Published in final edited form as:

J Am Soc Mass Spectrom. 2013 February ; 24(2): 193–201. doi:10.1007/s13361-012-0507-7.

Electron Capture Dissociation of Trivalent Metal Ion-Peptide Complexes

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Abstract

With electrospray ionization from aqueous solutions, trivalent metal ions readily adduct to small peptides resulting in formation of predominantly (peptide + $M_T - H$)²⁺, where M_T = La, Tm, Lu, Sm, Ho, Yb, Pm, Tb, or Eu, for peptides with molecular weights below ~1000 Da, and predominantly (peptide + M_T)³⁺ for larger peptides. ECD of (peptide + $M_T - H$)²⁺ results in extensive fragmentation from which nearly complete sequence information can be obtained, even for peptides for which only singly protonated ions are formed in the absence of the metal ions. ECD of these doubly charged complexes containing M_T results in significantly higher electron capture efficiency and sequence coverage than peptide-divalent metal ion complexes that have the same net charge. Formation of salt-bridge structures in which the metal ion coordinates to a carboxylate group are favored even for (peptide + M_T)³⁺. ECD of these latter complexes for large peptides results in electron capture by the protonation site located remotely from the metal ion and predominantly *c/z* fragments for all metals, except Eu³⁺, which undergoes a one electron reduction and only loss of small neutral molecules and *b/y* fragments are formed. These results indicate that solvation of the metal ion in these complexes is extensive, resulting in similar electrochemical properties of these metal ions both in the peptide environment and in water.

Introduction

Peptide sequencing by tandem mass spectrometry is widely used to identify proteins and locate sites of post-translational modifications [1–15]. Dissociation by electron-ion recombination methods, whether by capture of a free electron (electron capture dissociation; ECD) [4–7] or transfer of an electron from an anion (electron transfer dissociation; ETD) [8–11] or from an atom (electron capture induced dissociation; ECID) [16–18] produces characteristic *c* and *z* ion fragments and can often result in minimal loss of labile posttranslational modifications, such as phosphorylation [4], making these attractive methods for determining the locations of such chemically modified sites. The extent of fragmentation, electron capture efficiency, and recombination energy significantly increase with increasing ion charge state [19–25]. For example, at least 36% more unique peptides could be identified with ETD than collisional dissociation methods for both tryptic and Lys-C peptides with charge states greater than two [21].

One significant disadvantage of these ion-electron recombination methods for small peptides is that the charge state is reduced, making it challenging to apply these methods when only singly protonated ions are formed by electrospray ionization. With ECID, the reduced precursor of a singly protonated peptide can be reionized in a second atomic collision and

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the fragmentation induced by the neutralization step can be readily analyzed [16]. Electron detachment dissociation (EDD), where excited radical cations with one higher charge or anions with one lower charge are produced by irradiating trapped ions with electrons that have 10 eV or higher kinetic energy, results in similar fragmentation pathways as those obtained with ECD [26–29]. Although fragmentation can be more extensive with EDD than with ETD, the efficiency of the EDD process is relatively low [26–29].

Complexation of a divalent metal ion, M_D , to a small peptide can result in divalent ions that can readily dissociate upon ion-electron recombination [20,30–40]. Håkansson and coworkers found that ECD of (substance P + H + M_D)³⁺, where M_D = different alkaline earth metal ions, Mn, Fe, or Zn, results in similar sequence coverage to that obtained from ECD of (substance P + 3H)³⁺ [30]. Protonated *c* ions and complementary metal containing *z* ions are formed, which was attributed to the metal ion binding close to the C-terminus [30]. In contrast, ECD of Co²⁺ and Ni²⁺-bound peptides predominately cleaves C-terminal to methionine, the likely metal binding site, and lower sequence coverage than that from ECD of (substance P + 3H)³⁺ is obtained [30]. Zubarev and coworkers found fewer fragment ions are produced by ECD of (angiotensin II + Zn)²⁺ compared to ECD of the diprotonated ion, but the fragments provide complementary sequence information [20].

