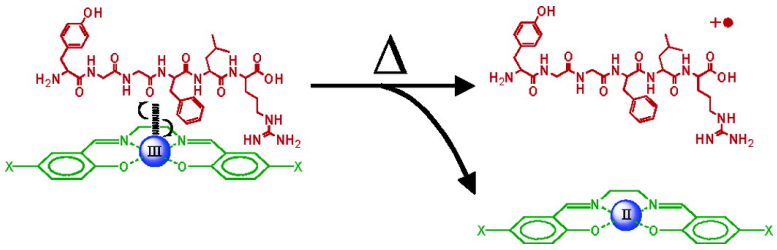
Article

Formation of Cationic Peptide Radicals by Gas-Phase Redox Reactions with Trivalent Chromium, Manganese, Iron, and Cobalt Complexes

Christopher K. Barlow, W. David McFadyen, and Richard A. J. O'Hair

J. Am. Chem. Soc., **2005**, 127 (16), 6109-6115• DOI: 10.1021/ja043088f • Publication Date (Web): 29 March 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 9 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 03/29/2005

Formation of Cationic Peptide Radicals by Gas-Phase Redox Reactions with Trivalent Chromium, Manganese, Iron, and Cobalt Complexes†

Christopher K. Barlow,‡,§ W. David McFadyen,*,‡ and Richard A. J. O'Hair*,‡,§

School of Chemistry and Bio21 Institute of Molecular Science and Biotechnology, The University of Melbourne, Victoria 3010, Australia

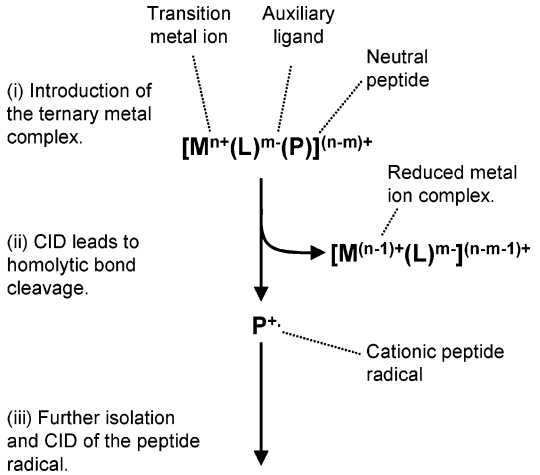
Received November 16, 2004; E-mail: rohair@unimelb.edu.au; wdmf@unimelb.edu.au

Abstract: The collision-induced dissociation (CID) of a series of gas-phase complexes $[M^{III}(salen)(P)]^+$ [where M = Cr, Mn, Fe, and Co; P = hexapeptides YGGFLR, WGGFLR, and GGGFLR; and salen = *N*,*N*′-ethylenebis(salicylideneaminato)] has been examined with respect to the ability of the complexes to form the corresponding cationic peptide radical ions, $P^{+\bullet}$, by homolytic cleavage of the metal peptide bond. This is the first example of the use of gas-phase ternary metal peptide complexes to produce the corresponding cationic peptide radical for a metal other than copper(II). The fragmentation reactions competing with radical formation are highly dependent on the metal ion used. In addition, examination of modified complexes in which the periphery of the salen was substituted allowed evaluation of electronic effects on the CID process, presumably without significant change in the geometry surrounding the metal. This substitution demonstrates that the ligand can be used to tune the dissociation chemistry to favor radical formation and suppress unwanted further fragmentation of the peptide radical that is typically observed immediately following its dissociation from the complex.

Introduction

Metalloproteins play a key role in electron-transfer processes in living systems. Metal−peptide complexes may serve as models systems for metalloproteins, and the advent of electrospray ionization (ESI) has facilitated the introduction of such complexes into the gas phase. By utilizing collision-induced dissociation (CID) of these complexes, it is possible to examine the chemistry that occurs due to redox processes. Redox chemistry has allowed the formation of gas-phase cationic peptide radical ions by the method outlined in Scheme 1: (i) A neutral peptide, P, is introduced into the gas-phase coordinated to a metal ion, M, with a bound auxiliary ligand, L. (ii) CID of the complex leads to dissociation of the peptide by homolytic bond cleavage, with concomitant reduction of the metal and oxidation of the peptide to form the cationic peptide radical, $P^{+\bullet}$. (iii) The peptide radical may then be further isolated and subjected to CID to probe its chemistry. In pioneering work by Siu and co-workers, the ternary complex $[Cu^{II}(dien)(YGGFLR)]^{2+}$ was shown to produce the $YGGFLR^{+\bullet}$ cationic peptide radical.[1] Stimulated by Siu's discovery, subsequent work has focused on the role of the auxiliary ligand in directing CID of peptide-containing complexes[2,3] and determination of which peptides are amenable to oxidation by this method.[4] Siu notes that peptides lacking either aromatic or basic residues generally fail to form cationic peptide radical ions, instead undergoing proton-transfer reactions to form the protonated peptide.[1,4] Recently, however, this shortcoming has been circumvented by the use of 12-crown-4 (1,4,7,10-tetraoxacyclododecane) as the auxiliary ligand, which promotes the formation of the cationic radical ion for peptides that contain only aliphatic residues.[2]

Scheme 1. Outline of a Method for Producing Cationic Peptide Radicals from Ternary Metal Complexes.



Despite advances made in examining the role of the auxiliary ligand, all work to date involving this method has utilized the

† Part 44 of the series "Gas-Phase Ion Chemistry of Biomolecules".
‡ School of Chemistry.
§ Bio21 Institute of Molecular Science and Biotechnology.

