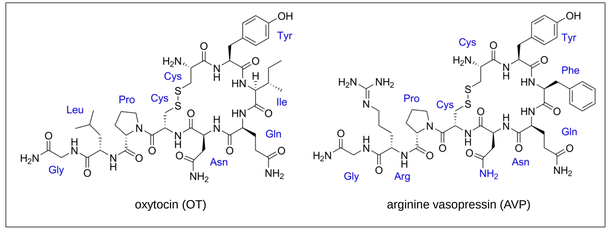
Using Differential Mobility Spectrometry to Measure the Interactions of Hormone Peptides with Divalent Metal Ions and Solvent Molecules

Chang Liu[1]; J. Larry Campbell[1]; J. C. Yves, Le Blanc[1]; Jefry Shields[2]; John S. Janiszewski[2]
[1]AB SCIEX, 71 Four Valley Drive, Concord, ON, L4K 4V8 Canada; [2]Pfizer Inc., 76 Eastern Point Road, Groton, CT 06340

INTRODUCTION

Oxytocin (OT) and arginine vasopressin (AVP), two cyclic nonapeptides containing an intramolecular disulfide bridge, are important neurohypophysical hormones.[1] The presence of divalent metal ions has been found to be essential for the specific binding of these hormones to their receptors. In fact, important characterization of these peptides, in both protonated and metal-adducted forms, have been done using classical ion mobility spectrometry (IMS).[2-3] To complement this work, we have employed differential mobility spectrometry (DMS) to investigate the interaction of OT, AVP, and their reduced forms with divalent metal ions, and in the presence of different chemical modifiers in the DMS cell. Such studies yield insights into the relationships between peptide ion forms, those structures, and their degrees of ion clustering/solvation.



oxytocin (OT) arginine vasopressin (AVP)

Figure 1. Chemical structure of oxytocin and arginine vasopressin.

MATERIALS AND METHODS

Sample Preparation:
Oxytocin and arginine vasopressin, metal salts, dithiothreitol (DTT), and ammonium bicarbonate, were obtained from Sigma-Aldrich (Oarkville ON, Canada). Oxytocin or arginine vasopressin (5 μg/mL) was mixed with calcium chloride, copper chloride, zinc sulfate, magnesium chloride, or iron(II) sulfate (50 μM) in the ESI solvent containing acetonitrile and water (50/50, v/v) with 0.1% formic acid. Reduced oxytocin were formed via reaction with DDT in ammonium bicarbonate buffer at 60 ° C for one hour.

DMS-MS Conditions:
A differential mobility spectrometer (SelexION™ device, AB SCIEX) system was mounted in the atmospheric region between the AB SCIEX QTRAP® 5500 system's sampling orifice and a Turbo V™ source (+5500 V) (Figure 2). The temperature of the DMS cell was maintained at 150 ° C, and the nitrogen curtain gas was operated at 20 psi. Chemical modifiers (water, dichloromethane, methanol, ethanol, acetonitrile, or isopropanol) were added into the nitrogen curtain flow at 1.5% (v/v). The fundamentals of the DMS device have been described elsewhere.[4-5] In this study, both separation voltage (SV) and compensation voltage (CV) were scanned synchronously. As the SV was stepped from 0 to 4000 V (in 250-V increments), CV was scanned from -40 V to +20 V in 0.15 V increments. These data were plotted as dispersion plots, with SV as the *x-axis*, and the optimal CV for ion transmission as the *y-axis*.

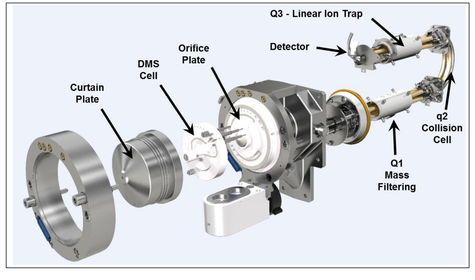


Figure 2. The instrument setup of the SelexION™-QTRAP® 5500 system.

RESULTS

The DMS analysis results of metal-adducted peptide hormones are shown in Figure 3 (OT), and Figure 4 (AVP). The ion solvation strength was weak (large positive CV values) with poor resolving power with the presence of water or dichloromethane in the curtain gas. However, the use of alcohols provided a greater degree of ion separation. For example, by adding ethanol or isopropanol into the DMS cell, we observed differing degrees of ion solvation between the zinc- and copper-adducted peptides, which was greater than the calcium-, magnesium-, or iron-adducted peptides. Interestingly, the use of acetonitrile provided an even higher degree of clustering/solvation (more negative CV values), albeit with less separation among the ions in CV-space.

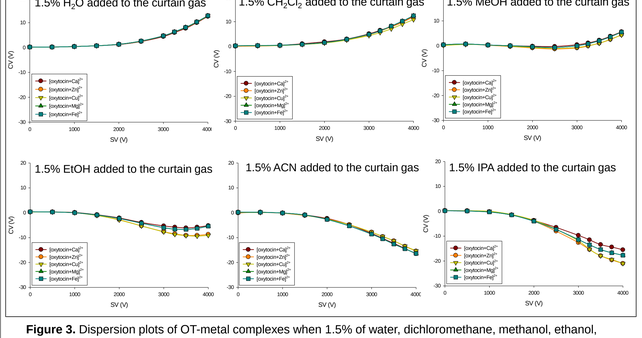


Figure 3. Dispersion plots of OT-metal complexes when 1.5% of water, dichloromethane, methanol, ethanol, acetonitrile, or isopropanol was added into the curtain gas.

