APPLICATION NOTE

On the Use of Scans at a Constant Ratio of B/E for Studying Decompositions of Peptide Metal(II)–Ion Complexes Formed by Electrospray Ionization

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The use of sector mass spectrometers to study metastable ion decompositions of peptide metal–ion complexes formed by electrospray ionization is discussed. Products that are formed by charge-separation reactions are characterized by large kinetic energy release distributions. This causes scans at a constant B/E to give incorrect product ion abundances and possibly incorrect mass assignments. Two instrumental methods exist that can be used either to detect the ions or to estimate relative ion abundances: a floated collision cell or mass-analyzed ion kinetic energy spectrometry (MIKES) scans. The floated collision cell, by virtue of an altered B/E scan law, however, discriminates against important metastable ion reactions that occur outside the cell. MIKES scans provide a clearer estimate of product ions that arise by metastable ion charge-separation reactions. Problems with pseudotandem (first field-free region) experiments are also discussed. *(J Am Soc Mass Spectrom 1995, 6, 608–610)*

Metastable ion and collision-induced dissociations (CID) are powerful techniques for structure determination of gas-phase complexes between peptides and metal(II) ions [1–3]. Recently, electrospray ionization (ESI) coupled to a forward-geometry sector instrument has been used to study the first field-free region CID of transition metal(II) complexes by using scans at a constant B/E [3]. An issue for all sector machines and scans at a constant of B/E, however, is instrumental discrimination against species that arise by charge-separation reactions. The discrimination is caused by the large kinetic energy release that results from coulombic repulsion in the reactions. This large kinetic energy release may result in unreliable relative ion abundances, decreased detection of ions formed by charge separation, and incorrect mass-to-charge ratio assignment in scans at a constant B/E.

Examples of this charge-separation phenomena are demonstrated here by using ESI and a JEOL (Peabody, MA) JMS-SX102/SX102A/E five-sector ($B_1E_1/B_2E_2/E_3$) tandem mass spectrometer, in which B = magnet and E = ESA or electrostatic analyzer [1f, 4]. Ions were formed in a JEOL Generation 2 ESI ion source at an accelerating voltage of 5 kV. Precursor ions were selected at a double-focusing mass-resolving power of 1000 (10% valley) by using B_1E_1. Products of metastable ion reactions that occurred in the third field-free region between B_1E_1 and B_2E_2/E_3 were detected by using a scan of B_2E_2/E_3 at a constant B/E up to 10 kV. The double-focusing mass-resolving power of B_2E_2 was 1000 (10% valley). The energy-resolving slit that follows E_3 was fully open so that E_3 only functioned as an ion guide [4]. Scans were acquired at a rate of 16 s per scan for a total time of 120 min. Mass-analyzed ion kinetic energy spectrometry (MIKES) of metastable ion reactions that occurred in the fifth field-free region between B_2E_2 and E_3 were acquired by scanning E_3 [4]. The 30-s scans were obtained over a period of 180 min. Narrow MIKES scans of E_3 to acquire kinetic energy release distributions (KERDs) were obtained after narrowing the energy-resolving slit that follows E_3. This gave a Gaussian-shaped (not flat-topped) precursor ion beam so that the kinetic energy distribution of the precursor ions was the limiting factor in energy-resolving power [4]. The 2-s narrow scans were summed over a period of 180 min.

Spectra acquired by using scans at a constant B/E of $[M + Me]^{2+}$ complexes of angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), in which Me = Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}, primarily reveal abundant doubly charged product ions (Figure 1). The nomenclature for the cleavage reactions is the same as in our previous papers [1], which unambiguously gives the exact mass of the product ions. Thus, doubly charged [b_6 + Co –

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$H]^{2+}$ ions (m/z 421.4 in Figure 1a) arise from $[M + Co]^{2+}$ precursor isomers by cleavage of the amide bond between His and Pro with hydrogen transfer from the ionic fragment to the neutral leaving group. Singly charged product ions of higher mass-to-charge ratio than the doubly charged precursors are either weakly abundant (Figure 1a, b, d) or are not detected (Figure 1c). The low-mass singly charged complementary ions are missing. Formation of singly charged products requires the decomposing precursor isomer to be a complex between a doubly charged metal ion and a zwitterionic peptide. This means that one site on the peptide is deprotonated and coordinated to the metal ion, whereas another site remote from the metal ion is protonated. Thus, singly charged $[y_6 + Co]^+$ ions (m/z 832.6 in Figure 1a) arise by cleavage of the amide bond between Arg and Val with hydrogen transfer from the protonated fragment to the fragment that contains the coordinated metal ion.

