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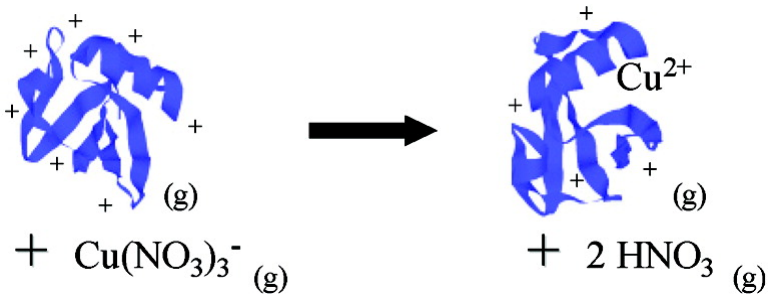

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Gas-Phase Peptide/Protein Cationizing Agent Switching via Ion/Ion Reactions

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Tandem mass spectrometry of cationized peptides and proteins in the gas phase is commonly used to determine primary structural information. This information is dependent on the number and identities of the cationizing species and their interactions with the peptide or protein.[1] By changing the cationizing reagent, complementary primary sequence information can be obtained. For example, collision-induced dissociation (CID) of a sodiated peptide leads to fragmentation adjacent to the C-terminal residue, while the protonated peptide usually fragments at various places along the peptide backbone.[1d] Most commonly, metal-cationized peptide/protein ions are introduced to the mass spectrometer by electrospray of solutions containing the peptide/protein of interest and a metal salt.[1] The mass spectrum of such a mixture is composed of peptide ions with various combinations of cationizing reagents. The presence and abundance of metal-cationized peptides is highly dependent upon solution composition and pH. Thus, in some cases, it may be difficult to obtain a signal for a specific metal-cationized ion of interest. We describe here an alternative strategy for generating metal-containing peptide/protein ions from protonated species via gas-phase ion/ion reactions. This approach allows exploration of a variety of metal/peptide interactions as well as the capability for formation of metal-containing ions that may not be produced directly by electrospray of solutions of metal salts and proteins.

The approach involves gas-phase ion/ion reactions in a quadrupole ion trap mass spectrometer equipped with multiple electrospray ionization sources.[2] In the case of ion/ion reactions used to generate metal-cationized peptide/protein ions in the gas phase, protonated peptide/protein ions were formed with one ion source, while anions of metal salts were formed from a second ion source.[3] The reactant ions are formed separately to allow for independent optimization of solution and electospray conditions. Therefore, this approach avoids signal suppression and dilution effects, which can be observed when polypeptide and metal salts are electrosprayed from the same solution. Another advantage of forming the reactant ions separately is that it facilitates the isolation of specific ions for participation in the ion/ion reactions. Products from the ion/ion reactions of selected reactants can be mass analyzed directly or isolated and subjected to CID prior to mass analysis.

Several reactions types have been observed involving multiply protonated peptides and metal-containing anions. For example, ions resulting from proton transfer have been noted, and the attachment of metal anion complexes to peptide ions has been observed.[4] However, reactions that lead to the incorporation of a metal into a peptide, P, via cation switching involving a multiply protonated peptide are emphasized in this report. The generic reaction for a doubly protonated peptide and singly charged metal with two counterions (X) is represented as:

$$[P+2H]^{2+} + [MetalX_2]^- \rightarrow [P+Metal]^+ + 2HX$$

More highly protonated polypeptides react to yield a metal-containing species with two fewer protons.