(1) Chu, I. K.; Rodriquez, C. F.; Lau, T. C.; Hopkinson, A. C.; Siu, K. W. M. *J. Phys. Chem. B* **2000**, *104*, 3393−3397.
(2) Chu, I. K.; Siu, S. O.; Lam, C. N. W.; Chan, J. C. Y.; Rodriquez, C. F. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 1798−1802.
(3) Barlow, C. K.; Wee, S.; McFadyen, W. D.; O'Hair, R. A. J. *Dalton Trans.* **2004**, 3199−3204.
(4) Chu, I. K.; Rodriguez, C. F.; Rodriguez, F.; Hopkinson, A. C.; Siu, K. W. M. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 1114−1119.

copper(II) ion, although Siu et al. have reported that CID of $[Ni^{II}(dien)(YGGFLR)]^{2+}$ failed to yield the $YGGFLR^{+\bullet}$ ion.[1] In contrast to other divalent metal ions, several examples exist in which copper(II) acts as an oxidant in complexes with peptides and amino acids.[5−9] It is notable in light of this selectivity that copper has the highest second ionization potential (IP) of any of the first-row transition metals (20.3 eV).[10] However, the third IPs of the first-row transition metals are greater than the second IP of copper. Accordingly, substitution of copper(II) by a trivalent metal ion may offer a more effective and widely applicable means of forming cationic peptide radicals. As a result, we have endeavored to substitute copper(II) for trivalent Cr, Mn, Fe, and Co ions (the third IP's of which are 31.0, 33.7, 30.7, and 33.5 eV, respectively)[10,11] in the hope of broadening the approach outlined in Scheme 1. Unfortunately, the transfer of small trivalent metal complexes in the +3 charge state to the gas phase proves difficult using ESI. Dissociation of solvent molecules from complexes below a critical size proceeds predominantly by fragmentation of the bound ligand or charge reductive pathways, either proton or electron transfer. Alternatively, ion pairing interactions with counterions can be maintained in the electrospray process, and this also leads to a lowering of the charge state of these complexes.[12,13] Indeed, our preliminary attempts to produce ternary peptide complexes in the +3 charge state proved unsuccessful.

To circumvent these problems, the current study adopts salen and 5,5′-disubstituted derivatives (Figure 1) as the auxiliary ligand with a range of trivalent first-row transition metal ions. On coordination of a metal ion, the $salenH_2X$ is deprotonated at both phenolic oxygens, making it a dianionic ligand, salenX. The resulting peptide-containing ternary complex is in the +1 charge state and is readily introduced into the gas phase using ESI. The tetradentate nature of the salen ligand is attractive, since it is unlikely to undergo competitive loss from the ternary complex and also leaves only two coordination sites available for binding between the metal and the peptide (assuming a six-coordinate geometry).[14] Indeed, the donor atoms in salen are typically planar, and consequently, we speculate that the peptide may only coordinate to a single axial site.[15] Restricting the coordination of the peptide to the metal has previously been shown to be important in facilitating the dissociation of the peptide from the complex.[3] Salen is also unlikely to act as an acid, which would promote dissociation via formation of the protonated peptide ion, a process that often competes with formation of the radical cation for other ligands.[16] The salen derivatives examined in this study are substituted at the 5,5′-position, which is remote from the metal center and should not alter the steric environment about the metal. This allows examination of electronic effects in a set of essentially isostructural complexes.[17] We report here the CID of the complexes $[M^{III}(salenX)(P)]^+$, where M^{III} are the trivalent chromium, manganese, iron, and cobalt ions, salenX are shown in Figure 1, and P are the hexapeptides YGGFLR, WGGFLR, and GGGFLR.

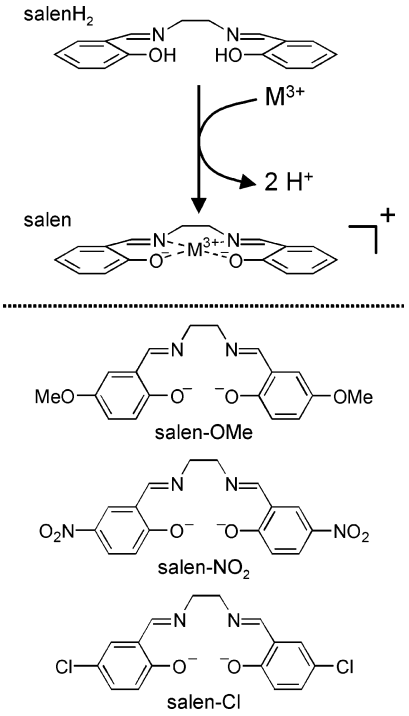


Figure 1. The salen ligands utilized in the current study are dianionic when coordinated to a metal ion. The substituted derivatives are shown below the dashed line.

Experimental Section

Mass Spectrometry. All experiments were conducted on a commercially available quadrupole ion trap mass spectrometer (Finnigan-MAT model LCQ, San Jose, CA) equipped with ESI. Samples were typically prepared by combining 100 μL of peptide stock solution (1

