# Examination of Various Metal Ion Sources for Reducing Nonspecific Zinc finger–$Zn^{2+}$ Complex Formation in ESI Mass Spectrometry

**Soojin Park**[†a], **Sunhee Park**[†a], **Joo Yeon Oh**[b], **Sang Yun Han**[b], **Kyubong Jo**[a], **and Han Bin Oh**[*a]

[a]*Department of Chemistry, Sogang University, Seoul 121-742, Korea*
[b]*Center for Nano-Bio Technology, Korea Research Institute of Standards and Science, Daejeon, 305-340, Korea*

*Received August 31, 2012; Revised September 17, 2012; Accepted September 17, 2012*
*First published on the web September 20, 2012; DOI: 10.5478/MSL.2012.3.3.82*

**Abstract:** The formation of zinc finger peptide–$Zn^{2+}$ complexes in electrospray ionization mass spectrometry (ESI-MS) was examined using three different metal ion sources: $ZnCl_2$, $Zn(CH_3COO)_2$, and $Zn(OOC(CHOH)_2COO)$. For the four zinc finger peptides (Sp1-1, Sp1-3, CF2II-4, and CF2II-6) that bind only a single $Zn^{2+}$ in the native condition, electrospray of apo-zinc finger in solution containing $ZnCl_2$ or $Zn(CH_3COO)_2$ resulted in the formation of zinc finger–$Zn^{2+}$ complexes with multiple zinc ions. This result suggests the formation of nonspecific zinc finger–$Zn^{2+}$ complexes. Zn(tartrate), $Zn(OOC(CHOH)_2COO)$, mainly produced specific zinc finger–$Zn^{2+}$ complexes with a single zinc ion. This study clearly indicates that tartrate is an excellent counter ion in ESI-MS studies of zinc finger–$Zn^{2+}$ complexes, which prevents the formation of nonspecific zinc finger–$Zn^{2+}$ complexes.

**Key words:** Zinc finger, Zinc ion, Electrospray ionization, Tartrate, Noncovalent complexes

## Introduction

Electrospray ionization mass spectrometry (ESI-MS) has been widely used to characterize specific noncovalent biological interactions in solution, including multi-protein assemblies, protein-/peptide-ligand, and DNA-/RNA-drug complexes.[1–9] In practice, ESI-MS has been used to determine binding stoichiometry,[5,10–12] and to measure relative[13,14] and absolute[15–17] association constants (binding affinities).

While ESI-MS is clearly a powerful bioanalytical tool, this technique does have certain limitations. One of its most notable limitations is related to the tendency of biological molecules to associate nonspecifically with other biomolecules, small molecules, or ions present in solution during the ESI process.[5] The formation of nonspecific noncovalent adducts with neutral or ionic species derived from buffer or impurities has been previously discussed.[18,19] The formation of nonspecific noncovalent complexes may lead to misinterpretation of ESI-MS results and obscure the binding stoichiometry of specific complexes. A variety of methods have been suggested to cope with the formation of nonspecific noncovalent complexes. In particular, dialysis,[20] reversed-phase high-performance liquid chromatography (HPLC),[21] and size exclusion chromatography[22] have been used to resolve nonspecific metal binding issues. However, these methods are limited because desalting might interfere with the protein metalation process. As a result, protein metal binding properties may not be properly reflected.

A few different metal sources that contain different counter ions have been evaluated for use in minimizing nonspecific protein-metal ion adduct formation in ESI-MS experiments. For example, Pan *et al*. used chloride ($Cl^-$), acetate ($CH_3COO^-$), and tartrate ($^-OOC\text{-}CH(OH)\text{-}CH(OH)\text{-}COO^-$) to examine which counter ion is most suitable for studying protein-metal ion interactions.[23] In particular, they studied $Ca^{2+}$ and $Zn^{2+}$ binding proteins. In their studies, tartrate, a weak chelator with a relatively high metal binding constant, was found to dramatically reduce nonspecific metalation. Tartrate was shown to be a particularly effective counter ion that produces metalation levels very close to those in bulk solution.