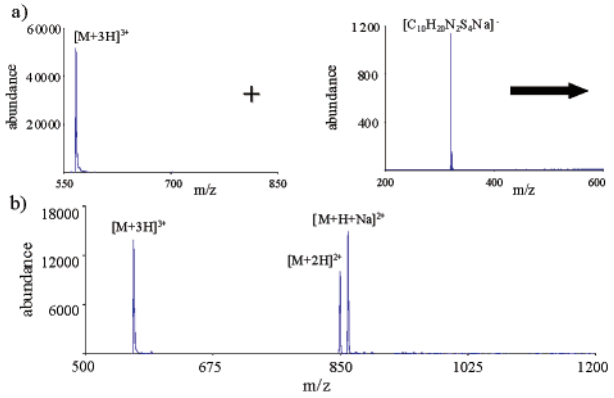

Figure 1. (a) Mass spectrum of isolated +3 *Trp*-11 neurotensin (left) and sodium diethyldithiocarbamate anions (right). (b) Ion/ion reaction product mass spectrum from mutual storage of the ions in (a).

An example of ion/ion reactions used to generate metal-cationized peptides in the gas phase by cation switching is the formation of sodiated *Trp*-11 neurotensin. Triply protonated neurotensin ions were isolated from the initial positive ion electrospray distribution (Figure 1a). While these neurotensin ions were stored in the quadrupole ion trap after isolation, anions from a sodium diethyldithiocarbamate solution (anion mass spectrum acquired in the absence of positive ions shown in Figure 1b) were introduced into the ion trap. Ion/ion reactions occurred during a period of simultaneous storage of triply protonated neurotensin ions and sodium diethyldithiocarbamate anions prior to mass analysis.

Positively charged products from this set of reactants arose from proton transfer[5] to yield the $[M + 2H]^{2+}$ ion, and cation exchange involving addition of sodium to the peptide to yield the $[M + H + Na]^{2+}$ ion (Figure 1c). The $[M + H + Na]^{2+}$ reaction product ions are likely to be formed via a relatively long-lived complex between the sodium-containing anions and protonated peptide ions. The complex breaks up after transfer of a sodium cation to the peptide and two protons to the diethyldithiocarbamate anion. The net effect is a "switching" of one sodium ion for two protons of the peptide ion. Complex formation upon ion/ion reactions is a known phenomenon and has recently been discussed.[6] The types of ion/ion reaction product ions observed in Figure 1 are representative of studies performed thus far with reactions between multiply protonated peptides and metal-containing anions. The generality of cation-switching ion/ion reactions to form a variety of metal-cationized ions of *Trp*-11 neurotensin is demonstrated in Figure 2. Triply protonated neurotensin ions were reacted with metal salt anions of calcium, silver, and nickel, respectively. The metal-containing anions were not isolated prior to the mutual storage period with the protonated peptide ions. Consequently, an assortment of product ions was generated for each of the ion/ion reactions.

In Figure 2, products resulting from exchange of protons for metal ions are observed in addition to proton-transfer products. These data reveal that gas-phase cation switching is not restricted

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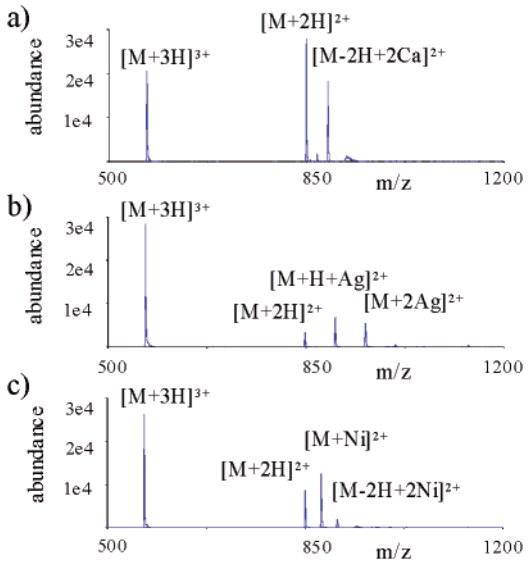

Figure 2. Product ion mass spectra for cation switching ion/ion reactions between triply protonated *Trp*-11 neurotensin and (a) calcium acetate anions, (b) silver nitrate anions, (c) nickel acetate anions.