(5) Hu, P.; Loo, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 11314−11319.
(6) Gatlin, C. L.; Turecek, F.; Vaisar, T. *J. Mass Spectrom.* **1995**, *30*, 1605−1616.
(7) Gatlin, C. L.; Turecek, F.; Vaisar, T. *J. Mass Spectrom.* **1995**, *30*, 1617−1627.
(8) Gatlin, C. L.; Rao, R. D.; Turecek, F.; Vaisar, T. *Anal. Chem.* **1996**, *68*, 263−270.
(9) Vaisar, T.; Gatlin, C. L.; Turecek, F. *Int. J. Mass Spectrom. Ion Processes* **1997**, *162*, 77−87.
(10) Lide, D. R., Ed. *CRC Handbook of Chemistry and Physics*, 84th ed.; Lide, D. R., Ed.; CRC Press: Boca Raton, FL, 2004.
(11) Ligation will modulate these IPs, but few gas-phase studies have examine the influence of anionic ligands: (a) McCullough, S. M.; Jones, A. D.; Lebrilla, C. B. *Int. J. Mass Spectrom. Ion Processes* **1991**, *107*, 545−552. (b) Dai, P.; McCullough-Catalano, S.; Bolton, M.; Jones, A. D.; Lebrilla, C. B. *Int. J. Mass Spectrom. Ion Processes* **1995**, *144*, 67−77. (c) Schroder, D.; Barsch, S.; Schwarz, H. *Int. J. Mass Spectrom.* **1999**, *192*, 125−139. (d) Schroeder, D.; Baersch, S.; Schwarz, H. *J. Phys. Chem. A* **2000**, *104*, 5101−5110.
(12) Blades, A. T.; Jayaweera, P.; Ikonomou, M. G.; Kebarle, P. *Int. J. Mass Spectrom. Ion Processes* **1990**, *101*, 325−336.
(13) Shvartsburg, A. A. *Chem. Phys. Lett.* **2002**, *360*, 479−486.
(14) The gas-phase coordination chemistry of metal−salen complexes has been examined: Chromium(III)−salen complexes can coordinate an additional two ligands: Kumar, M. K.; Prabhakar, S.; Kumar, M. R.; Reddy, T. J.; Premsingh, S.; Rajagopal, S.; Vairamani, M. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 1103−1108. Manganese(III)−salen complexes coordinate a single additional ligand: Plattner, D. A.; Feichtinger, D.; El-Bahraoui, J.; Wiest, O. *Int. J. Mass Spectrom.* **2000**, *195/196*, 351−362.
(15) The condensed phase coordination environment of transition metal salen complexes is quite diverse and can depend on the structures of the ancillary ligand(s). For reviews, see: (a) Calligaris, M.; Nardin, G.; Randaccio, L. *Coord. Chem. Rev.* **1972**, *7*, 385−403. (b) Yamada, S. *Coord. Chem. Rev.* **1999**, *190−192*, 537−555. Hobday, M. D.; Smith, T. D. *Coord. Chem. Rev.* **1973**, *9*, 311−337.
(16) Unfortunately, the gas phase thermochemistry associated with the dissociation reactions of these complexes is unknown. Their solution phase electrochemistry is of limited value in making gas phase predictions on IPs, since they are dependent on both the solvent and the coordinating ligand. (a) for Co systems, see: Eichhorn, E.; Rieker, A.; Speiser, B. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1215−1217. (b) For Fe systems, see: (b) Ranchet, D.; Tommasino, J. B.; Vittori, O.; Fabre, P. L. *J. Sol. Chem.* **1998**, *27*, 979−992. Co−R bond dissociation energies of salen complexes are significantly effected by the nature of the trans ligand: (c) Li, G.; Zhang, F. F.; Chen, H.; Yin, H. F.; Chen, H. L.; Zhang, S. Y. *Dalton Trans.* **2002**, 105−110.
(17) The salen ligand has been electronically tuned through the use of substituents to influence the dissociation reactions of manganese(V)−salen complexes: Plattner, D. A. *Top. Current Chem.* **2003**, *225*, 153−203.

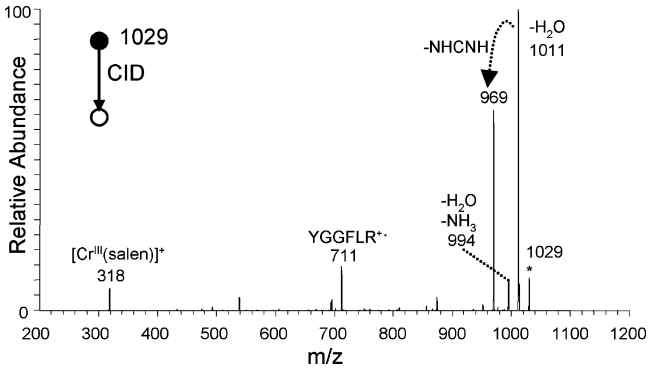


Figure 2. The CID spectrum of $[Cr^{III}(salen)(YGGFLR)]^+$ is characteristic of all the spectra in the set of $[Cr^{III}(salen)(P)]^+$. Note that CID proceeds primarily via fragmentation of the bound peptide. The $YGGFLR^{+\bullet}$ radical and $[Cr^{III}(salen)]^+$ ions are only minor products. The * designates the parent ion.

mg/mL) and 100 μL of the metal complex stock solution (1 mg/mL) (metal complexes used are listed below) to a total volume of 1 mL in methanol. Many metal complexes proved only partially soluble at 1 mg/mL in methanol, so 100 μL of the saturated solution was used instead. Under these conditions, the proportion of $[M(salen)(P)]^+$ to other ions formed in the ESI-MS was variable but in all cases sufficient to permit mass selection of the complex and subsequent formation of the peptide radical cation by CID.

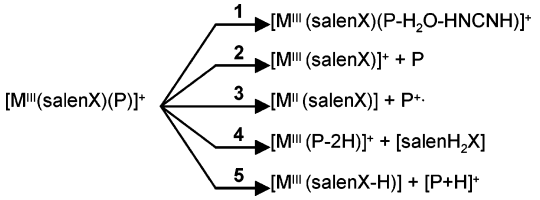
Once prepared, samples were immediately introduced into the mass spectrometer at 3.0 μL/min via the ESI source. The typical ranges of source conditions used were as follows: spray voltage, 3.5−5.0 kV; capillary temperature, 100−250 °C; nitrogen sheath pressure, 0 psi, capillary voltage, −135 to +135 V; tube lens offset voltage, −200 to +200 V. CID experiments were performed utilizing the advanced scan functions of the LCQ instrument. The isolation window was minimized while still allowing the mass selection of the ion; accordingly, the widths are specific to each sample investigated. As far as practicable, monoisotopic selection of the parent ion was achieved. Activation voltage was similarly varied for each ion with a constant activation time of 30 ms.

