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# **High Resolution Electrospray Mass Spectrometry with a Magnetic Sector Instrument:** Accurate Mass Measurement and Peptide Sequencing

BY

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Defence Research Establishment Suffield

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Protein Engineering Network of Centres of Excellence
University of Alberta

JANUARY 1995

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## ABSTRACT

The accurate molecular weights for a series of thirty-seven unknown synthetic peptides, used in research studies involving synthetic vaccines, antibacterial peptides or the de novo design of helical peptides and proteins, were determined with a magnetic sector instrument. All data were obtained with external calibration over a wide mass range during magnetic scanning. Errors between observed and theoretical monoisotopic molecular weights were typically in the 5 to 60 ppm range for the unknowns at sector resolutions between 2500 and 9000 (10% valley). Isotopic clusters for charge states up to +10 were resolved through the use of high sector resolution. Collisional activated dissociation in the electrospray interface afforded production of product ions that enabled either full or partial sequencing of most unknown peptides below 2000 Da. The complete primary sequence for one peptide was determined and the importance of high resolution was demonstrated by the differentiation of Lysine from Glutamine, two amino acids differing in residue mass by only 0.0364 Da. Two other peptides, with identical monoisotopic masses, but different primary sequences, were differentiated based on their CAD/MS data.

## INTRODUCTION

The Canadian Forces (CF) may be called on to perform peacekeeping or battlefield operations in regions of the world where there is a significant threat of chemical/biological warfare (CBW) agent use. To operate effectively in these theatres the CF must be able to identify the exact nature of the chemical/biological agent(s). This requirement is being actively addressed at DRES by conducting research and development into new methods for the identification of chemical/biological warfare agents. DRES has adopted a multi-disciplinary approach that includes instrumental analytical techniques, immunological methods and other technologies in order to identify as wide a variety of CB agents as possible.

This research encompasses the detection, identification and confirmation of CW agents, toxins and BW agents of concern. In order to meet these objectives a research effort was initiated with the following general aim:

> **To provide the CF with an independent chemical, toxin and biological agent identification and confirmation capability**

To meet this aim, a strong capability must exist in core identification technologies. This will allow the laboratory to respond quickly and provide the CF with the ability to unambiguously detect and confirm the presence of known and novel chemical, toxin and biological agents in samples suspected to contain these materials.

The principal CB identification research effort focuses on the development of new techniques for the identification and confirmation of toxins and BW agents. Recent advances in biotechnology, including, the isolation and production of peptides using solid-phase synthesis and recombinant DNA-modified microorganisms, has opened up new avenues for the preparation of militarily significant quantities of agents in the "mid-spectrum" between classical chemical and classical biological warfare agents. Mid-spectrum agents, including toxic peptides, proteins and bioregulators, have emerged as a real threat. The current lack of appropriate identification methods makes confirmation of allegations of use of mid-spectrum agents extremely difficult and would likely result in inconclusive evidence. Development of suitable instrumental analytical methods for the identification and confirmation of these threat compounds must be addressed. A number of instrumental analytical technologies including mass spectrometry, liquid chromatography and supercritical fluid extraction, have been targeted as candidate analytical technologies for the identification and confirmation of novel mid-spectrum agents.

The application of mass spectrometry to the analysis of biomolecules of this type has undergone tremendous growth due to the recent development of the electrospray interface, a technique that allows rapid identification and characterization of high molecular weight peptides and proteins. When employed with collisional activated dissociation mass spectrometry (CAD/MS) or tandem mass spectrometry (MS/MS) positive identification is possible for complete unknowns.

Electrospray mass spectrometry will be used at DRES for the identification of both target toxins and novel unknown toxins. The large number of potential toxins precludes the development of individual methods for all the candidate toxins. Therefore, a general approach that will provide maximal structural information and enable identification of toxins in general has been adopted. Briefly, the electrospray mass spectrometry identification strategy follows:

i) Determination of the monoisotopic or average molecular weight for toxic peptides or proteins (tentative identification) and
ii) Determination of the primary sequence, based on residue mass losses, of toxic peptides or proteins (positive identification). Full confirmation would require the comparison of acquired spectrometric data with that obtained for authentic reference material.

