# Dehydration of Peptide $[M + H]^+$ Ions in the Gas Phase

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The loss of water from protonated peptides was studied using [$^{18}$O]-labeling of the C-terminal carboxyl group. The structures (including the location of the isotopic label) of first-generation product ions were examined by sequential product ion scanning ($MS^3$ and $MS^4$) using a hybrid sector/quadrupole mass spectrometer. Water loss may involve carboxylic acid groups, side-chain hydroxyls, or peptide backbone oxygens. Although one of these three pathways often predominates, more than one dehydration route can be operative for a single peptide structure. When peptide backbone oxygen is lost, the dehydration can occur at one or two primary sites along the backbone, with the location of the site(s) varying among peptides. When water loss involves the C-terminal carboxyl group, the resulting ion may undergo extensive intraionic oxygen isotope exchange. This evidence for complex intraionic interactions further emphasizes the significance of gas-phase conformation in determining the fragmentations of peptide ions. *(J Am Soc Mass Spectrom 1993, 4, 477–481)*

A frequently observed fragmentation of peptide $[M + H]^+$ ions during tandem mass spectrometric analyses involves the loss of water. Conventional product ion spectra often exhibit the loss of 18 u from the $[M + H]^+$ precursor species and may show the loss of 18 u from various product ions as well. There are three possible sources for the oxygen lost during the dehydration of peptides. The first and perhaps most obvious of these is the carboxylic acid group, including the C-terminus itself and any acidic side chains present in the peptide. A second likely source is the hydroxyl group present in peptides containing the alcoholic residues serine and threonine and, possibly (but less likely), the phenolic tyrosine. The third possible source is the peptide backbone itself, which is essentially a polyamide structure. Given all of these possibilities, the observation of an $[M + H - 18]^+$ fragment ion in the product ion spectrum of a peptide is not particularly informative from the point of view of a detailed structural analysis. A consideration of this easily ignored reaction may, however, be informative with regard to the fundamental behavior of peptide $[M + H]^+$ ions in the gas phase.

Carboxylic acid groups can be readily labeled with two $^{18}$O atoms through acid-catalyzed $^{18}$O exchange and other procedures [1]. When the C-terminus of a peptide is [$^{18}O_2$]-labeled, C-terminal fragment ions in the conventional product ion spectrum can be identified by comparison with the corresponding spectrum of the unlabeled peptide [2]. C-terminally [$^{18}O_2$]-labeled peptides were used in this laboratory during studies of complex rearrangement and intraionic isotope exchange processes involving the C-terminal oxygens [3–6]. An incidental finding (and not further explored) during those previous studies [3–6] was considerable variability among peptides in the dehydration reaction. Some [$^{18}O_2$]-labeled peptides exhibited almost exclusively the loss of $H_2{}^{18}O$, whereas others showed primarily the loss of $H_2{}^{16}O$. Still others exhibited the loss of both labeled and unlabeled water with nearly equal facility.

Several recent studies have indicated the importance of the conformations adopted by peptides and other ions in the gas phase [4–10]. Other studies have emphasized the importance of the site or sites of charge localization in influencing the fragmentation pathways of cationized species [10–16]. Either or both of these factors could be involved in determining the differences among peptides in the nature of the dehydration reaction. In light of these considerations, the present studies were undertaken to investigate further the dehydration of peptide $[M + H]^+$ ions, using the techniques of tandem mass spectrometry ($MS^2$) and sequential mass spectrometry ($MS^3$ and $MS^4$).

## Experimental

### *Materials*

The peptides bradykinin (1–5) (RPPGF), antikentsin (TRKR), leucine–enkephalin (YGGFL), and delta-sleep-

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inducing peptide (WAGGDASGE) were purchased from Bachem (Torrance, CA) and were used as received. Some peptides were labeled at their C-termini with $^{18}O$ by acid-catalyzed isotopic exchange, as previously described [4]. The dimethylpyrimidylornithyl (DMPO) derivatives of arginyl peptides, methyl esters, and trifluoroacetyl derivatives were generated as previously described [4]. An aliquot corresponding to 5–10 μg of each peptide or peptide derivative was used for tandem mass spectrometry analysis.