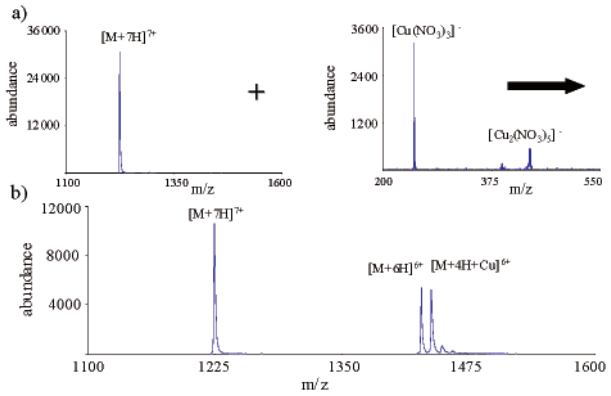

Figure 3. (a) Mass spectrum of isolated +7 ubiquitin (left) and nonisolated copper nitrate anions (right). (b) Ion/ion reaction product mass spectrum from mutual storage of the ions in (a).

to alkali metals and can occur for alkaline earth metals as well as singly and multiply charged transition metals. Some of the product ions in the ion/ion reaction spectra shown in Figure 2 have more than one of the respective metal ions as cationizing agents. These products are formed from reactions with anions containing multiple metal and counterions for a given metal and are not the result of consecutive reactions of first-generation ion/ion reaction products. Consecutive reactions would yield products of lower-charge states since each reaction with a singly charged anion results in products with one less charge.

Metal ions can also be inserted into proteins. Formation of copper-cationized ubiquitin ions via gas-phase ion/ion reactions is shown in Figure 3. Copper nitrate anions were reacted with $[M + 7H]^{7+}$ ubiquitin ions. The mass spectra of the reactant ions are given in Figure 3a. In addition to the proton-transfer product, $[M + 6H]^{6+}$, a product resulting from cation exchange to the protonated ubiquitin ions, $[M + 4H + Cu]^{6+}$, was observed (Figure 3b). Gas-phase formation of metal-containing protein ions by this method offers a high degree of flexibility in regard to the metals and proteins examined and allows for specificity that cannot be obtained by directly electrospraying a metal salt and protein mixture.

The primary focus of these studies to date has been on characterization and optimization of the reactions rather than on investigation of reaction product ions. However, comparisons of CID behavior of ions formed in the gas phase with that of ions formed directly from solution are also of obvious interest. CID was therefore performed on selected ion/ion reaction products. For comparative purposes, selected metal and peptide solutions were combined and nanoelectrosprayed. There was no evidence for loss of the metal ion upon activation of the metal-cationized peptides formed in the gas phase. (Data not shown.) Rather, fragmentation along the peptide backbone was observed that often provided complementary sequence information relative to the fragmentation of the protonated species. In studies to date, the fragmentation patterns of metal-cationized ions formed in the gas phase via ion/ion reactions have been similar to those of metal-cationized peptides ions generated from solution. (Data not shown.)

In summary, cation switching ion/ion reactions offer a novel way to generate metal-cationized peptides/proteins in the gas phase from multiply protonated species. The capability of this technique to form a wide variety of metal-cationized ions of a given peptide has been demonstrated. The same or similar procedures can be performed using any peptide/protein that can be formed as a doubly or more highly charged ion. The specificity afforded by effecting the reactions within the context of a tandem mass spectrometry experiment, whereby the reactants are mass-selected prior to reaction, is a particular advantage in forming metal-containing product ions of interest. Far less control is afforded by attempting to form ions via electrospray of solutions comprised of a mixture of peptides/proteins and metal-containing salts. The ability to form a variety of peptide/protein ions with various cationizing reagents in the gas phase is attractive both for the study of intrinsic interactions of metal ions with polypeptides and for maximizing the structural information available from tandem mass spectrometry of peptides and proteins.

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