In the early 1980's Barber and co-workers revolutionized mass spectrometry by demonstrating the use of fast atom bombardment (FAB) mass spectrometry for the analysis of peptides (1). In the ensuing years, the useful mass range of mass spectrometry for biomolecule applications increased, particularly with the demonstration by Fenn and co-workers that electrospray ionization could be used to form multiply charged gaseous ions from large biomolecules (2,3). Biemann (4) has reviewed the mass spectrometry of peptides and proteins and the current status of biological mass spectrometry was reviewed in 1994 by Burlingame, Boyd and Gaskell (5). Numerous references, reviews and books are cited in these reviews and they serve as a good starting point for researchers interested in assessing the potential of biological mass spectrometry.

The electrospray interface was initially interfaced to a quadrupole mass spectrometer (2,3) and most of the applications in the literature deal with this type of instrumentation. Many users in the mass spectrometry community conduct research with higher resolution instruments, in large part because of the accurate mass measurement capabilities of these instruments. High resolution data has been collected following electrospray introduction of biomolecules into Fourier transform mass spectrometers and this topic was recently reviewed by Buchanan and Hettich (6). Di- or tri- sector geometry mass spectrometers, although not capable of the resolution of Fourier transform mass spectrometers, are more common and have been used extensively for the acquisition of high resolution data.

Development of suitable electrospray interfaces for high resolution sector use (7) was in large part driven by the potential to increase mass measurement accuracy. Use of high resolution enables the assignment of charge state to multiply charged isotope clusters and aids in the interpretation of peptide primary sequence data during collisional activated dissociation in the region between the capillary exit and skimmer in the electrospray interface (8-14). Particularly valuable is the ability to determine monoisotopic molecular weight, as these values are independent of $^{12}C/^{13}C$ variations. High resolution analysis of multiply charged molecular and product isotopic clusters enables immediate assignment of charge state.

Relatively low resolution CAD/MS spectra, yielding characteristic $a_n$- $b_n$- and $y_n$-series ions, have been acquired in the region between the capillary exit and the skimmer region in an electrospray interface at a resolution of 1000 to 1500 (10% valley) for three model peptides (8). Starrett and DiDonato, working at a resolution of 5000 (5% valley) under voltage scanning conditions over a narrow mass range concentrated on the accurate mass measurement of product ions generated during CAD/MS (14). Use of an internal calibrant minimized differences between the theoretical and observed product ion masses to about 5 ppm for several peptides including, angiotensin II and Substance P (1-9). In one case the full primary sequence of a peptide, human renin substrate, was determined with the exception that the Leu (L) and Ile (I) isomers could not be determined (8). The value of high resolution for the assignment of charge state for CAD/MS product ions was demonstrated by Loo's group in a paper focusing on the determination of protein structural information following electrospray introduction (13).

Larsen and McEwen employed resolutions of 5000 and 10000 (10% valley) for accurate molecular weight determination and found that errors seldom exceeded 25 ppm for several pure peptides. Calibration was done internally and the isotopic cluster for the +5 charge state of insulin was resolved (9). Similar ppm errors were reported in a second paper for a number of standard peptides and proteins under lower resolution conditions during average molecular weight determinations. Average molecular weight accuracy was sufficient to allow for the differentiation of a single point modification differing by only 1 Da for biomolecules up to 20000 Da (11).

High resolution separation of a $(M+9H)^{9+}$ isotopic cluster was demonstrated by Cody, Tamura and Musselman for lysozyme at resolution of 10000 (10% valley). Errors associated with these measurements were in the 5 to 20 ppm range when internal calibration was employed (12). Higher errors, typically in the 5 to 90 ppm range, were observed during average molecular weight determinations when external calibration was used over a 10 hour period.

Examples of accurate mass measurement by high resolution electrospray mass spectrometry with a magnetic sector instrument, are limited and all examples dealt with the analysis of pure peptide standards, generally obtained from a commercial supplier. The accuracies reported represent a improvement over quadrupole data, where 100 ppm errors have been reported (15).