### *Mass Spectrometry*

The various tandem mass spectrometry experiments were performed with a VG ZAB-SEQ (VG Analytical Ltd., Manchester, UK) hybrid instrument of BEqQ configuration (where B is the magnetic sector, E the electric sector, q the radio frequency-only quadrupole, and Q the quadrupole mass filter). Ionization was by fast-atom bombardment using 8-keV xenon for the primary atom beam. The liquid matrix was a 1:1 mixture of thioglycerol and 2,2'-dithiodiethanol, saturated with oxalic acid. Scan data were acquired using the multichannel analyzer mode of the VG 11/250J data system, with 8–15 scans typically accumulated. It should be noted that the apparent resolution of full-scan plots of multichannel analyzer data may appear artifactually degraded owing to compression algorithms incorporated in the plotting routines of the data system.

Conventional first-generation product ion spectra were obtained by manual selection of the precursor ion with the double-focusing portion of the instrument (resolution 1200–1500, 10% valley), with subsequent scanning of Q (1–1.5 mass-to-charge ratio resolution) to detect the first-generation product ions formed in q. Collisionally activated decomposition (CAD) conditions were used for these experiments, with argon used as the collision gas at an estimated pressure in the collision region of $1.8 \times 10^{-4}$ mbar (approximately 50–60% reduction of the main beam) and with a collision energy of 8–30 eV.

For second-generation product ion spectra ($MS^3$), appropriate settings of B and E were manually established to select first-generation product ions (effective mass-to-charge ratio resolution 800) formed in the first field-free region (FFR1) of the mass spectrometer (in the absence of added collision gas) using a previously described instrument modification that permits independent control of the accelerating and electric sector voltages [3]. First-generation product ions so selected were subjected to low-energy CAD in q as described above, with subsequent mass-to-charge ratio analysis of the second-generation product ions with Q. Similarly, for third-generation product ion spectra ($MS^4$), B was set to transmit first-generation product ions formed in FFR1, and E was set to transmit second-generation product ions formed in the second field-free region (FFR2). Third generation products formed in q were analyzed by scanning Q. For these analyses no collision gas was added to either FFR1 or FFR2, and low-energy CAD conditions were used in q, as described above.

## Results and Discussion

### *Water Loss Involving Carboxylic Acid Groups*

The conventional first-generation product ion spectrum of the $[M + H]^+$ ion of a C-terminally $[^{18}O_2]$-labeled peptide can reveal whether or not the C-terminal carboxylic acid group is involved in the dehydration reaction for that particular peptide. An example of this is shown in Figure 1, which depicts an expanded portion of the conventional product ion spectrum of $[^{18}O_2]$antikentsin (TRKR). (The symbols used here in Figures 1–9 for designating the type of mass spectrometric experiment are those recommended by Cooks and co-workers [17, 18].) In this spectrum, the major signal representing dehydration corresponds to the loss of $H_2{}^{18}O$, indicating that the C-terminal carboxylic acid group contributes the oxygen for the primary route of dehydration in this peptide. The minor signal corresponding to the loss of $H_2{}^{16}O$ indicates that either the threonine hydroxyl or the peptide backbone contributes to a minor secondary dehydration pathway.

### *Water Loss Involving Side-Chain Hydroxyls*

A very different situation exists with the tris-methyl ester derivative of delta-sleep-inducing peptide (WAGGDASGE). Figure 2 presents the conventional product ion spectrum of this compound, which exhibits a strong water loss despite its lack of free carboxylic acid groups. Several of the C-terminal ($Y_n''$ type, according to the Roepstorff and Fohlman [19] nomenclature for peptide fragmentations) and N-terminal fragment ions ($B_n$ type) present in this spectrum also exhibit the loss of water, but it is not clear from this spectrum whether