Synthesis of Metal Complexes. Salen and substituted derivatives were prepared by condensation of ethylenediamine and the corresponding commercially available salicylaldehyde in ethanol, as reported previously.[18a] The syntheses of [Fe(salen)]Cl,[18b] [Mn(salen)]OAc,[18a] $[Cr(salen)(H_2O)_2]Cl$,[18c] and [Co(salen)]OAc,[18d] have been described previously, and these procedures were used with substitution of the appropriate derivative in place of salen.

Results

Chromium(III). The CID spectrum of $[Cr^{III}(salen)(YGGFLR)]^+$ is shown in Figure 2 and is typical of all the complexes in the set $[Cr^{III}(salen)(P)]^+$. These complexes primarily undergo CID via small molecule loss from the bound peptide. Losses of 18, 35, and 60 mass units are assigned to H_2O, $H_2O + NH_3$, and H_2O + NHCNH, respectively. The last of these losses is characteristic of peptides containing a C-terminal arginine residue (the NHCNH coming from the arginine side chain) and has been observed to occur in both protonated and metalated peptides (reaction 1, Scheme 2).[19−21] Products relating to the dissociation of the peptide as a neutral to yield the $[Cr^{III}(salen)]^+$ ion (reaction 2, Scheme 2) and a cationic radical, $P^{+\bullet}$ (reaction 3, Scheme 2), are evident in low abundance. CID of the substituted salen derivatives led to only minor differences in the CID of the complexes. CID of the $[Cr^{III}(salenNO_2)(P)]^+$ complexes produced sufficient $P^{+\bullet}$ to allow it to be subjected to CID, although $P^{+\bullet}$ remained a minor product. Conversely, CID of the $[Cr^{III}(salenOMe)(P)]^+$ complexes proceeds via reaction 2 to a greater extent than the other chromium complexes.

Scheme 2. Dissociation Pathways of the Metal Complexes Examined[a]



[a] The reaction number is given in bold above the arrow.

Manganese(III). The chemistry of the manganese(III) complexes differs considerably from that of chromium(III). Fragmentation of the bound peptide via loss of 60 mass units (H_2O + NHCNH) is no longer observed, although water loss typically operates as a minor pathway. CID of the $[Mn^{III}(salenX)(P)]^+$ occurs predominantly via reactions 2 and 3 (Scheme 2). In addition, an ion corresponding to the $[Mn^{III}(P\text{-}2H)]^+$ complex is also present, indicating loss of the doubly protonated, neutral $salenH_2X$ (reaction 4, Scheme 2).[22] The extent to which each of these pathways operates is mediated primarily by X, the substituent on the salen ligand, and to a lesser extent the peptide. Figure 3, shows the CID of the series of ions, $[Mn^{III}(salenX)(YGGFLR)]^+$. Dissociation of the $[Mn^{III}(salenNO_2)(P)]^+$ ions (Figure 3A) produces only the peptide radical and associated further fragment ions (Scheme 3). Dissociation of the $[Mn^{III}(salenCl)(P)]^+$ ions is similar, although $[Mn^{III}(salenCl)]^+$ and $[Mn^{III}(P\text{-}2H)]^+$ are present at low abundance (Figure 3B). CID of the $[Mn^{III}(salen)(P)]^+$ ions leads to $[Mn^{III}(salen)]^+$ and $P^{+\bullet}$ as the two most prominent ions, and in addition, the $[Mn^{III}(P\text{-}2H)]^+$ ion is also clearly evident (Figure 3C). The CID of the $[Mn^{III}(salenOMe)P]^+$ ions is dominated by the $[Mn^{III}(salenOMe)]^+$ ion, while the $P^{+\bullet}$ and $[Mn^{III}(P\text{-}2H)]^+$ ions are only minor products (Figure 3D). It is evident that competition between reactions 2 and 3 of Scheme 2 is related to the electron-withdrawing capacity of X. Specifically, increasing the electron-withdrawing capacity of X, NO_2 > Cl > H > OMe, favors dissociation by reaction 3, that is, radical formation, rather than reaction 2, which corresponds to loss of the neutral peptide. In addition, for the $[Mn^{III}(salen)(P)]^+$ complexes, the relative intensities of the $[Mn^{III}(salen)]^+$ and $P^{+\bullet}$ ions show a dependence on P. The loss of the neutral peptide versus radical formation is enhanced when P = GGGFLR rather than YGGFLR or WGGFLR.

(18) (a) Gravert, D. J.; Griffin, J. H. *Inorg. Chem.* **1996**, 35, 4837−4847. (b) Gerloch, M.; Lewis, J.; Mabbs, F. E.; Richards, A. *J. Chem. Soc. A* **1968**, 112−116. (c) Mabbs, F. E.; Coggon, P.; McPhail, A. T.; Richards, A.; Thornley, A. S. *J. Chem. Soc. A* **1970**, 3296−3303.(d) Bailes, R. H.; Calvin, M. *J. Am. Chem. Soc.* **1947**, *69*, 1886−1893.
(19) Deery, M. J.; Summerfield, S. G.; Buzy, A.; Jennings, K. R. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 253−261.
(20) Lin, T.; Payne, A. H.; Glish, G. L. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 497−504.
(21) Zhao, H.; Reiter, A.; Teesch, L. M.; Adams, J. *J. Am. Chem. Soc.* **1993**, *115*, 2854−2863.
(22) The formation of $salenH_2X$ is not unexpected, due to the presence of both the basic imine nitrogen and the phenoxide oxygen. Protonation of the phenoxide oxygen will be supported by hydrogen bonding to the imine nitrogen, enhancing its gas-phase anion proton affinity (APA). Although the APA of $salenH_2$ is unknown, condensed phase studies reveal that it is a weak diprotic acid. Lloret, F.; Mollar, M.; Faus, J.; Julve, M.; Castro, I.; Diaz, W. *Inorg. Chim. Acta* **1991**, *189*, 195−206.