In the present study, we extend the previous study by Pan *et al*. to zinc finger peptides that have high $Zn^{2+}$ binding affinity. Zinc fingers belong to an important protein family that has attracted extensive attention due to its intrinsic biological significance.[24–26] Zinc fingers can recognize a specific DNA double-stranded sequence. Their unique specificity and DNA binding affinity give zinc fingers promising potential as DNA recognition motifs in artificial restriction enzymes. In our previous study, we showed that ESI-MS can be successfully used to examine the binding specificity of zinc finger peptides to DNA with a cognate sequence.[26] In this study, we demonstrate that tartrate is most effective in reducing nonspecific $Zn^{2+}$ adduction for zinc finger peptides, i.e., minimizing the formation of zinc finger–$Zn^{2+}$ complexes with multiple zinc ions.

[†]Both authors equally contributed to this work.
*Reprint requests to Dr. Han Bin Oh
E-mail: hanbinoh@sogang.ac.kr

**Table 1**. Sequences and masses of zinc finger peptides used in the present study.

| Peptide | Sequence | Monoisotopic mass |
|---|---|---|
| Sp1-1 | QHICH IQGCG KVYGK TSHLR AHLRW HTGER | 3378.7 |
| Sp1-3 | KFACP ECPKR FMRSD HLSKH IKTHQ NKK | 3393.7 |
| CF2II-4 | PYTCS YCGKS FTQSN TLKQH TRIHT GEK | 3214.5 |
| CF2II-6 | PYTCP YCDKR FTQRS ALTVH TTKLH PL | 3175.6 |
| Melittin | GIGAV LKVLT TGLPA LISWI KRKRQ Q | 2846.7 |

## Experimental

Zinc finger peptides were all custom-synthesized from Peptron Inc. (Daejeon, Korea) using Fmoc solid phase chemistry. Melittin was available from Sigma (Seoul, Korea). The sequences and monoisotopic masses of zinc finger peptides used in the present study are shown in Table 1.[27–29] Zinc chloride ($ZnCl_2$), zinc acetate ($Zn(CH_3COO)_2$), and ammonium tartrate were purchased from Sigma (Seoul, Korea). Zinc tartrate ($Zn(OOC(CHOH)_2COO)$) was prepared by mixing equimolar amounts of ammonium tartrate and zinc acetate. All peptide samples were dissolved at a peptide concentration of 20 μM, and the pH was adjusted to pH 7.5 using aqueous $NH_4OAc$ buffered solution.

All mass spectrometry measurements were made on an ion-trap mass spectrometer (LCQ Deca, Thermo Finnigan, San Joes, CA) operated in the positive ion mode (needle voltage = +3.5 kV). The capillary was heated to 200 °C and applied potential was +10 V. The tube lens offset was maintained at +20 V. A solution of 100 mM aqueous $NH_4OAc$ buffer was infused at 3 μl $min^{-1}$. Full-scan mass spectra were recorded in the range of *m*/*z* 200–2000.

## Results and Discussion

Three different zinc metal source compounds were added to electrospray solutions to evaluate the effects of various zinc-ion sources on the formation of zinc finger–$Zn^{2+}$ complexes. Zinc chloride ($ZnCl_2$), zinc acetate ($Zn(CH_3COO)_2$), and zinc tartrate ($Zn(OOC(CHOH)_2COO)$) were used as zinc metal sources.[23]

Figure 1 shows ESI mass spectra of Sp1-3, CF2II-6, and melittin obtained by co-spraying with zinc chloride, zinc