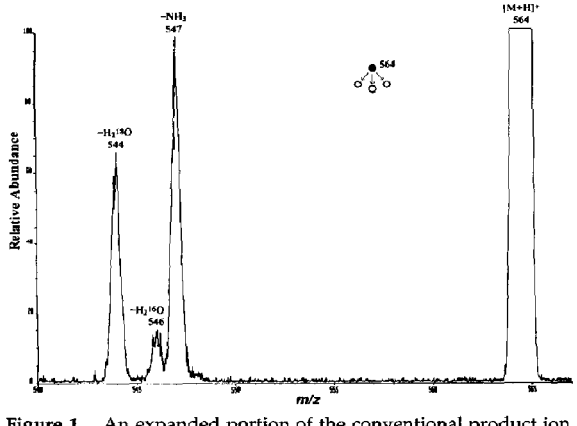


Figure 1. An expanded portion of the conventional product ion spectrum of the $[M + H]^+$ ion of $[^{18}O_2]$antikentsin (TRKR), showing the region corresponding to the loss of water from the precursor ion.

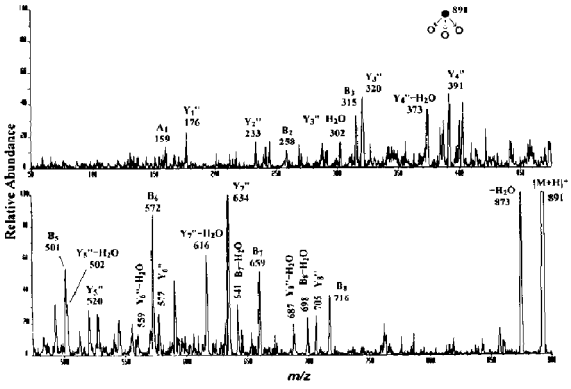


**Figure 2.** Conventional product ion spectrum of the $[M + H]^+$ ion of the tris-methyl ester derivative of delta-sleep-inducing peptide (WAGGDASGE).

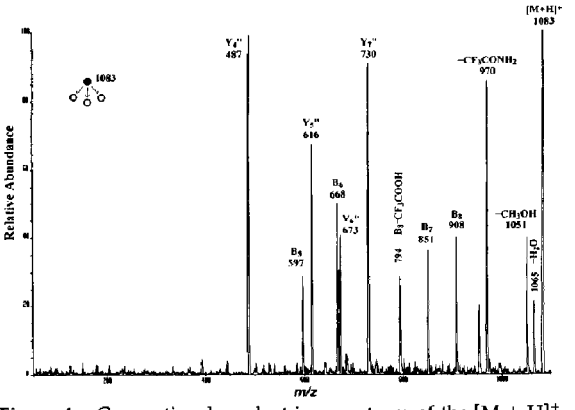


**Figure 4.** Conventional product ion spectrum of the $[M + H]^+$ ion of the tris-methylated, bis-trifluoroacetylated derivative of delta-sleep-inducing peptide (WAGGDASGE).

the dehydration occurs at a single site or at multiple sites. Figure 3, which shows the second-generation product ion spectrum depicting the $[M + H]^+ \rightarrow [M + H - H_2O]^+ \rightarrow$ products reaction sequence for this peptide, is more informative in this regard. The $Y_n''$-series ions from $Y_3''$–$Y_8''$ are present only as the dehydrated species, but $Y_1''$ is not dehydrated ($Y_2''$ is not observed). The N-terminal ions $B_2$, $B_5$, and $B_6$ are not dehydrated, but $B_7$ and $B_8$ have lost water. These data strongly suggest that the serine residue is the site of dehydration, but the spectrum in Figure 3 does not permit distinguishing between the involvement of the serine side-chain hydroxyl and that of the carbonyl contributed to the peptide backbone by the serine residue. Figure 4, which presents the conventional product ion spectrum of the same peptide as the tris-methylated, bis-trifluoroacetylated derivative, facilitates this distinction. Protecting the serine hydroxyl group dramatically reduces the propensity to water loss (compared with other fragmentation pathways; compare Figure 2) and abolishes the loss of water from the B and Y″ ions. Collectively, the data are consistent with the involvement of the serine hydroxyl in the dehydration of the tris-methyl ester derivative of delta-sleep-inducing peptide.