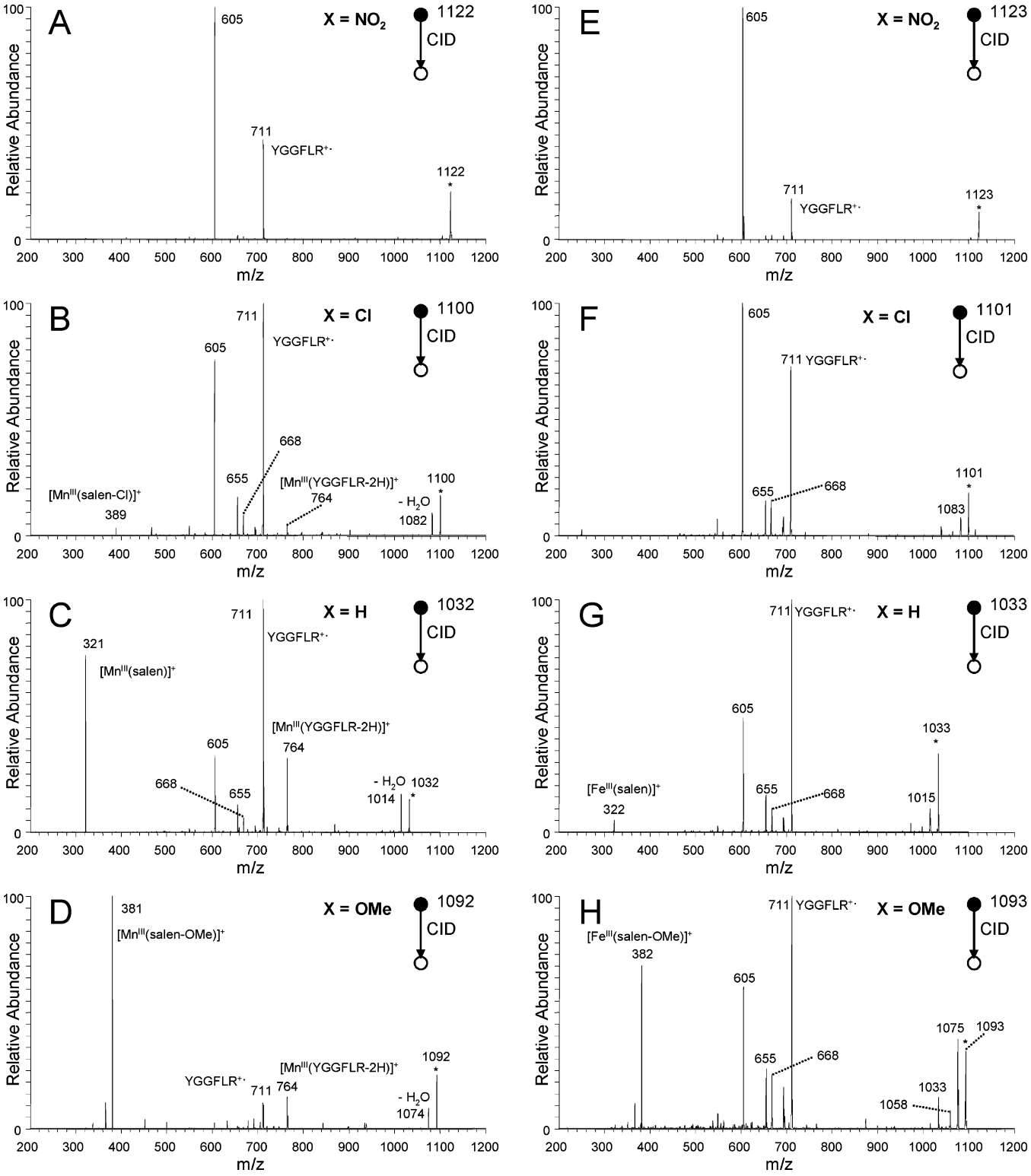
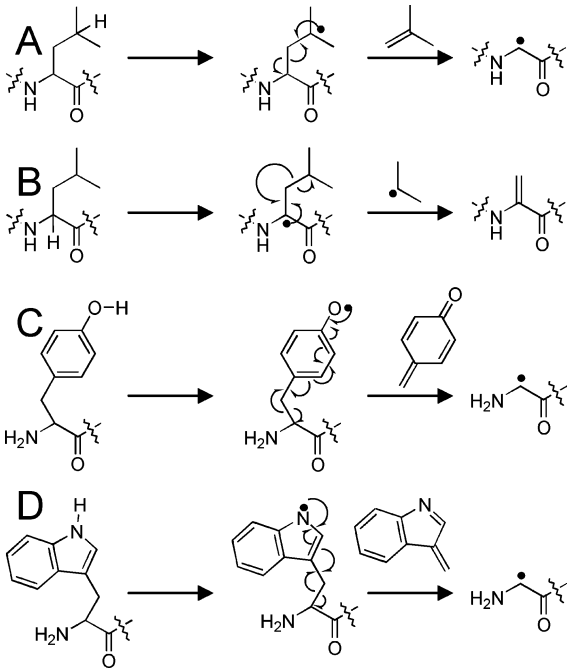


Figure 3. CID spectra of the $[Mn^{III}(salenX)(YGGFLR)]^+$ complexes, left (A−D), and $[Fe^{III}(salenX)(YGGLFR)]^+$ complexes, right (E−H), listed in order of increasing electron-donating capacity of X. The * designates the parent ion. Peaks at m/z = 605, 655, and 668 represent loss of *p*-quinomethide, 2-methylpropene, and the $\bullet C_3H_7$ from the peptide radical respectively, Scheme 3.