We report accurate molecular weight data for a series of thirty-seven unknown synthetic peptides used in research studies involving synthetic vaccines, antibacterial peptides or the de novo design of helical peptides and proteins (16-20). All data were obtained with external calibration over a wide mass range during magnetic scanning. Error between observed and theoretical monoisotopic molecular weights were typically in the 5 to 60 ppm range for the unknowns at sector resolutions between 2500 and 9000 (10% valley) and isotopic clusters for charge states up to +10 were resolved. Electrospray conditions were selected to afford production of product ions that enabled either full or partial sequencing of most unknown peptides below 2000 Da. The complete primary sequence for one peptide was determined and the importance of high resolution was demonstrated by the differentiation of Lys (K) from Gln (Q), two amino acids differing in residue mass by only 0.0364 Da. Two other peptides, with identical monoisotopic masses, but different primary sequences, were differentiated based on their CAD/MS data.

## EXPERIMENTAL

### Samples

Thirty-seven peptides, synthesized by Dr. R. Hodges' Group (member of Protein Engineering Network of Centres of Excellence, University of Alberta, Edmonton), were received as unknowns for analysis by electrospray MS. The peptides, synthesized in support of research studies, were shipped and received at Defence Research Establishment Suffield as frozen aqueous solutions (0.5 mL) in the 0.1 to 1.5 mg/mL range. All samples were kept frozen at -18°C until just prior to electrospray MS analysis, at which time individual samples were either analysed directly or diluted with distilled water to give a concentration of approximately 0.1 mg/mL.

### Instrumental

All electrospray mass spectra were acquired using a VG (Fisons) Autospec-Q mass spectrometer (with EBEQQ geometry) equipped with the recently released VG (Fisons) Mark II electrospray interface. The electrospray needle was operated at 7.8 kV and ions were accelerated into the mass spectrometer at 4 kV. The effect of varying sampling cone voltage (0 to 250 V) was investigated during earlier analyses of bioregulators and toxic peptides in the 500 to 2000 molecular weight range, including bombesin, bradykinins, Substance P and its fragments. A good compromise between molecular weight and fragmentation ion information was found at about 100 V and this voltage was used for all electrospray analyses of the peptide unknowns. Nitrogen (Very Dry, Liquid Carbonic Inc.) bath gas was introduced into the interface (80°C) at a flow rate of 500 L/hr. Nitrogen nebulizer gas was introduced at a flow rate of 14 L/hr. The electrospray interface was pumped with both a rotary and a turbomolecular pump, which enabled maintenance of a $2 \times 10^{-6}$ and $3 \times 10^{-8}$ Torr within the source and analyser regions of the instrument, respectively.

Electrospray data were acquired in the continuum mode by scanning the magnet from 1550 to 400 Da or 1000 to 400 Da exponentially at a scan rate of 25 and 30 sec/decade, respectively. Five to ten scans were averaged to enhance the signal-to-noise ratio and the data were smoothed using VG (Fisons) OPUS software, with a smooth number of 2 and a window of 7. Monoisotopic molecular weights for all but two samples (9 and 30) were calculated in triplicate from $(M+nH)^{n+}$ and/or $(M+nH+mNa)^{(n+m)+}$ ions, where the charge states were determined by the isotopic cluster spacings. Average molecular weights were determined in a similar manner in triplicate for the two higher molecular weight peptide samples, 9 and 30, due to incomplete resolution of the isotope clusters. The following resolutions, based on the 10% valley definition, were employed:

| Peptide Mol. Wt. | Resolution Employed |
|---|---|
| 1000-2000 | 2500 |
| 2000-3000 | 3000 |
| 3000-4000 | 4500 |
| 4000-6000 | 6000 |
| 7000-8000 | 8000 |
| > 8000 | 9000 |

External calibrations were performed prior to sample analysis with a polyethylene glycol (PEG) standard containing both PEG 600 and PEG 1540 (0.5 mg/mL in distilled water).

Loop injections of the samples were made under conditions that simulated those employed during liquid chromatographic separations. An Applied Biosystems dual syringe pump was used to mix and deliver 20 µL/min of a 1:1 mixture of A and B: A, 100% water with 0.05% trifluoroacetic acid and B, 20% water/80% acetonitrile with 0.05% trifluoroacetic acid. Two Rheodyne 8125 sample injectors were placed in series with a 7 µm, 10 cm x 1 mm I.D. Aquapore RP300 column (Applied Biosystems) located after the first injector and prior to the second. The principal advantage of this liquid chromatographic configuration was that it allowed the analyst the flexibility of either sample introduction through the first injector and column or loop injection. Data were acquired following a 50 µL injection of the peptide or calibration sample into the second injector.