## *Water Loss Involving Peptide Backbone Oxygen*

Conventional product ion spectra of $[^{18}O_2]$bradykinin (1–5) (RPPGF) [5] indicated that this pentapeptide dehydrates primarily through the loss of $H_2{}^{16}O$ despite the presence of a free carboxy terminus. This indicated the involvement of one or more of the oxygens of the peptide backbone in the dehydration of this peptide, which has no side-chain hydroxyls.

Figure 5 presents the second-generation product ion spectrum representing the $[M + H]^+ \rightarrow [M + H - H_2{}^{16}O]^+ \rightarrow$ products sequence for this peptide. The oxygen at either of the four peptide bonds could be

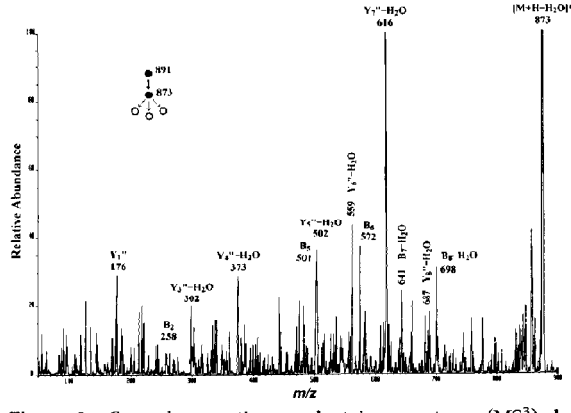


**Figure 3.** Second-generation product ion spectrum ($MS^3$) depicting the $[M + H]^+ \rightarrow [M + H - H_2O]^+ \rightarrow$ products reaction sequence for the tris-methyl ester derivative of delta-sleep-inducing peptide (WAGGDASGE).

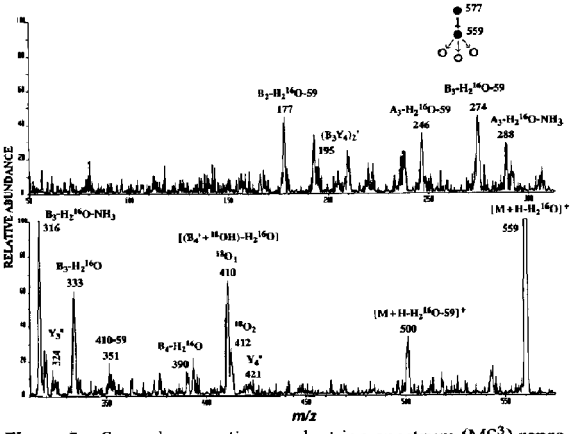


**Figure 5.** Second-generation product ion spectrum ($MS^3$) representing the $[M + H]^+ \rightarrow [M + H - H_2{}^{16}O]^+ \rightarrow$ products reaction sequence for $[^{18}O_2]$bradykinin(1–5) (RPPGF).

involved in this reaction. In the sequential product ion spectrum shown in Figure 5, all of the observed N-terminal ions (A and B series and related ions) are present as the dehydrated species. The intact C-terminal $Y_3''$ and $Y_4''$ ions are (reproducibly) observed, with no corresponding dehydrated species detected. The relative abundance of the $Y_4''$ ion in Figure 5 is dramatically reduced compared with that in the conventional product ion spectrum [5]. The fragmentation pattern in Figure 5 strongly suggests that the primary site of water loss involves the carbonyl oxygen of the peptide bond nearest the amino terminus. Similar spectra for the unlabeled peptide from both the BEqQ hybrid and a four-sector instrument have been published previously and exhibited similar fragmentation patterns [20], as did spectra from the hybrid (not shown) for the methyl ester, DMPO, and DMPO-methyl ester derivatives of this peptide.

