Gonzalez, C., Pople, J.A.: Gaussian 03, Revision D.01. Gaussian, Inc, Wallingford CT (2004)
46. Becke, A.D.A.: New mixing of hartree-fock and local density-functional theories. *J. Chem. Phys.***98**, 1372–1377 (1993)
47. Becke, A.D.: Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.***98**, 5648–5652 (1993)
48. Lee, C., Yang, W., Parr, R.C.: Development of the colle-salvetti correction-energy formula into a function of the electron density. *Phys. Rev. B***37**, 785–789 (1988)
49. Hay, P.J., Wadt, W.R.: Ab intio effective core potentials for molecular calculations. Potentials for the transition metal atoms Sc to Hg. *J. Chem. Phys.***82**, 270–283 (1985)
50. Wadt, W.R., Hay, P.J.: Ab initio effective core potentials for molecular calculations. Potentials for main group elements Na to Bi. *J. Chem. Phys.***82**, 284–298 (1985)
51. Hay, P.J., Wadt, W.R.: Ab intio effective core potentials for molecular calculations. Potentials for K to Au including the outermost core orbitals. *J. Chem. Phys.***82**, 299–310 (1985)
52. Hopkinson, A.C.: Radical cations of amino acids and peptides: structures and stabilities. *Mass Spectrom. Rev.***28**, 655–671 (2009)
53. Wee, S., O'Hair, R.A.J., McFadyen, W.D.: Comparing the gas-phase fragmentation reactions of protonated and radical cations of the tripeptide GXR. *Int. J. Mass Spectrom.***234**, 101–122 (2004)
54. Wee, S., O'Hair, R.A.J., McFadyen, W.D.: The role of the position of the basic residue in the generation of peptide radical cations. *Int. J. Mass Spectrom.***249**, 171–183 (2006)

55. Chu, I.K., Lam, C.N.W.: Generation of peptide radical dications via low-energy collision-induced dissociation of $[Cu^{II}(terpy)(M+H)]^{3+}$. *J. Am. Soc. Mass Spectrom.***16**, 1795–1804 (2005)
56. Deery, M.J., Summerfield, S.G., Buzy, A.B., Jennings, K.R.: A mechanism for the loss of 60 u from peptides containing an arginine residue at the C-terminus. *J. Am. Soc. Mass Spectrom.***8**, 253–261 (1997)
57. Millefiori, S., Alparone, A., Millefiori, A., Vanella, A.: Electronic and vibrational polarizabilities of the 20 naturally occurring amino acids. *Biophys. Chem.***132**, 139–147 (2008)
58. Wesendrup, R., Schalley, C.A., Schröder, D., Schwarz, H.: Bimolecular gas-phase reactions of d-block transition-metal cations with dimethyl peroxide: trends across the periodic table. *Chem. Eur. J.***1**, 608–613 (1995)
59. Caraiman, D., Koyanagi, G.K., Bohme, D.K.: Gas-phase reactions of transition-metal ions with hexafluorobenzene: room-temperature kinetics and periodicities in reactivity. *J. Phys. Chem. A***108**, 978–986 (2004)
60. Koyanagi, G.K., Bohme, D.K.: Kinetics and thermodynamics for the bonding of benzene to 20 main-group atomic cations: formation of half-sandwiches, full-sandwiches, and beyond. *Int. J. Mass Spectrom.***227**, 563–575 (2003)
61. Zhao, X., Flaim, E., Huynh, L., Jarvis, M.J.Y., Cheng, P., Lavrov, V.V., Blagojevic, V., Koyanagi, G.K., Bohme, D.K.: Electron transfer and ligand addition to atomic mercury cations in the gas phase: kinetic and equilibrium studies at 295 K. *Inorg. Chem.***45**, 9646–9653 (2006)
62. Ling, S., Yu, W., Huang, Z., Lin, Z., Haranczyk, M., Gutowski, M.: Gaseous arginine conformers and their unique intramolecular interactions. *J. Phys. Chem. A***110**, 12282–12291 (2006)
63. Sovago, I., Osz, K.: Metal ion selectivity of oligopeptides. *Dalton Trans.*, 3841–3854 (2006).
64. Sigel, H., Martin, R.B.: Coordinating properties of the amide bond-stability and structure of metal–ion complexes of peptides and related ligands. *Chem. Rev.***82**, 385–426 (1982)
65. Wong, C.K.L., Chan, T.W.D.: Cationization processes in matrix-assisted laser desorption/ionization mass spectrometry: attachment of divalent and trivalent metal ions. *Rapid Commun. Mass Spectrom.***11**, 513–519 (1997)
66. Rulisek, L., Vondrasek, J.: Coordination geometries of selected transition metal ions (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+}) in metalloproteins. *J. Inorg. Biochem.***71**, 115–127 (1998)