ECD of Cu²⁺-bound peptides results in mostly *b/y* fragments and few or no *c/z* ions are typically formed [30–34]. However, *c/z* ions are formed by ECD of Cu²⁺-bound peptides when there are a sufficient number of coordinating residues [35,36], an effect attributed to a lowering of the electron-metal ion recombination energy making electron capture at a metal remote site more favorable [35]. The electronic configuration of metal ions has also been reported to play a role in whether metal ion reduction occurs [34]. Chan and coworkers have investigated ECD of many different metal ion-peptide complexes [34,37,38]. For alkali earth metals and some transition metals, ECD results in metallated and non-metallated *c/z* fragment ions, a result attributed to a zwitterionic peptide structure in which a carboxylate group is deprotonated and fragmentation is driven by a remote protonation site [34,37,38]. For other transition metal ions, *a/b/y* ions were formed, which was attributed to reduction of the metal ion in a charge-solvated structure [34,37,38].

Trivalent metal ion (M_T)-peptide complexes can be readily formed by electrospray ionization (ESI), and complexation of metal ions to small peptides can increase the charge state of the molecular ions [41–47]. Attachment of trivalent metal ions to small peptides for which singly protonated ions would typically be formed resulted in deprotonated, doubly charged ions, where the metal ion displaces a proton at an acidic site to form a salt-bridge structure [41–43]. Results from infrared multiphoton dissociation spectroscopy of M_T -polyalanine complexes, (Ala_{*n*} – H + M_T)²⁺, *n* = 2 – 5, indicate that these ions exist in compact salt-bridge conformations in which all carbonyl oxygen atoms of the peptide backbone coordinate to M_T , but coordination to other side chains, such as the Tyr or Phe aromatic rings, is favorable for other peptides [41]. For larger peptides, (peptide + M_T)³⁺ is primarily formed, even for peptides for which only doubly protonated ions are typically formed by ESI [43–45]. ECD of lanthanide metal-heptadentate ligands complexed with phosphopeptides resulted in extensive fragmentation of the peptide backbone and the phosphorylation site could be readily identified [47].

Here, results for ECD of peptides complexed with lanthanide ions are reported for peptides with molecular weights below ~2,000 Da and are compared to those for the multiply protonated ions formed directly by ESI. These results indicate that cationization of peptides with trivalent metal ions can lead to significant increases in both the fragmentation and electron capture efficiency for small peptides, but is not so advantageous for larger peptides for which triply protonated ions can be formed. These results also indicate that the metal ion

solvation by the peptide is extensive in the larger peptides, resulting in similar electrochemical properties of these metal ions in both the peptide environment and in aqueous solution.

Experimental

All experiments were performed using a 9.4 T Fourier-transform ion cyclotron resonance mass spectrometer that is described elsewhere [48]. Ions are generated by nanoESI using borosilicate capillaries that are pulled so that the tips have a $\sim 2\ \mu\text{m}$ inner diameter (model P-87 capillary puller, Sutter Instruments, Novato, CA). Approximately 2 to 10 μL of sample is loaded into the capillary, and a platinum wire is inserted into the solution. The capillary is positioned $\sim 2\ \text{mm}$ from the source inlet, and a potential difference of 800 to 1200 V between the platinum wire and source inlet is applied. Ions are accumulated in an external hexapole ion trap for 0.5 to 4.0 s, and are subsequently injected into the ion cell. For tandem MS experiments, the precursor ion of interest is isolated using stored waveform inverse Fourier transforms (SWIFTs) followed by a 100 ms delay. For ECD, electrons from a heated dispenser cathode (Heatwave Labs, Watsonville, CA) that is mounted axially from the cell center are introduced into the ion cell by changing the cathode housing potential from +10 V to $-1.4\ \text{V}$ for 100 ms. Ions are detected 1.0 s after electron irradiation. For sustained off-resonance irradiation collisionally activated dissociation (SORI-CAD), the precursor is excited using a single frequency waveform ($4.0\ \text{V}_{\text{peak-to-peak}}$, 2500 Hz offset) for 0.5 s, resulting in a maximum lab-frame translational energy of 0.5 eV, and collided with N_2 gas that is introduced to a pressure of $\sim 10^{-6}$ Torr using a pulsed piezoelectric valve. All peptides and salts were obtained from Sigma Aldrich (St. Louis, MO). Solutions were prepared at a concentration of 10 to 100 μM peptide with 100 μM to 1 mM metal salt using 18 M Ω water (Milli-Q, Millipore, Billerica, MA) with a peptide to metal ratio of 1:10 for all solutions. A pH of ~ 7 was measured for all sample solutions using pH indicator strips.