Iron(III). CID of the iron(III) complexes proceeds primarily by the formation of the cationic peptide radical (reaction 3, Scheme 2), and the peptide radical or one of its further fragmentation products is the primary product in all spectra. In particular, the loss of *p*-quinomethide (p-qm) from YGGFLR and 3-methyleneindolenine (3-MeIn) from WGGFLR appears very facile, as noted previously with copper(II) complexes (e.g., Figure 5B).[1,4] The Fe(III) complexes differ from their Mn(III) counterparts in that they are more prone to dissociation of the peptide by radical-forming homolytic cleavage (reaction 3) rather than heterolytic bond cleavage (reaction 2) for a given ligand and peptide (Figure 3). In contrast to the manganese(III) complexes, no $[M^{III}(P\text{-}2H)]^+$ ion is evident for M = Fe, demonstrating that reaction 4 does not occur for iron(III). Losses

Scheme 3. Further Fragmentation of the Peptide Radical Preceded by Migration of a Hydrogen Atom from the Site of the Reaction, Followed by Radical-Directed Fragmentation[a]



of water and 60 mass units (H_2O + NHCNH) (reaction 1) are observed, however, for the iron(III) complexes. The trend observed for the manganese complexes with respect to the electron-withdrawing properties of X and competition between reactions 2 and 3 is maintained in the iron complexes. It is also apparent that the extent to which further fragmentation proceeds is also a function of the electron-withdrawing properties of X. Specifically, greater further fragmentation of the peptide radical is observed as X becomes more electron withdrawing. Similarly, the $[Mn^{III}(salenNO_2)(P)]^+$ ions also show greater secondary fragmentation of $P^{+\bullet}$ than for either $[Mn^{III}(salenCl)(P)]^+$ or $[Mn^{III}(salen)(P)]^+$ ions.

Cobalt(III). The chemistry of the cobalt complexes examined here differs from that observed with the other metal ions in a number of respects. In particular, CID is sensitive to the identity of the peptide to a much greater extent than observed for the other metals, and competitive dissociation by formation of the protonated peptide is evident (reaction 5, Scheme 2). Figure 4 shows the CID of the set of $[Co^{III}(salenOMe)(P)]^+$ ions. For P = YGGFLR (Figure 4A), the $[Co^{III}(salenX)(YGGFLR)]^+$ complexes appear to dissociate almost exclusively via reaction 3, to provide the $YGGFLR^{+\bullet}$ ion as the main fragment ion. The other ions present are due to the further fragmentation of the $YGGFLR^{+\bullet}$ ion. In addition to the familiar loss of *p*-quinomethide, further fragmentation proceeds by losses of 44 and 45 mass units from the radical. These latter fragment ions are not observed with the other metal complexes and are attributed to loss of CO_2 and $\bullet CO_2H$, respectively. In contrast to P = YGGFLR, for P = GGGFLR, CID of the $[Co^{III}(salenX)(GGGFLR)]^+$ complexes fails to produce any of the $GGGFLR^{+\bullet}$ ion (Figure 4B). It appears that the radical is formed transiently before fragmenting further to yield the $[M - CO_2]^{+\bullet}$ and $[M - \bullet CO_2H]^+$ ions. The complexes in which P = WGGFLR act as an intermediate case; $WGGFLR^{+\bullet}$ is evident but undergoes extensive fragmentation by loss of 3-methyleneindolenine, CO_2 and $\bullet CO_2H$. The protonated ion is also readily formed,