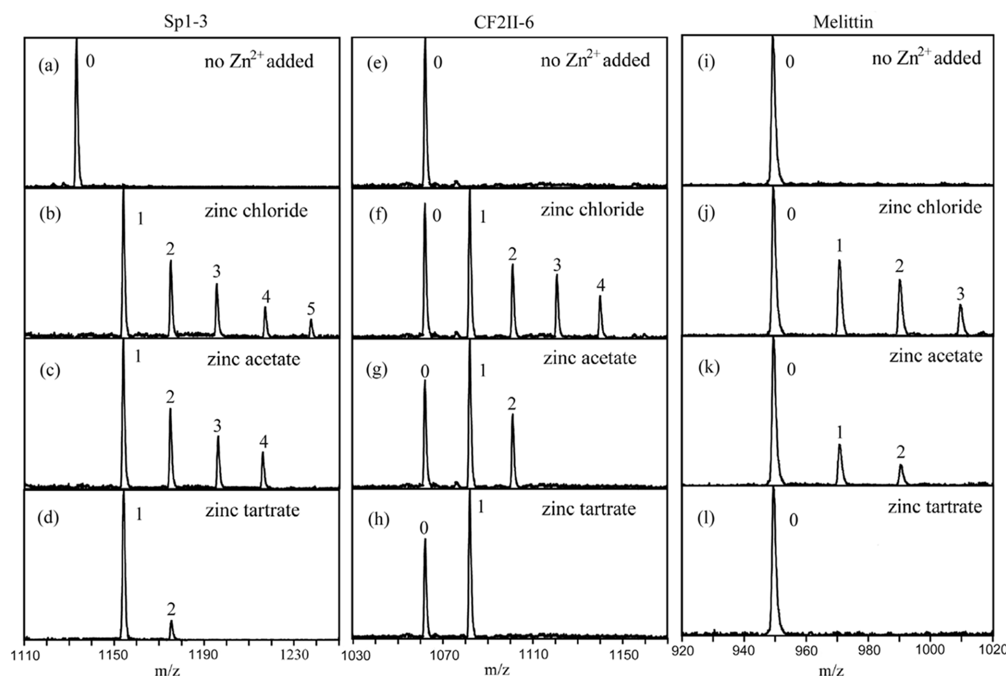


**Figure 1.** ESI mass spectra of three peptides recorded using different zinc ion sources in positive ion mode: first column, Sp1-3; second column, CF2II-6; third column, melittin. Peaks shown here represent a series of +3 ions: $(M+3H)^{3+}$, $(M+H+Zn^{2+})^{3+}$, $(M-H+2Zn^{2+})^{3+}$, $(M-3H+3Zn^{3+})^{3+}$, $(M-5H+4Zn^{2+})^{3+}$, and $(M-7H+5Zn^{2+})^{3+}$. Numbers denoted close to peaks represent the number of zinc ions contained in the peak. Mass spectra in the first row were recorded in the absence of zinc salts. Spectra in the second to fourth rows were acquired in the presence of $ZnCl_2$, $Zn(CH_3COO)_2$, and $Zn(OOC(CHOH)_2COO)$, respectively.

acetate, and zinc tartrate, respectively. Figure 1 also shows the control ESI mass spectra obtained without any $Zn^{2+}$ source. In Figure 1, only the m/z region corresponding to +3 ions is shown. Figure 1(a) and (e) show Sp1-3 and CF2II-6, respectively, when no $Zn^{2+}$ source was added. Only protonated molecular ions, such as $(M+3H)^{3+}$, were observed. When $Zn^{2+}$ was added, $Zn^{2+}$-containing molecular ions appeared. For example, when $ZnCl_2$ was added to the ESI solution for Sp1-3 at 200 μM, the following $Zn^{2+}$-adduct molecular ions were observed: $(M+H+Zn^{2+})^{3+}$, $(M–H+2Zn^{2+})^{3+}$, $(M–3H+3Zn^{2+})^{3+}$, $(M–5H+4Zn^{2+})^{3+}$, and $(M–7H+5Zn^{2+})^{3+}$ (see Figure 1(b)). Sp1-3 zinc finger peptide ions with a single $Zn^{2+}$, i.e., $(M+H+Zn^{2+})^{3+}$, reflect the native zinc finger metal binding stoichiometry. In addition to these ions, $Zn^{2+}$-adducts with multiple $Zn^{2+}$ ions were observed: $(M–H+2Zn^{2+})^{3+}$, $(M–3H+3Zn^{2+})^{3+}$, $(M–5H+4Zn^{2+})^{3+}$, and $(M–7H+5Zn^{2+})^{3+}$. Among many $Zn^{2+}$ ions contained in $[M+(3–2n)H+nZn^{2+}]^{3+}$ molecular ions, one structural $Zn^{2+}$ ion is very likely to coordinate in a native-like metal ion binding site while the other $Zn^{2+}$ ions are likely to be adducted. In other words, zinc finger peptide ions with multiple $Zn^{2+}$ ions are likely to form nonspecific complexes, which should be avoided in this experiment. The addition of $Zn(CH_3COO)_2$ yielded very similar results. As shown in Figure 1(c), $Zn^{2+}$-adducts with multiple $Zn^{2+}$ ions were observed. In contrast, when Zn(tartrate) was added at 200 μM, Sp1-3 zinc finger peptide ions with a single $Zn^{2+}$, i.e., $(M+H+Zn^{2+})^{3+}$, were dominant with a low abundance of $(M–H+2Zn^{2+})^{3+}$ ions containing two $Zn^{2+}$ ions (see Figure 1(d)). This result clearly indicates that when Zn(tartrate) was used, the formation of nonspecific complexes with multiple $Zn^{2+}$ ions was significantly reduced, consistent with the previous study by Pan *et al*.[23]