Results and Discussion

Attachment of Trivalent Metal Ions

ESI of an aqueous solution of 10 μM leucine enkephalin (LeEnk) with 100 μM LaCl_3 results in the formation of both $(\text{LeEnk} + \text{H})^+$ and $(\text{LeEnk} - \text{H} + \text{La})^{2+}$ (Fig 1a). Only protonated amidated LeEnk, $(\text{LeEnk-NH}_2 + \text{H})^+$, is formed with these same conditions; no ($<0.004\%$) cationization by La^{3+} occurs (Fig 1a, inset). This indicates that La^{3+} binds to the carboxylate group of LeEnk, consistent with a salt-bridge structure identified for this ion by IRPD spectroscopy [40]. In contrast, cationization of substance P (SP), which has an amidated C-terminus and no acidic residues, with La^{3+} readily occurs, and the abundance of $(\text{SP} + \text{La})^{3+}$ is a factor of $3\times$ greater than that of $(\text{SP} + 3\text{H})^{3+}$. These results indicate that a salt-bridge structure is not essential for La^{3+} adduction to all peptides. Stabilization of La^{3+} can also occur through interactions with polarizable atoms, such as carbonyl oxygen atoms on the peptide backbone or side-chain hetero atoms or phenyl groups, resulting in a charge-solvated structure.

The relative abundances of $(\text{peptide} + \text{M}_T - \text{H})^{2+}$ and $(\text{peptide} + \text{M}_T)^{3+}$ depend predominantly on peptide size. For example, in contrast to the results for leucine enkephalin, $(\text{peptide} + \text{La})^{3+}$ is the most abundant form of SFLLRNPNDKYEPF (SFLLR), and this ion is $\sim 8\times$ more abundant than $(\text{peptide} + 3\text{H})^{3+}$ (Fig 1b). Both $(\text{SFLLR} - \text{H} + \text{La})^{2+}$ and $(\text{SFLLR} - 3\text{H} + 2\text{La})^{3+}$ are also formed, but are approximately a factor of 70 and 3 less abundant, respectively, than $(\text{SFLLR} + \text{La})^{3+}$. The relative abundances of $(\text{peptide} + \text{M}_T - \text{H})^{2+}$ and $(\text{peptide} + \text{M}_T)^{3+}$ as a function of molecular weight (MW) for various peptides complexed to La^{3+} are shown in Fig 1c. For peptides with MW's below $\sim 1,000\ \text{Da}$, $(\text{peptide} - \text{H} + \text{La})^{2+}$ is preferentially formed, whereas $(\text{peptide} + \text{La})^{3+}$ is the most abundant ion for

peptides with MW > 1,000 Da. There is no significant dependence of the abundance of (peptide + M_T)³⁺ on the 3rd ionization energy of the trivalent metal for any of the peptides or metal ions investigated here. The transition to (peptide + La)³⁺ becoming the most abundant form of the molecular ion above ~1,000 Da (or more than approximately eight residues) indicates that there is a critical peptide size where charge-solvation structures, or salt-bridge structures in which a proton is displaced to another residue, become most stable.

ECD of (peptide – H + M_T)²⁺

ESI of an aqueous solution that contains a trivalent metal ion and small peptide (MW < 800 Da) for which multiply protonated ions are not typically formed results in the formation of (peptide – H + M_T)²⁺ for peptides with an acidic site. The same charge state can also be formed by attachment of divalent metal ions. To compare dissociation of divalent peptide ions formed by attachment of trivalent metal ions and divalent metal ions, ECD spectra of the small peptides LeEnk, Ala₆, and FLEEL were acquired for (peptide – H + M_T)²⁺, where M_T = La³⁺, Tm³⁺, Eu³⁺, or Yb³⁺, and compared to ECD results for divalent metal ion-peptide complexes, (peptide + M_D)²⁺, where M_D = Mg²⁺, Ca²⁺, Ba²⁺, or Sr²⁺. In general, the electron capture (EC) efficiency and sequence coverage is greater (by more than 6% and 25%, respectively) for (peptide – H + M_T)²⁺ compared to (peptide + M_D)²⁺. Representative ECD spectra for both (LeEnk + Ca)²⁺ and (LeEnk – H + La)²⁺ are shown in Fig 2 with the relative fragment abundances as a function of cleavage site inset. All fragments retain the metal ion for both precursors, but the sequence coverage and EC efficiency is 2× and ~6% greater, respectively, for (LeEnk – H + La)²⁺, for which complete sequence coverage is obtained. Complete sequence coverage is also obtained for Yb³⁺ and Tm³⁺, whereas the maximum sequence coverage obtained with the divalent metal ions was only 75% for (LeEnk + Mg)²⁺ (Table S1). Similarly, sequence coverage is 40 to 50% greater for ECD of (peptide – H + M_T)²⁺ (peptide = FLEEL and Ala₆) for all trivalent metal ions, except for Eu³⁺, compared to results for (peptide + M_D)²⁺ (Fig S1 and S2). ECD of (peptide – H + Eu)³⁺ also results in c/z fragment ions, but a minimum of 25% lower sequence coverage is obtained for all peptides than with the other trivalent metal ions. The EC efficiency was 6 to 34% greater for (peptide – H + M_T)²⁺ compared to (peptide + M_D)²⁺ for all trivalent metal ions, even Eu³⁺, a result that may be attributed to higher localized charge on the peptide with the trivalent metal ion. These results indicate that formation of (peptide – H + M_T)²⁺ for small peptides substantially increase the EC efficiency and the structural information obtained with ECD.