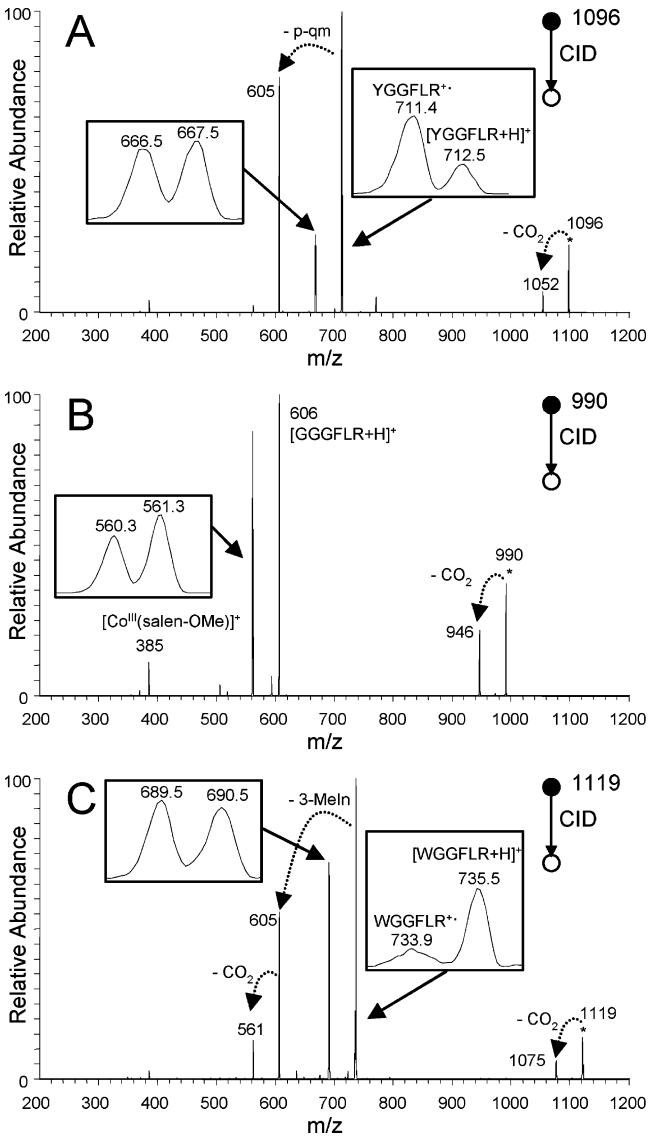


Figure 4. CID of the $[Co^{III}(salen\text{-}OMe)(P)]^+$ complexes: (A) P = YGGFLR, (B) P = GGGFLR, and (C) P = WGGFLR. Note that the $[YGGFLR + H]^+$ peak is only significant for X = OMe.

demonstrating competitive dissociation by reaction 5. For those complexes for which X = OMe, loss of CO_2 from the ternary complex and dissociation of the neutral peptide are also observed as minor fragmentation pathways (Figure 4).

Discussion

It is significant that for each of the transition metal ions utilized in this study formation of cationic peptide radicals was achieved to some extent, supporting the basic premise that trivalent metal ions may be used in place of copper(II) for the method outlined in Scheme 1. The manganese and iron complexes are significantly better at producing the cationic peptide radical than either the cobalt and chromium complexes. In the case of chromium, dissociation occurs by both reactions 2 and 3; however, fragmentation of the bound peptide is far more prominent. This suggests that a greater barrier exists toward dissociation from the chromium than for the iron and manganese complexes. This is not surprising, as it is known that in the gas phase the rate constant for the dissociation of

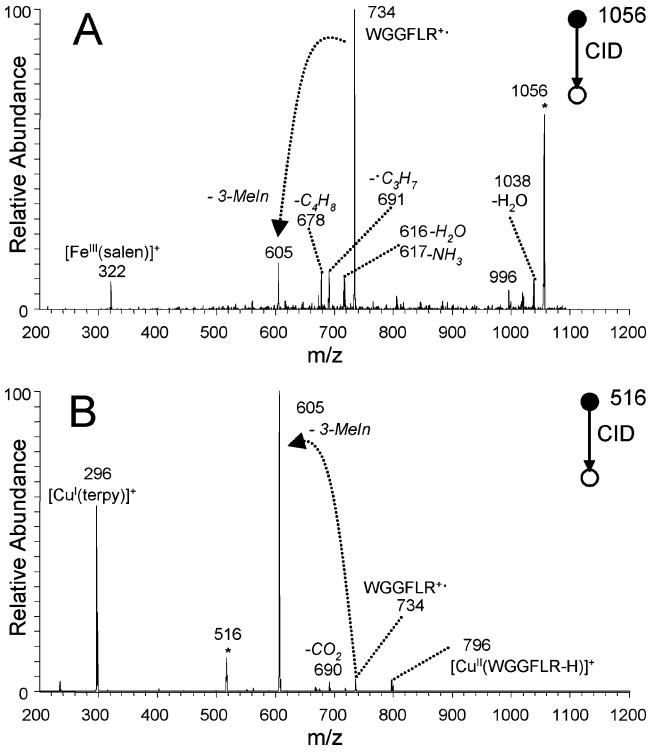


Figure 5. CID spectra of $[Fe^{III}(salen)(WGGFLR)]^+$ (A) and $[Cu^{II}(terpy)(WGGFLR)]^{2+}$ (B).[3] The further fragmentation of the $WGGFLR^{+\bullet}$ ion is far less pronounced from the iron than the copper complex. The further fragment ions are labeled in italics, 3-MeIn = 3-methyleneindolenine and C_4H_8 = 2-methylpropene. The * designates the parent ion.

water from the $[M^{III}(salen)(H_2O)]^+$ complex is approximately an order of magnitude lower when M = Cr than for M = Mn or Co.[23]

The manganese and iron complexes demonstrate very similar CID, proceeding mainly by reactions 2 and 3, the products of which differ only by a single electron. Substitution at the 5,5′-position of the salen ligand ought to perturb the electronic structure of the ligand, ultimately altering the capacity of the ligand to delocalize the positive charge from the metal onto the ligand. For example, when X is electron-donating, as in X = OMe, the capacity of the salen ligand to delocalize the positive charge of the metal onto the ligand ought to increase. As a consequence, the ligand is better able to support a higher oxidation state i.e., the metal is harder to reduce, and so dissociation by loss of the neutral rather than the cationic radical of the peptide is favored. Conversely, as we observe, the presence of an electron-withdrawing X favors cationic radical formation. In addition, in the ternary complex the presence of the electron-donating group ought to make the metal appear more electronegative and so decrease the strength of the metal−peptide bond. The converse arguments may be made with respect to the electron-withdrawing X's, X = NO_2 and Cl. These substituents ought to lead to a more electropositive metal center and, consequently, a stronger metal peptide bond. CID in an ion trap proceeds by multiple low-energy collisions, and the energy is randomized throughout all the degrees of freedom of the complex.[24] Consequently, further fragmentation of the peptide radical may be used as an indication of the extent of excitation necessary to cleave the metal−peptide bond. As anticipated by the above argument, further fragmentation of the peptide radical observed immediately following dissociation (i.e. in the MS^2 spectrum) is greater for electron-withdrawing X.