For CF2II-6, the above-described $Zn^{2+}$-adduct formation tendency was also observed. ESI solutions prepared with either $ZnCl_2$ or $Zn(CH_3COO)_2$ produced significant amounts of nonspecific zinc finger–$Zn^{2+}$ complexes (see Figure 1(f) and (g)). Specifically, the ESI solution with $ZnCl_2$ showed ESI mass distribution of $(M+3H)^{3+}$, $(M+H+Zn^{2+})^{3+}$, $(M–H+2Zn^{2+})^{3+}$, $(M–3H+3Zn^{2+})^{3+}$, and $(M–5H+4Zn^{2+})^{3+}$. The ESI solution with $Zn(CH_3COO)_2$ yielded a distribution with $(M+3H)^{3+}$, $(M+H+Zn^{2+})^{3+}$, and $(M–H+2Zn^{2+})^{3+}$. As described above, zinc finger–$Zn^{2+}$ complexes with multiple zinc ions are simple $Zn^{2+}$ adducts that do not reflect the stoichiometry of native zinc finger peptides. ESI-MS results obtained with $Zn(CH_3COO)_2$ were very similar to those obtained with $ZnCl_2$. However, for $Zn(CH_3COO)_2$, the production of $(M–3H+3Zn^{2+})^{3+}$ and $(M–5H+4Zn^{2+})^{3+}$ was completely quenched. When Zn(tartrate) was used as a metal source, the generation of nonspecific zinc finger–$Zn^{2+}$ complexes was not observed at all. As shown in Figure 1(h), only $(M+3H)^{3+}$ and $(M+H+Zn^{2+})^{3+}$ were observed, indicating that the formation of nonspecific complexes with multiple $Zn^{2+}$ ions was completely inhibited. It is also noteworthy to note that when $Zn^{2+}$ was added, Sp1-3 did not show any $(M+3H)^{3+}$, while CF2II-6 showed abundant $(M+3H)^{3+}$ peaks. This different complex formation behavior may be due to differences in the $Zn^{2+}$ binding constants of the two peptides. In our unpublished data, the $Zn^{2+}$ binding constant of Sp1-3 was higher than that of CF2II-6 by approximately two orders of magnitude.

To confirm whether Zn(tartrate) generally reduces the formation of nonspecific zinc finger–$Zn^{2+}$ complexes, the same approach was repeated for other peptides, such as Sp1-1 and CF2II-4. The formation of nonspecific complexes was significantly reduced (spectra not shown) for the other peptides. We also performed another control experiment with melittin, which has a mass similar to those of other zinc finger peptides but does not have any $Zn^{2+}$ binding properties. Figure 1 (i)-(l) shows the resulting ESI mass spectra. As expected, the ESI solution with Zn(tartrate) did not produce any zinc finger–$Zn^{2+}$ complex, while the addition of $ZnCl_2$ or $Zn(CH_3COO)_2$ caused the formation of zinc finger–$Zn^{2+}$ complexes with multiple $Zn^{2+}$ ion complexes. These control experiments clearly suggest that Zn(tartrate) is an appropriate zinc ion source that minimizes the formation of nonspecific zinc finger–$Zn^{2+}$ complexes.

A detailed mechanistic study on why tartrate reduces the formation of nonspecific complexes between metal binding peptides and metal ions is still required.[23] However, the relatively high $Zn^{2+}$ binding affinity of tartrate was previously suggested to play a certain role in reducing nonspecific complexes. Acetate ion, which has a relatively higher $Zn^{2+}$ binding ability than chloride ion, was shown to produce less nonspecific complexes than chloride ions did (see Figure 1).

## Acknowledgments

The Korea Ministry of Environment supported this work through “The Environment Health Action Program”.