ECD of (peptide + M)³⁺

ECD spectra were also acquired for (peptide + M_T)³⁺, where M_T = La³⁺, Eu³⁺, Yb³⁺, Pm³⁺, Tb³⁺, Sm³⁺, Tm³⁺, Ho³⁺, or Lu³⁺, for seven peptides with MW's > 900 Da, and these results are compared to ECD data for the fully protonated ion with the highest charge state formed by ESI from a purely aqueous solution. ECD of (peptide + M_T)³⁺ typically results in similar or lower sequence coverage compared to ECD of (peptide + 3H)³⁺ or (peptide + 2H)²⁺. For example, the ECD products for (ranakinin + 3H)³⁺ and (ranakinin + M_T)³⁺ where M_T = La³⁺, Eu³⁺, or Yb³⁺ are shown in Fig 3. These trivalent metal ions span the lowest (La³⁺) and highest (Eu³⁺ and Yb³⁺) 3rd ionization energies of the lanthanides investigated. The same sequence coverage (100%) and similar EC efficiency (~65%) was obtained from ECD of (ranakinin + M_T)³⁺ with La³⁺ and Yb³⁺ compared to (ranakinin + 3H)³⁺, whereas (ranakinin + Eu)³⁺ resulted in only 50% sequence coverage. Cleavage at the N-C_α peptide bond N-terminal to proline is not detected either because it does not occur or because the N and C_α atoms in the proline residue remain linked by the side chain methylene units after cleavage of the N-C_α bond. However, b/y ions are formed due to amide bond cleavage N-terminal to the proline residue, likely a result of vibrational excitation of the charge reduced precursor or ECD fragment ions [49,50]. As was the case

for smaller peptides, the results for Eu^{3+} are significantly different than those obtained for the other trivalent metal ions.

ECD data for (peptide + La^{3+}) where the peptide is SFLLR, neurotensin, neurokinin, or histatin 8 resulted in 23 – 33% lower sequence coverage than (peptide + 3H^{3+}). ($\text{SP} + \text{La}^{3+}$) was the only ion where higher sequence coverage (by 10%) was obtained for (peptide + M_T^{3+}) vs. (peptide + 3H^{3+}). For both angiotensin II and bradykinin 2 – 9, (peptide + 3H^{3+}) could not be formed, so ECD data of (peptide + M_T^{3+}) is compared to that for (peptide + 2H^{2+}). The EC efficiency was 19 to 44% higher for the (peptide + M_T^{3+}) for both angiotensin II and bradykinin 2–9 compared to (peptide + 2H^{2+}), but the sequence coverage was the same for all trivalent metal ions, except for Eu^{3+} , compared to the protonated ions. These results indicate that ECD of (peptide + M_T^{3+}) does not improve the sequence coverage compared to (peptide + 2H^{2+}) or (peptide + 3H^{3+}), but can result in a significant improvement in the EC efficiency if complexation of the trivalent metal ion to the peptide results in an increase in the charge state compared to the fully protonated form.