Water was distilled in glass and filtered through a 0.45 µm Millipore filter prior to use in the mobile phase or for diluting the provided peptide samples. Acetonitrile was Burdick and Jackson UV grade and trifluoroacetic acid was HPLC Grade (Pierce, USA).

## RESULTS AND DISCUSSION

Thirty-seven synthetic peptides, used in research studies involving synthetic vaccines, antibacterial peptides or the de novo design of helical peptides and proteins (16-20), were provided as unknowns to DRES for electrospray MS analysis. Electrospray MS data provides primarily molecular weight information but when used with higher cone voltage setting or under tandem mass spectrometric (MS/MS) conditions full or partial primary sequence data may be accessed. The acquisition of both accurate molecular weight and primary sequence data for a given peptide enables the analyst to identify the presence of that peptide with a level of certainty that is only exceeded by the analysis of an authentic standard under identical experimental conditions. Given the Canadian Forces requirement to be able to identify toxins or mid-spectrum agents, in suspect samples, electrospray MS and electrospray MS/MS offer a tremendous opportunity for fulfilling this requirement.

The principal objective of this study was the accurate determination of the unknown peptide monoisotopic molecular weights, through the use of high resolution electrospray MS. Secondary to this goal, was the determination of full or partial primary sequence information for the unknown peptides. This general approach to unknown peptide identification typifies the process that would be followed for the identification of known or unknown toxic peptides and as such shapes the capabilities of electrospray MS for the Canadian Forces identification requirement.

Each peptide was analysed under electrospray MS with a cone voltage setting of 100 V. This condition resulted in the formation of the $(M+nH)^{n+}$ series ions for each peptide, in some instances sodium adduct ions, (e.g., $(M+nH+mNa)^{(n+m)+}$) and structurally significant product ions. The resolution was set at a level such that the isotopic cluster ions were well separated. The spacing between ions in each cluster relates directly to charge state of the cluster, where the spacing on the m/z scale in an electrospray mass spectrum for $(M+nH)^{n+}$ ions would be 1/n Da. For example for a triply charged cluster, $(M+3H)^{3+}$, the spacing between each ion will be 1/3 Da. The lowest mass (first) ion, A, in a $(M+nH)^{n+}$ cluster (containing only $^{12}C$), may then be used to calculate the peptide's monoisotopic molecular weight, M, using the following equation:

$$M = n\,(A - 1.007825)$$

where 1.007825 is the mass of hydrogen

Table I contains the observed monoisotopic molecular weight data for all but two of the unknown peptides analysed and compares the acquired data with the theoretical values. Average molecular weight data were calculated for these two peptides, as the higher molecular weights of these compounds resulted in incomplete resolution of the isotopic clusters. Monoisotopic molecular weights were calculated from three different ion clusters in the electrospray mass spectrum, to indicate the degree of precision that could be expected during typical analyses and all analyses were externally calibrated. With the exception of peptide samples 14, 17, 19, 20 and 21 the accuracies, indicated in the difference column, were typically in the 5 to 60 ppm range.

Following submission of the observed monoisotopic molecular weight data, the University of Alberta provided DRES with the peptide primary sequences and theoretical monoisotopic molecular weight data (Appendix II). Subsequent inspection of the raw electrospray MS data indicated that the five peptide samples 14, 17, 19, 20 and 21 were all acquired in the same data set, using the same external calibration file. No other peptide samples were acquired in this data set. Review of the raw data indicated more variability in the acquired data for the PEG standard, likely due to minor magnetic scanning fluctuations during the course of acquisition. This resulted in a poorer than expected calibration for these five peptides and all monoisotopic molecular weight differences were about 0.2 Da (80 to 140 ppm) above theoretical values. Following the study one of the these peptides, 21, was selected for re-analysis with a resolution of 2500. External calibration was performed and the monoisotopic molecular weight differences for both analyses were consistent with the 5 to 60 ppm range stated above. Increased accuracy would be possible if internal calibration under voltage scanning conditions were employed (14). However, this means of calibration increases analysis times, making it less suitable for routine screening of unknowns over a large mass scale.