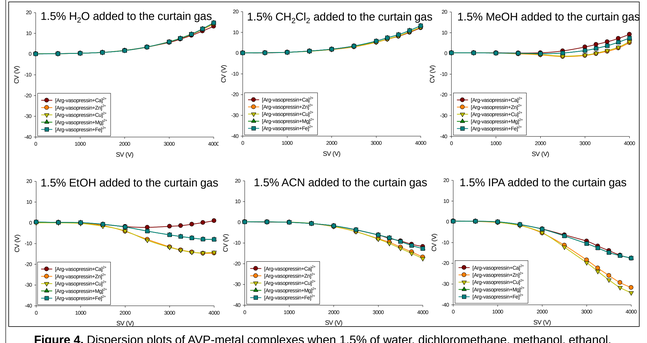


Figure 4. Dispersion plots of AVP-metal complexes when 1.5% of water, dichloromethane, methanol, ethanol, acetonitrile, or isopropanol was added into the curtain gas.

The impact of the intramolecular disulfide bond in the metal binding and solvation behavior of OT was also assessed. We did this by cleaving the disulfide bond and adding only 2 Da in mass (Figure 5). The most obvious difference between OT and reduced OT was observed when ethanol was used as the chemical modifier, where the optimum CV of zinc- and copper-adducted reduced OT were lower than the corresponding OT complexes (Figure 6).

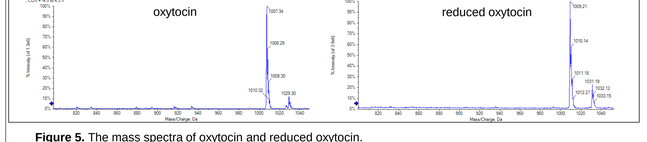


Figure 5. The mass spectra of oxytocin and reduced oxytocin.

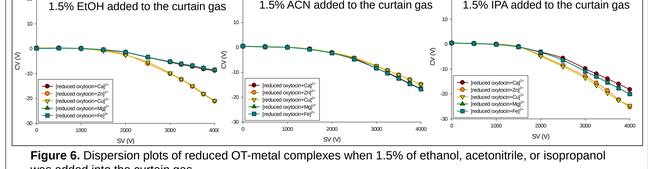


Figure 6. Dispersion plots of reduced OT-metal complexes when 1.5% of ethanol, acetonitrile, or isopropanol was added into the curtain gas.

The DMS behavior of copper-adducted AVP was found to be pH dependent (Figure 7). More negative CVs were necessary for the ion transmission through the DMS cell when $[AVP+Cu]^{2+}$ ions were generated from acidic ESI solutions (1% acidic acid) , compared to more positive CVs for the same ions generated from basic solutions (1% ammonium hydroxide). This suggests that, at lower pH, zwitterionic forms of the $[AVP+Cu]^{2+}$ ion may present multiple locations of charging that yield ions of the same m/z but of very different mobilities.

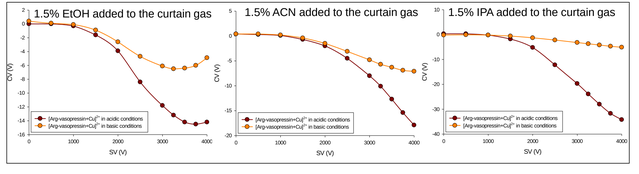


Figure 7. Dispersion plots of $[AVP+Cu]^{2+}$ at acidic and basic condition, when 1.5% of ethanol, acetonitrile, or isopropanol was added into the curtain gas.

CONCLUSIONS

Differential mobility spectrometry was used to study the interaction strength of divalent metal ion adducted hormone peptides with volatile solvent molecules. With the addition of alcohols in the curtain gas as the chemical modifier, differing degrees of ion solvation strength was observed. The presence of the disulfide bond reduced the solvation strength of OT compared with the corresponding reduced forms. Interestingly, the DMS behavior of copper adducted AVP was found to be pH dependent. Its clustering strength with chemical modifiers was stronger in the acid conditions, indicating that there may be multiple locations of charging that yield ions of the same m/z but very different mobilities.

REFERENCES

1. Barberis, C.; Tribollet, E. *Crit. Rev. Neurobiol.* **1996**, *10*, 119-154.
2. Liu, D.; Seuthe, A. B.; Ehrler, O. T.; Zhang, X.; Wyttenbach, T.; Hsu, J. F.; Bowers, M. T. *J. Am. Chem. Soc.* **2005**, *127*, 2024-2025.
3. Wyttenbach, T.; Liu, D.; Bowers, M. T. *J. Am. Chem. Soc.* **2008**, *130*, 5993-6000.
4. Schneider, B. B.; Covey, T. R.; Coy, S. L.; Krylov, E. V.; Nazarov, E. G. *Int. J. Mass Spectrom.* **2010**, *298*, 45-54.
5. Schneider, B. B.; Covey, T. R.; Coy, S. L.; Krylov, E. V.; Nazarov, E. G. *Anal. Chem.* **2010**, *82*, 1867-1880.

TRADEMARKS/LICENSING