Metal Binding to Acidic Sites

ECD of (peptide + M_T^{3+}) results in both metal-attached fragment ions as well as fragments that do not bind the metal ion. Metal attached fragment ions formed by ECD of (ranakinin + M_T^{3+}) all contain the glutamic acid residue, whereas no protonated fragments with this residue are formed (Fig 3), indicating that the metal ion binds to glutamic acid in this peptide. Similar results were obtained for other peptides with acidic sites. ECD fragmentation of (neurokinin + La^{3+}), (SFLLR + La^{3+}), and (angiotensin II + La^{3+}) are shown in Fig 4. Neurokinin is amidated on the C-terminus, and has one acidic residue, D4. All metal containing fragments include D4, and those that do not contain this residue do not have the metal ion. Similar results are also obtained when the trivalent metal ion is Eu^{3+} or Yb^{3+} (Fig S3). All ECD fragments of (SFLLR + La^{3+}) that retain La^{3+} contain both D9 and E12 (Fig 4b). No fragments between these residues are formed, suggesting that the metal ion may coordinate to both acidic residues. Similar results are obtained when the trivalent metal ion is Yb^{3+} , Lu^{3+} , Ho^{3+} , Sm^{3+} , Tm^{3+} , Tb^{3+} , or Pm^{3+} (Fig S4). For (angiotensin II + La^{3+}) (Fig 4c), there are two acidic sites, the C-terminus and D9. Both N- and C-terminal fragments retain La^{3+} , indicating that either site likely coordinates to La^{3+} and both forms of these adducted ions are present.

These data indicate that for peptides with acidic sites, the trivalent metals ions coordinate specifically to these sites, and that the salt-bridge form of the trivalent ion in which the metal ion is bound to a carboxylate group and a proton is bound elsewhere in the peptide is more stable than the entirely charge-solvated form. In contrast, other studies indicate divalent metal ions bind to locations other than acidic sites in the peptide depending on metal ion identity [30]. The propensity for trivalent metal binding at acidic sites should make it possible to more readily determine the position of acidic residues in small peptides even when fragmentation is incomplete. Sequence information can also be obtained from the accurate mass, but there are instances where isobaric fragments cannot be determined from accurate mass alone at a given mass measuring accuracy [51]. The most common nominal isobar arises from the difference between CH_4 and O (36 mDa). For example, the combined residue mass of Tyr and Leu is only 36 mDa higher than the combined residue mass of Phe and Glu [51]. In these instances, this method could be used to provide additional sequence information to aid peptide identification.

Eu^{3+} as an Electron Trap

ECD of (peptide + Eu^{3+}) generally resulted in less than half the sequence coverage compared to the other trivalent metal ions and the extent of fragmentation decreases with

increasing peptide size. For example, there are no *c/z* fragments in the ECD spectra of (angiotensin II + Eu)³⁺; *b/y* ions and ions corresponding to small neutral losses from the charge reduced precursor are formed instead (Fig 5). The sequence coverage obtained from the ECD spectrum of (angiotensin II + Eu)³⁺ is only 29%, whereas that for (angiotensin II + 2H)²⁺ and (angiotensin II + M_T)³⁺, where M_T = La³⁺ or Yb³⁺, is 100%.

For even larger peptides, i.e., > ~1560 Da (histatin 8, neurotensin, and SFLLR), the charge reduced precursor, (peptide + Eu)²⁺, accounts for >93% of the total product ions formed from ECD, and predominantly small neutral loss products or *b/y* ions are formed. For example, the electron capture dissociation spectra for (SFLLR + La)³⁺ and (SFLLR + Eu)³⁺ are shown in Fig 6a–b. Whereas, electron capture by (SFLLR + La)³⁺ results mostly in the formation *c/z* ions (69% sequence coverage) and the charged reduced precursor accounts for only 7% of the total product ions (Fig 6a), for (SFLLR + Eu)³⁺, the charge reduced precursor accounts for 96% of the total product ions (no sequence coverage), and only a small neutral loss and a *b* fragment is observed (Fig 6b). Significantly more extensive fragmentation occurs for many lanthanides (e.g., La, Lu, Sm, Ho, Pm, and Tb) that have lower 3rd ionization energies than Eu, which has one of the highest. Despite the significantly different fragmentation, there is essentially no difference (<2%) in the ECD efficiency for these two ions. To determine if *c/z* ions are formed from ECD of (SFLLR + Eu)³⁺, but remain noncovalently bound in the reduced precursor, the charge reduced precursor, (SFLLR + Eu)²⁺, was activated by SORI-CAD (Fig 6c). Only *b/y* fragments, and no *c/z* ions, are formed.