## References

1. Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, 246, 64.
2. Katta, V.; Chait, B. T. *J. Am. Chem. Soc.* **1991**, 113, 8534.
3. Lim, H. K. Hsieh, Y. L. Ganem, B.; Henion, J. D. *J. Mass Spectrom.* **1995**, 30, 708.
4. Cheng, X.; Chen, R.; Bruce, J. E.; Schwartz, B. L.; Anderson, G. A.; Hofstadler, S. A.; Gale, D. C.; Smith, R. D. *J. Am. Chem. Soc.* **1995**, 117, 8859.
5. Loo, J. A. *Mass Spectrom. Rev.* **1997**, 16, 1.
6. Rostom, A. A.; Robinson, C. V. *Curr. Opin. Struct. Biol.* **1999**, 9, 135.
7. Oh, H. B.; Breuker, K.; Sze, S. K.; Ying, G.; Carpenter, B. K.; McLafferty, F. W. *Proc. Nat'l. Acad. Sci. U.S.A.* **2002**, 99, 15863.
8. Lee, S. Y.; Park, S. J. Ahn, S. H.; Oh, H. B. *Int. J. Mass Spectrom.* **2009**, 279, 47.

9. Seong, Y. M.; Han, S. Y.; Jo. S. C.; Oh, H. B. *Mass Spectrom. Lett.* **2011**, 2, 73.
10. Hutchens, T. W.; Allen, M. H.; Li, C. M.; Yip, T. T. *Febs Lett.* **1992**, 309, 170.
11. Hu, P. F.; Ye, Q. Z.; Loo, J. A. *Anal. Chem.* **1994**, 66, 4190.
12. Feng, R.; Castelhano, A. L.; Billedeau, R.; Yuan, Z. Y. *J. Am. Soc. Mass Spectrom.* **1995**, 6, 1105.
13. Cheng, X.; Chen, R.; Bruce, J. E.; Schwartz, B. L.; Anderson, G. A.; Hofstadler, S. A.; Gale, D. C.; Smith, R. D.; Gao, J.; Sigal, G. B.; Mammen, M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1995**, 117, 8859.
14. Rostom, A. A.; Tame, J. R. H.; Ladbury, J. E.; Robinson. C. V. *J. Mol. Biol.* **2000**, 296, 269.
15. Kempen, E. C.; Brodbelt, J. S. *Anal. Chem.* **2000**, 72, 5411.
16. Wang, W.; Kitova, E. N.; Klassen, J. S. *Anal. Chem.* **2003**, 75, 4945.
17. Gabelica, V.; Galic, N.; Rosu, F.; Houssier, C.; De Pauw, E. *J. Mass Spectrom.* **2003**, 38, 491.
18. Speir, J. P.; Senko, M. W.; Little. D. P.; Loo, J. A.; McLafferty, F. W. *J. Mass Spectrom*. **1995**, 30, 39.
19. Tolić, L. P.; Bruce, J. E.; Lei, Q. P.; Anderson, G. A.; Smith, R. D. *Anal. Chem.* **1998**, 70, 405.
20. Yi, S.; Boys, B. L.; Brickenden, A.; Konermann, L.; Choy, W. Y. *Biochemistry* **2007**, 46, 13120.
21. Niessen, W. M. *J. Chromatogr. A* **1999**, 856, 179.
22. Cavanagh, J.; Benson, L. M.; Thompson, R.; Naylor, S. *Anal. Chem.* **2003**, 75, 3281.
23. Pan, J.; Xu, K.; Yang, X.; Choy, W.-Y.; Konermann, L. *Anal. Chem.* **2009**, 81, 5008.
24. Miller, J.; McLachlan, A. D.; Klug, A. *EMBO J.* **1985**, 4, 1609.
25. Jamieson, A. C.; Miller, J. C.; Pabo, C. O. *Nature Reviews Drug Discovery* **2003**, 2, 361.
26. Park, S. J.; Jo, K. B.; Oh, H. B. *Analyst* **2011**, 136, 3739.
27. Kriwacki, R. W.; Schultz, S. C.; Steitz, T. A.; Caradonna, J. P. *Proc. Nat'l. Acad. Sci. U.S.A.* **1992**, 89, 9759.
28. Hsu, T.; Gogos, J. A.; Kirsh, S. A.; Kafatos, F. C. *Science* **1992**, 257, 1946.
29. Gogos, J. A.; Jin, J.; Wan, H.; Kokkinidis, M.; Kafatos, F. C. *Proc. Nat'l. Acad. Sci. U.S.A.* **1996**, 93, 2159.