In contrast, MIKES experiments give spectra that show the presence of the singly charged product ions (Figure 2). The high-mass, singly charged ions for all precursors are detected as broad peaks that have a low signal-to-noise ratio. The low-mass complementary ions are significantly less abundant. Narrow, signal-averaged kinetic energy scans across the high- and low-mass peaks reveal that the ions are formed by reactions that have a large kinetic energy release (Figure 3). The kinetic energy release distributions (KERDs) give a most probable kinetic energy release (KER) of

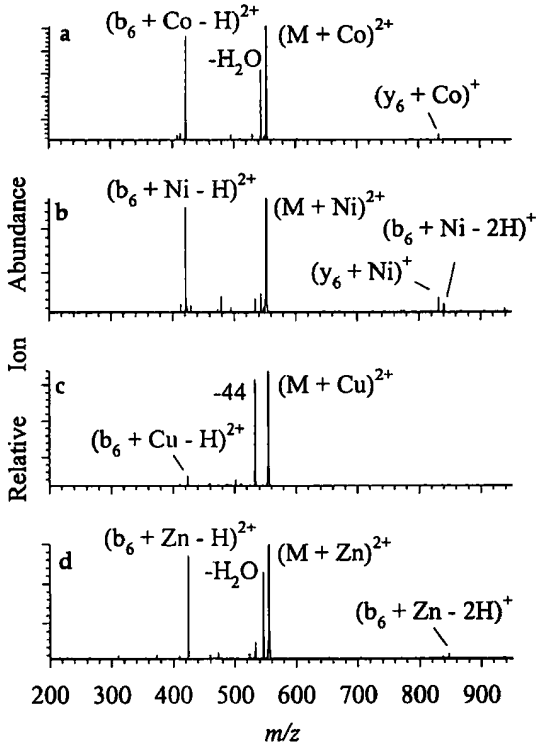

Figure 1. Metastable ion decomposition spectra of (a) $[M + Co]^{2+}$, (b) $[M + Ni]^{2+}$, (c) $[M + Cu]^{2+}$, and (d) $[M + Zn]^{2+}$ complexes of angiotensin II produced by ESI. Spectra were acquired by using scans at a constant B/E.

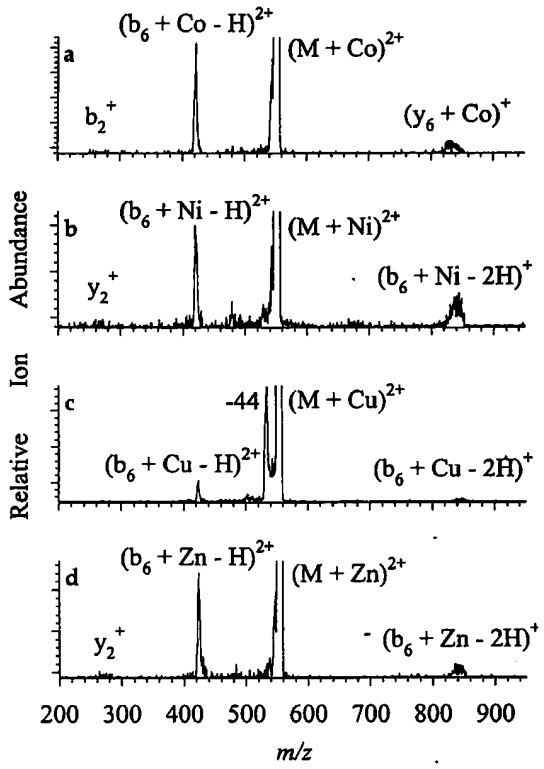

Figure 2. Metastable ion decomposition spectra of (a) $[M + Co]^{2+}$, (b) $[M + Ni]^{2+}$, (c) $[M + Cu]^{2+}$, and (d) $[M + Zn]^{2+}$ complexes of angiotensin II produced by ESI. Spectra were acquired by using MIKES scans.