An ion of particular note in Figure 5 is that detected at *m/z* 410, corresponding to the dehydrated rearrangement ion $[(B_4' + {}^{18}OH) - H_2{}^{16}O]$. This ion is nominally equivalent to the mono-[$^{18}$O]-labeled form of the $[M + H]^+$ ion of the tetrapeptide RPPG, which has undergone dehydration through the loss of $H_2{}^{16}O$. Figure 6 presents a mass spectrum of this ion as the third-generation product ion spectrum ($MS^4$) representing the $[M + H]^+ \rightarrow [M + H - H_2{}^{16}O]^+ \rightarrow [(B_4' + {}^{18}OH) - H_2{}^{16}O] \rightarrow$ products reaction sequence for [$^{18}O_2$]bradykinin (1–5). This spectrum reinforces the finding that the oxygen of the peptide bond nearest the N-terminus is the primary site of dehydration. The internal cleavage ions $[(B_4Y_3)_2'' + {}^{18}OH]$ and particularly $[(B_4Y_4)_3'' + {}^{18}OH]$ are intact; in contrast, the $A_2$ ion, which includes only the carbonyl of the first peptide bond, is present as the $[A_2 - H_2{}^{16}O]$ species. It should be noted that several of the ions observed in Figure 6 were not detected in either the second-generation product ion spectrum (Figure 5) or the first-generation spectrum [5]. This is an example where the higher order [18] mass spectrometric experiment ($MS^4$) reveals fragmentation pathways that were not evident in the lower order experiments ($MS^3$ and $MS^2$).

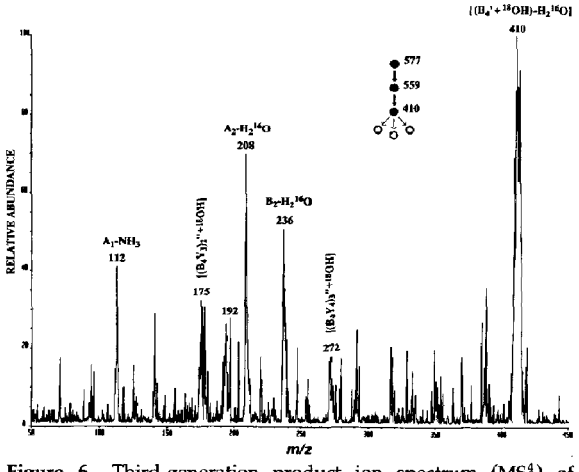


**Figure 6.** Third-generation product ion spectrum ($MS^4$) of [$^{18}O_2$]bradykinin(1–5) (RPPGF), representing the $[M + H]^+ \rightarrow [M + H - H_2{}^{16}O]^+ \rightarrow [(B_4' + {}^{18}OH) - H_2{}^{16}O] \rightarrow$ products reaction sequence.

## *More Than One Pathway in the Same Peptide*

Although the peptides discussed above each dehydrate primarily at a single site, it is certainly possible for peptides to dehydrate at more than one location. This situation is exemplified by leucine–enkephalin (YGGFL). An expanded portion of the conventional product ion spectrum of [$^{18}O_2$]leucine–enkephalin is shown in Figure 7. This peptide exhibits the loss of both $H_2{}^{16}O$ and $H_2{}^{18}O$, indicating that this peptide can dehydrate through at least two major routes; one involves the C-terminus, and the other involves either peptide backbone oxygen or the tyrosine hydroxyl (although the latter possibility is unlikely owing to the aromaticity of the phenolic tyrosine). The structure of each of these dehydrated ions can again be examined through sequential mass spectrometry. Figure 8 presents the second-generation product ion spectrum representing the $[M + H]^+ \rightarrow [M + H - H_2{}^{16}O]^+ \rightarrow$ products sequence for this peptide. Both the intact and the dehydrated forms of the $Y_3''$ ion are present; the $Y_2''$ ion loses ammonia but not water, and the $B_2$, $B_3$, $B_4$, and $Y_4''$ ions are present only in dehydrated form. These data suggest that the backbone oxygen contributed by either the first or the second glycine residue is involved in the loss of $H_2{}^{16}O$ (both reactions occur).