The absence of *c/z* fragments formed from ECD of (peptide + Eu)³⁺ or by SORI-CAD of the reduced precursor for larger peptides indicates that a direct one-electron reduction of Eu³⁺ occurs to form an ion where there is no radical site, (peptide + Eu)²⁺ (Scheme 1; Site A). In contrast, electron capture is directed by the protonation site for the other trivalent metal ion-peptide complexes to form a more conventional odd electron ECD product ion, (peptide + M_T)^{2+•} that dissociates through typical ECD pathways (Scheme 1; Site B). The recombination enthalpy of direct trivalent metal ion reduction, ΔH_{red} , depends on the 3rd ionization energy of the metal ion, $\Delta H(\text{III})$, and the difference in solvation energies of the trivalent, $\Delta H_{\text{solv}}(3+)$, and divalent metal ion, $\Delta H_{\text{solv}}(2+)$, that correspond to the enthalpy change upon solvation of the respective metal ions by the peptide, eq. 1 [24].

$$\Delta H_{\text{red}} = -\Delta H(\text{III}) - \Delta H_{\text{solv}}(3+) + \Delta H_{\text{solv}}(2+) \quad (1)$$

The complete absence of *c/z* fragments from ECD of (peptide + Eu)³⁺ for large peptides indicates that the 3rd ionization energy of Eu³⁺ is sufficiently large to overcome both the change in solvation energy that results when the metal ion is reduced and the reduction energy at a site remote from the metal ion, resulting in direct reduction of Eu³⁺. The *b/y* fragments and small neutral losses from the charge reduced precursor that are formed from ECD of (peptide + Eu)³⁺ are a result of the recombination energy associated with reduction of Eu³⁺ to Eu²⁺ being redistributed throughout the peptide, and subsequent fragmentation of the even electron peptide ion. The decrease in these fragments with increasing peptide size is attributable to a degrees of freedom effect. These results are consistent with results for electron capture by Eu(H₂O)_n³⁺ that show direct reduction of Eu³⁺ occurs, whereas ion-electron pairs are formed for the other solvated trivalent metal ions (and some divalent metal ions) at large cluster size [24,52–55]. One electron reduction of Eu³⁺ occurs in aqueous solution, but not for the other trivalent metal ions investigated here, with the exception of Yb³⁺ which has a significantly lower reduction potential [ref].

Similar results are obtained for most other triply charged ions where 3+ to 2+ reduction occurs in aqueous solution. ECD spectra of (SFLLR + Co(NH₃)₆)³⁺ and (SFLLR + Ru(NH₃)₆)³⁺ were measured (Fig S5), and the primary product ion is the reduced precursor that has lost six NH₃ molecules. No *c/z* ions are formed. These results indicate that electron capture reduces the metal complex to its 2+ form, as occurs upon electron capture by these same metal ion complexes in aqueous nanodrops [56]. Part of the recombination energy goes into the expulsion of the six NH₃ molecules located at the site of electron capture.

It is interesting that for Yb, which has the highest third ionization energy of the lanthanides investigated here, ECD of peptides complexed to Yb³⁺ results in *c/z* ion formation. These results indicate that Yb³⁺ is not directly reduced in these experiments. In aqueous solution, the one-electron reduction potential of Yb³⁺ is lower than that of Eu³⁺, Ru(NH₃)₆³⁺, and Co(NH₃)₆³⁺ by at least 0.8 V (0.8 eV) [57–61]. Thus, Eu³⁺, Ru(NH₃)₆³⁺, and Co(NH₃)₆³⁺ are significantly more readily reduced than Yb³⁺ in water, despite Yb having a higher third ionization energy than Eu in isolation. Thus, solvation can profoundly affect the relative reduction energies of metals. For ECD of metalated peptides, the reduction enthalpy of Yb³⁺ is not sufficiently high to overcome the solvation enthalpy difference of the corresponding 3+ and 2+ ions to reduce the metal ion directly and suppress radical directed fragmentation of the peptide (eq. 1), which is consistent with the results for these ions when fully solvated in solution. These results indicate that the electrochemical properties of the metal ions when solvated by larger peptides are more similar to those of the same ions in solution than those of the metal ions in isolation.