Partial or full primary sequencing was possible for most of the unknown peptides with molecular weights below 2000 Da. The structurally significant product ions observed were the result of collisional activated dissociation (CAD) in the electrospray interface. Selection of a relatively high voltage difference of 100 V, increased the residence time for the multiply charged ions formed during the electrospray process. This increased residence time resulted in increased fragmentation, due to more opportunities for collision, or near collision, with neutrals in this region. Characteristic ions of the $b_n$- or $y_n$- series type were generally detected.

For example, peptide unknown 27 (Figure 1, Table II) could be completely sequenced based on the acquired CAD/MS data acquired with the exception that the Leu (L) and Ile (I) isomers could not be distinguished. Prior to receiving the sequence data it was possible to assign all the amino acids in the primary sequence, but some uncertainty as to the nature of the N-terminal blocking group existed. The benzoyl benzoyl (BB) could only be confidently assigned following receipt of the actual primary sequence data.

Figure 1 illustrates the electrospray MS acquired under cone voltage conditions that facilitated production of diagnostic product ions. Overlap of the $b_n$- and $y_n$- series singly charged fragmentation ions enabled identification of this unknown as a peptide with the following primary sequence, BB-GGDEQFIPK-$NH_2$. High resolution enabled confident assignment of the charge state of all the ions in the electrospray mass spectrum, based on the isotopic cluster spacings. The residue masses for the amino acids (Appendix I) was sufficiently accurate to distinguish between Lys (K) and Gln (Q), two amino acids that differ by only 0.0364 Da. This finding was confirmed in both the $b_n$- and $y_n$- series ions, and both Gln (Q) and Lys (K) were correctly assigned. The alternate possibility in each case, given in parenthesis in Table II, exceeds by a factor of three to four the typical amino acid residue mass measurement errors (0.008 ± 0.006 Da, n=11) obtained during analysis of this peptide sample.

Two of the unknown peptides, 5 and 6, exhibited within experimental uncertainty the same monoisotopic molecular weight (1682.06 and 1682.04 Da, respectively). The two peptides differ only in the relative positions of the amino acids, Glu (E) and Ala (A) at the third and fourth, and tenth and eleventh amino acid positions, where the first position refers to the N-terminal amino acid. Both peptides could be differentiated, based on the interpretation of the multiply charged $y_n^{2+}$ and $y_n^{3+}$ series ions observed (Figure 2, Table III). The charge state of the characteristic series ions could be confidently assigned, based on the charge spacing (1/2 Da or 1/3 Da) between the isotopic cluster ions for each. In both cases the first four amino acids in the sequence, beginning from the N-terminal were confirmed at a resolution of 2500. Errors were consistent with the previous example, where the difference between the observed and theoretical amino acid residue losses were 0.009±0.004 Da (n=6) and 0.005±0.004 Da (n=6) for peptide samples 5 and 6, respectively.

Figures 3, 4, 5, 6 and 7 illustrate the utility of high resolution electrospray MS for assigning the charge state of molecular ion isotopic clusters. Figure 3 illustrates the electrospray MS data obtained for peptide sample 7 at a resolution of 2500, sufficient for the assignment of the charge state of all ion clusters in this mass spectrum. Ions with 1/2 Da spacing were observed for the $(M+2H)^{2+}$ isotopic cluster and an impurity was postulated based on the observation of a second $(M+2H)^{2+}$ cluster (and its sodium adducts) at m/z 656.916. The mass difference between the principal component, 7, and the impurity was 128.098 Da. Lys (K) has a monoisotopic mass of 128.09496 and this residue occupies several positions in the sequence in a repeating manner. It seems probable, based on the high resolution evidence, that during peptide synthesis one Lys (K) was not incorporated in the sequence. Further LC-MS experiments at the University of Alberta indicated that the impurity coelutes with the analyte.

Increased resolution is required with increasing mass to resolve the isotopic clusters formed during the electrospray process. Figures 4, 5, 6 and 7 illustrate the electrospray mass spectra for four peptides, ranging from 3500 to 7500 Da, at resolutions of 4500, 6000, 6000 and 8000, respectively. Resolution of $(M+nH)^{n+}$ cluster ions differing in mass by 1/4, 1/5, 1/8 and 1/10 Da were possible and have been expanded and illustrated below the full electrospray mass spectra obtained for each of the four examples. Determination of charge state may be made based solely on the spacing, or by simply counting the number of ions over a 1 Da range in the expanded portions of the electrospray mass spectra.