The identity of the N-terminal residue also affects the CID chemistry, particularly for the cobalt complexes. Dissociation to form the radical cation is most heavily favored for YGGFLR and least favored for GGGFLR, with WGGFLR being intermediate. Consistent with our observations here, it has been noted previously that the presence of either tyrosine or tryptophan is often important in promoting the formation of the corresponding radical cation. Indeed, Siu and co-workers have previously demonstrated that the corresponding cationic radical may be formed from the single amino acid tyrosine or tryptophan by utilizing the copper(II)−dien complex.[25]

The dissociative pathways that compete with the formation of the peptide radical typically observed for the copper(II) complexes differ from those observed in the current study. It is difficult to compare the gas-phase chemistry of the previously reported copper(II) complexes and those utilized in the present study for two main reasons: (i) the copper(II) complexes examined have used ligands different than those employed here with trivalent metal ions; (ii) more importantly, there is a difference in charge state between the copper(II) and trivalent metal complexes. Whereas the copper(II) complexes have always utilized neutral ligands and consequently been in the +2 charge state, the complexes of trivalent metal ions coordinated to the dianionic salen carry only a single positive charge. The importance of the charge state is borne out in differences in the competing dissociation pathways for the two types of complexes. For example, for the singly charged complexes in the current study, loss of the neutral peptide is often the most prevalent pathway competing with radical formation. In contrast, loss of the neutral peptide is rarely observed from the doubly charged copper complexes; indeed, the most prominent competitive dissociative pathways is loss of the protonated peptide, $[P + H]^+$ (cf. reaction 5, Scheme 2). The driving force for both radical formation and this competitive pathway for the doubly charged complexes is the separation of charge that can be achieved by dissociation via two +1 fragments, rather than a neutral and +2 fragment. By judicious choice of ligand, the loss of the protonated peptide may be circumvented. The conclusions from this observation would appear to be clear: complexes with greater charge state ought to be used to generate radical cations. However, the singly charged complexes examined in this work have an advantage over the previously studied copper(II) complexes in that they suppress the further fragmentation of the peptide radical once formed. This is most clearly demonstrated in the formation of the radical cation $WGGFLR^{+\bullet}$ using singly charged complexes of this study that had previously proved to be difficult with copper(II) complexes (Figure 5). This advantage may also lead to broader applicability of our work in, for example, bioanalytical applications,[26] where relative yield of desired radical species is an important consideration.

Conclusions

The use of transition metal complexes for the formation of cationic peptide radicals by the method outlined in Scheme 1

(23) Lee, S.-W.; Chang, S.; Kossakovski, D.; Cox, H.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1999**, *121*, 10152−10156.
(24) McLuckey, S. A.; Goeringer, D. E. *J. Mass Spectrom.* **1997**, *32*, 461−474.
(25) Bagheri-Majdi, E.; Ke, Y.; Orlova, G.; Chu, I. K.; Hopkinson, A. C.; Siu, K. W. M. *J. Phys. Chem. B* **2004**, *108*, 11170−11181.

is not solely applicable to copper(II). In this report, we have demonstrated that the trivalent transition metal ions Cr^{III}, Mn^{III}, Fe^{III}, and Co^{III} may all be utilized to perform this gas-phase redox chemistry. Although complexes containing each of these metal ions formed the peptide radical ion successfully, the Fe^{III} and Mn^{III} complexes proved to be of greater utility in performing this task. The chemistry of these complexes was modulated by both substitution on the auxiliary salen ligand and the identity of the peptide. In particular, competition between dissociation of the neutral and cationic radical peptide was controlled to a significant extent by substitution on salen. When X was electron-withdrawing, homolytic cleavage of the metal peptide bond was favored, producing radical peptide ions. Conversely, with an electron-donating X, heterolytic cleavage was favored, leading to the loss of the neutral peptide. In addition, X influenced the degree of further fragmentation of the peptide radical once formed. When X is electron-withdrawing, the metal peptide bond is presumably stronger, and consequently, the energy required to achieve dissociation is greater. As a result, the peptide radical appears to be more excited immediately following dissociation and thus has a greater propensity toward further fragmentation.[27] This suggests that the selection of the ligand is of great importance in influencing the CID chemistry of these complexes, and a balance must be achieved between promoting homolytic bond cleavage while ensuring dissociation proceeds readily enough to prevent excessive further fragmentation of the peptide radical thus formed.

Acknowledgment. R.A.J.O and W.D.M. thank the Australian Research Council for financial support (Grant# DP0344145). C.K.B. acknowledges an Australian Postgraduate Research Award.

JA043088F

(26) The development of metal complexes of peptides as a bioanlytical tool hinges upon a number of condensed and gas phase factors. The ideal ternary metal complex would (i) readily form in the condensed phase, (ii) be sequence independent in its binding of peptides, (iii) readily transfer to the gas phase under ESI conditions without undergoing charge reduction or fragmentation, and (iv) fragment to give abundant peptide cation radicals. In our work we have solely focused on the gas phase fragmentation behavior of the ternary complexes and have not tried to optimize their condensed phase behavior. We note that, in general, Fe, Co, and Mn readily formed the ternary complexes in high abundance.

(27) The subsequent fragmentation of the peptide cation radical may be a result of electronic excitation. Schlag and co-workers have demonstrated that tetrapeptides are characterized by many and densely spaced excited electronic states and that these provide opportunities for charge migration and fragmentation in peptides: Remacle, F.; Levine, R. D.; Schlag, E. W.; Weinkauf, R. *J. Phys. Chem. A* **1999**, *103*, 10149−10158.