Figure 9 shows the corresponding second-generation product ion spectrum representing the $[M + H]^+ \rightarrow [M + H - H_2{}^{18}O]^+ \rightarrow$ products sequence (i.e., the loss of C-terminal oxygen) for [$^{18}O_2$]leucine–enkephalin. The fragmentation pattern in this spectrum is very different from that in Figure 8, further confirming that the two dehydrated species are structurally distinct. Also in contrast to Figure 8, many of the product ions present in Figure 9 show evidence for the occurrence

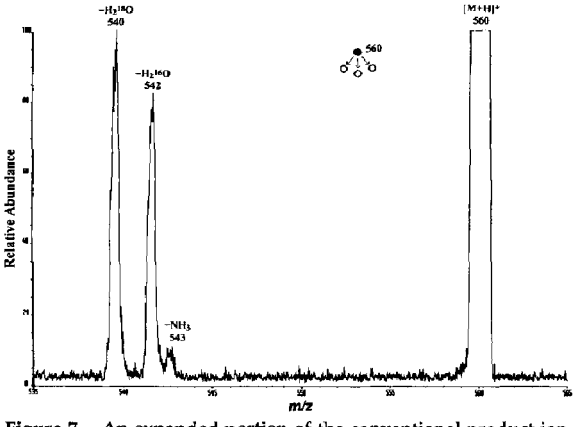


**Figure 7.** An expanded portion of the conventional product ion spectrum of the $[M + H]^+$ ion of [$^{18}O_2$]leucine–enkephalin (YGGFL), showing the region corresponding to the loss of water from the precursor ion.

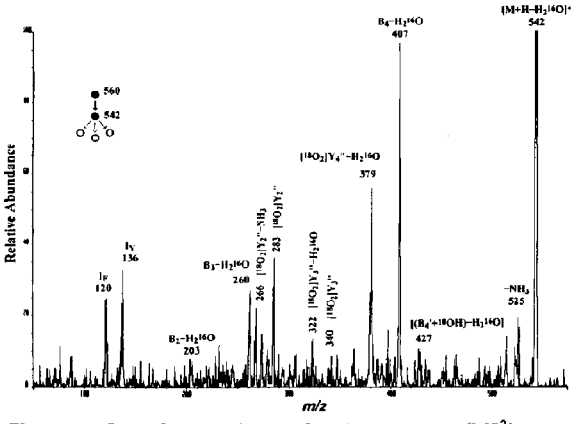


**Figure 8.** Second-generation product ion spectrum ($MS^3$) representing the $[M + H]^+ \rightarrow [M + H - H_2{}^{16}O]^+ \rightarrow$ products reaction sequence for $[^{18}O_2]$leucine–enkephalin (YGGFL).

of intraionic isotope exchange [5]. Extreme examples of this are the loss of the $[^{18}O]$-label from approximately one-fourth of the dehydrated $Y_4''$ ions and retention of an $[^{18}O]$-label in roughly one-half of the $(B_4Y_4)_3$ internal cleavage ions. Signals corresponding to the loss of both labeled and unlabeled carbon monoxide are present. It is clear that the intraionic interactions leading to this isotope exchange are very complex and must necessarily involve the three-dimensional interaction of the C-terminal portion with the rest of the ion. It is not evident from the data, however, whether the different conformations were adopted before or after the dehydration occurred. It is also not clear whether the different conformations involve or are influenced by alternative sites of protonation.

## Conclusions

The dehydration of peptide $[M + H]^+$ ions varied considerably among the peptides studied, involving either the loss of carboxylic acid oxygen, side-chain hydroxyl oxygen, or peptide backbone oxygen. With some examples, only one of these pathways predominated, although more than one of these routes can be active for a single peptide. For the examples where peptide backbone oxygen was lost, the dehydration occurred at one or two primary sites along the backbone, with the location of the site (or sites) varying among the peptides. When C-terminal $^{18}O$ was lost from an $[^{18}O_2]$-labeled peptide, the resulting product ion was strongly predisposed to undergo intraionic isotope exchange. It is clear that complex intraionic interactions occur during the dehydration of peptide $[M + H]^+$ ions. It is likely that the observed differences among the model peptides in the routes of dehydration reflect the particular three-dimensional conformations adopted by each peptide $[M + H]^+$ ion in the gas phase.