Recent studies indicate that electron capture by Cd²⁺ and Hg²⁺ adducted peptides results in mostly *a*-type fragment ions and small neutral side chain losses from the reduced precursor, whereas typical *c/z* ions are formed for Zn²⁺ adducted peptides [38]. These results were attributed to Cd²⁺ and Hg²⁺-peptide complexes adopting a charge-solvated structure, and Zn²⁺ adducted peptides forming salt-bridge structures [38]. Our results indicate that the electrochemical properties of the metal ions in the peptide environment determine whether or not electron capture occurs at the metal ion or at a remote protonation site in the peptide.

Conclusions

Attachment of trivalent metal ions to small peptides for which only singly protonated ions are formed by ESI can result in higher charge state ions, making this method to increase charge effective for peptide sequencing with electron capture methods. Divalent ions, (peptide – H + M_T)²⁺, are formed for small peptides with acidic sites, whereas (peptide + M_T)³⁺ are formed for larger peptides. The trivalent metal ions preferentially bind to acidic sites resulting in salt-bridge structures, although entirely charge-solvated structures can be formed for large peptides without acidic sites. For the larger peptides, electron capture does not result in the direct reduction of the trivalent metal ion for all the lanthanide metals investigated except Eu. Reduction is driven by the protonation site located remotely from metal ion binding and results in *c/z* ions. In contrast, Eu³⁺ is directly reduced and *b/y* ions are formed instead. The electrochemical properties of these metal ions bound to the larger peptides is the same as that observed for these same ions in large aqueous nanodrops and in solution. This indicates that the solvation environment provided by these gaseous peptides results in high solvation energies comparable to those in water, which produces similar electrochemical properties of these ions both in the gas phase and in solution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors gratefully acknowledge financial support from the National Institutes of Health (grant no: R01GM096097).

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Fig 1.

(a) ESI mass spectrum of an aqueous solution containing 10 μM leucine enkephalin (LeEnk) and 100 μM LaCl_3 . Inset is an ESI mass spectrum of an aqueous solution containing 10 μM amidated leucine enkephalin (LeEnk-NH₂) and 100 μM LaCl_3 with a $\times 50$ expansion of the spectral region where $(\text{LeEnk-NH}_2 - H + \text{La})^{2+}$ would be located. (b) ESI mass spectrum of an aqueous solution containing 10 μM SFLLRNPNDKYEPF and 100 μM LaCl_3 . (c) Relative ion abundances of $(\text{peptide} - H + \text{La})^{2+}$ and $(\text{peptide} + \text{La})^{3+}$ as a function of peptide molecular weight

Fig 2.
ECD spectra of $(\text{LeEnk} + \text{Ca})^{2+}$ and (b) $(\text{LeEnk} - \text{H} + \text{M})^{3+}$ with abundance of sequence-specific fragments inset

Fig 3. Relative ECD fragment ion abundances at each cleavage site for (a) (ranakinin + $3H$) $^{3+}$, (b) (ranakinin + La) $^{3+}$, (c) (ranakinin + Eu) $^{3+}$, and (ranakinin + Yb) $^{3+}$. Grey bars correspond to fragments that retain M_T , whereas black bars correspond to non-metallated fragments

Fig 4. Relative ECD fragment ion abundances at each cleavage site for (a) (neurokinin + La)³⁺, (b) (SFLLR + La)³⁺, and (c) (angiotensin II + La)³⁺. Grey bars correspond to fragments that retain M_T, whereas black bars correspond to non-metallated fragments

Fig 5.
ECD spectrum of (angiotensin II + Eu)³⁺

Fig 6.

(a) ECD spectrum of $(\text{SFLLRNPNDKYEPF} + \text{La})^{3+}$ with relative ECD fragment ion abundances at each cleavage site inset. (b) ECD spectrum of $(\text{SFLLRNPNDKYEPF} + \text{Eu})^{3+}$. (c) SORI-CAD spectrum of the charge reduced precursor, $(\text{SFLLRNPNDKYEPF} + \text{Eu})^{2+}$, generated from ECD of $(\text{SFLLRNPNDKYEPF} + \text{Eu})^{3+}$

For $M^{3+} = La^{3+}, Yb^{3+}, Lu^{3+}, Ho^{3+}, Sm^{3+}, Tm^{3+}, Tb^{3+}, Pm^{3+}$

For $M^{3+} = Eu^{3+}, Ru(NH_3)_6^{3+}, Co(NH_3)_6^{3+}$

Scheme 1.