0.986 and 0.939 eV (0.962 ± 0.033 eV). (The most probable KER is the maximum of the KERD. The KERDs were calculated from the peak shapes by accounting for y- and z-axial discrimination according to the methods in [5].) The identical nature of the KERDs

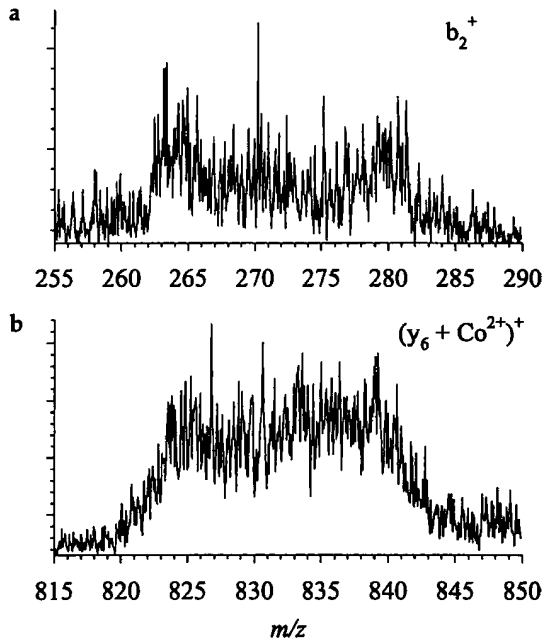

Figure 3. Narrow-range MIKES scans of (a) b_2^+ and (b) $(y_6 + Co)^+$ complementary product ions that arise from dissociation of $[M + Co]^{2+}$ precursor complexes.

indicates that the two ions are indeed a complementary pair. Increased dishing and decreased detectability of the low-mass (low kinetic energy) ions occur because instrumental discrimination in the *y*- and *z*-axial directions is greater for lower kinetic energy ions. This is caused by two instrumental factors. One is that the energy bandpass of the ESA is proportional to ion kinetic energy. The second is that the high kinetic energy release (high velocity) components in the *y*- and *z*-axial directions are proportionately larger for low-mass ions.

There are at least two important implications of these results. Mass spectra acquired by using scans at a constant B/E give seriously misleading relative abundances for product ions formed by charge-separation reactions. MIKES experiments are one method that may be used either to detect the ions or to estimate relative ion abundances. The reliability of estimation, however, is limited severely by the impact of instrumental discrimination that causes significantly reduced transmission of product ions. The large KER also could cause a potential problem in scans at a constant B/E if magnet calibration were imprecise, so that linkage between the magnet and ESA were slightly imperfect. If only an edge, or "horn," of a dished peak (Figure 3a) were double-focused in a B/E scan, an error in mass assignment could occur. The problem with ion transmission will be important in all sector instruments, whereas incorrect mass assignment will be instrument-dependent. Accurate mass calibration is vital to minimize the latter effect.

A second method that may be used to improve detectability of ions that arise from charge-separation reactions is a floated collision cell. Detrimental effects of kinetic energy release on product ion transmission are minimized [6]. Use of a floated collision cell, however, will prevent transmission of metastable product ions that are formed outside the cell because of an altered B/E scan law. Products from metastable ion reactions that occur in the cell are only weakly abundant compared to products formed throughout the entire field-free region. For complexes between peptide and transition metal(II) ions, metastable ion decompositions are critical [1f]. They occur from low-energy reacting configurations of the precursor complexes, configurations that undergo structurally revealing, low-energy charge-induced cleavages in proximity to the metal–ion binding site. Consequently, the use of a floated collision cell may have limitations in elucidating binding sites in gas-phase peptide transition metal(II)–ion complexes.

Pseudotandem mass spectrometric experiments (first field-free region) have several drawbacks in the study of complexes between peptides and metal ions by using scans at a constant B/E. Reactions that occur in the first field-free region can be the result of CID, not metastable ion dissociation. Thus, the structures of the low-energy reacting complexes that contain an intact metal–ion binding site may not be revealed. Additionally, poor precursor ion selection leads to two significant problems. Precursor ions that do not contain the metal ion may be transmitted, which can result in artifact ions and unreliable relative ion abundances. This may be partially alleviated by background subtraction [1a–e]. Of equal significance is that transmission of multiple metal–ion isotopes can cause problems in unambiguously assigning nomenclature to product ions that differ by small mass-to-charge ratio values. Correct nomenclature assignment is essential to elucidate reaction mechanisms and product ion structures. In addition to these issues, the problem of severe discrimination against product ions that arise from charge-separation reactions still persists. This also may result in unreliable relative ion abundances and inaccurate mass-to-charge ratio assignments.

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