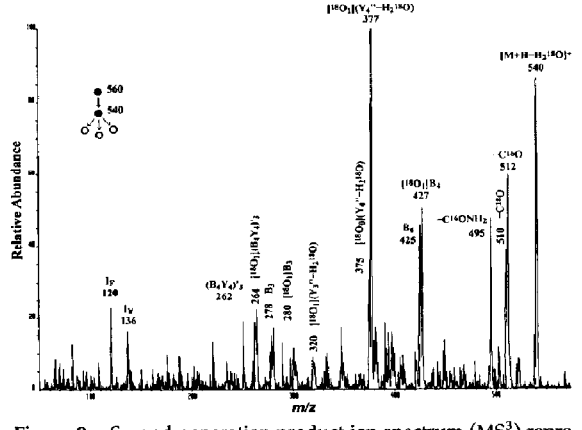


**Figure 9.** Second-generation product ion spectrum ($MS^3$) representing the $[M + H]^+ \rightarrow [M + H - H_2{}^{18}O]^+ \rightarrow$ products reaction sequence for $[^{18}O_2]$leucine–enkephalin (YGGFL).

## Acknowledgment

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## References

1. Murphy, R. C.; Clay, K. L. *Methods Enzymol.* **1990**, *193*, 338–348.
2. Scoble, H. A.; Martin, S. A. *Methods Enzymol.* **1990**, *193*, 519–536.
3. Thorne, G. C.; Gaskell, S. J. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 217–221.
4. Thorne, G. C.; Ballard, K. D.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 249–257.
5. Ballard, K. D.; Gaskell, S. J. *J. Am. Chem. Soc.* **1992**, *114*, 64–71.
6. Russell, D. H.; McGlohon, E. S.; Mallis, L. M. *Anal. Chem.* **1988**, *60*, 1818–1824.
7. Teesch, L. M.; Adams, J. *J. Am. Chem. Soc.* **1990**, *112*, 4110–4120.
8. Teesch, L. M.; Adams, J. *J. Am. Chem. Soc.* **1991**, *113*, 812–820.
9. Leary, J. A.; Zhou, Z.; Ogden, S.; Williams, T. D. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 473–480.
10. Antoine, M.; Adams, J. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 776–778.
11. Hunt, D. F.; Yates, J. R. III; Shabanowitz, J.; Winston, S.; Hauer, C. R. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 6233–6237.
12. Contado, M. J.; Adams, J.; Jenson, N. J.; Gross, M. L. *J. Am. Soc. Mass Spectrom.* **1991**, *2*, 180–183.
13. Wysocki, V. H.; Ross, M. M. *Int. J. Mass Spectrom. Ion Processes* **1991**, *104*, 179–211.
14. Burlet, O.; Yang, C.-Y.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 337–344.
15. Burlet, O.; Orkiszewski, R. S.; Ballard, K. D.; Gaskell, S. J. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 658–662.
16. Tang, X.-J.; Boyd, R. K. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 651–657.
17. Louris, J. N.; Wright, L. G.; Cooks, R. G.; Schoen, A. E. *Anal. Chem.* **1985**, *57*, 2918–2924.
18. Schwartz, J. C.; Wade, A. P.; Enke, C. G.; Cooks, R. G. *Anal. Chem.* **1990**, *62*, 1809–1818.
19. Roepstorff, P.; Fohlman, J. *Biomed. Mass Spectrom.* **1984**, *11*, 601.
20. Ballard, K. D.; Gaskell, S. J.; Jennings, R. C. K.; Scrivens, J. H.; Vickers, R. G. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 553–559.