The resolution of the mass spectrometer was always set with a PEG ion in the 700 Da range to a value above that theoretically required to produce a 10% valley. However, particularly in the case of the $(M+8H)^{8+}$ (Figure 6b) and $(M+10H)^{10+}$ (Figure 7b) isotopic clusters the observed valley approached 50%. With increased resolution, the transmission decreases such that a transmission of 10% is typically observed at 9000 to 10000 resolution. The reduced signal, relative to the background, could be a contributing factor to the observation of a higher than expected valley between ions. Alternately slight energy spreading during the electrospray process may be leading to some ion broadening. Finally the fact that several weaker electrospray spectra were summed could lead to some unanticipated ion broadening. From an empirical standpoint, about 25% more resolution, than calculated by the software for a PEG ion, would be required to resolve isotopic clusters to the 10% valley definition.

The monoisotopic molecular weights of two peptides, above 8000 Da, could not be confidently assigned and for this reason average molecular weight data under high resolution conditions were reported for both peptide samples 9 and 30. This was in large part due to the fact that with increasing molecular weight the relative intensity of the ion used for the monoisotopic molecular ion calculations (containing only $^{12}C$) decreases until it is not easily resolved from the background. For example the relative intensity of the ion used for this purpose for peptide sample 32 (monoisotopic molecular weight, 7368.36), at m/z 737.814 exhibited a relative intensity of about 10% (refer to Figure 7b). This compares to 100% relative intensity at lower molecular weights, (e.g., 1439.92 Da, Figure 3b). This fact along with reduced transmission, with increasing resolution, made confident assignment of these ions for peptides above 8000 Da impossible under the experimental conditions employed.

Figure 8 illustrates the electrospray mass spectrum obtained for the highest mass synthetic peptide. Ions with up to 16 charges were detected and the charge state for the ions in this mass spectrum were determined by solving two simultaneous equations and checking that all the observed $(M+nH)^{n+}$ were consistent with the calculated average molecular weight. Errors between the theoretical and observed average molecular weight were within the 5 to 60 ppm window generally observed for externally calibrated monoisotopic molecular weight data, where the average molecular weight was calculated by centroiding the mass envelope. The envelop apex was selected and, although slightly skewed, was approximately gaussian. More accurate means of centroid assignment would likely improve the accuracy of this measurement.

## CONCLUSIONS

The accurate molecular weights for a series of thirty-seven unknown synthetic peptides, used in research studies involving synthetic vaccines, antibacterial peptides, or the de novo design of helical peptides and proteins, were determined with a magnetic sector instrument. All data were obtained with external calibration over a wide mass range during magnetic scanning. Errors between observed and theoretical monoisotopic molecular weights were typically in the 5 to 60 ppm range for the unknowns at sector resolutions between 2500 and 9000 (10% valley). Isotopic clusters for charge states up to +10 were resolved through the use of high sector resolution. Collisional activated dissociation in the electrospray interface afforded production of product ions that enabled either full or partial sequencing of most unknown peptides below 2000 Da. The complete primary sequence for one peptide was determined and the value of high resolution was demonstrated by the differentiation of Lys (K) from Gln (Q), two amino acids differing in residue mass by only 0.0364 Da. Two other peptides, with identical monoisotopic masses, but different primary sequences, were differentiated based on their CAD/MS data.

The molecular weight range of the unknown peptides studied were representative of peptides that might be collected during intelligence gathering by the Canadian Forces. High resolution electrospray mass spectrometry enabled the determination of unknown peptide molecular weight and full or partial sequence information were obtained for lower molecular weight peptides. This represents a significant step towards achieving the level of identification currently available for classical chemical warfare agents and demonstrates the usefulness of electrospray MS for the future identification of toxic peptides and proteins contained in the CB Threat Spectrum.

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## REFERENCES

1. Barber, M., Bordoli, R.S., Sedgwick, R.D. and Tyler, A.N., J. Chem. Soc. Chem. Commun., 325, 1981.

2. Meng, C.K., Mann, M. and Fenn, J.B., Z. Phys. D, **10**, 361, 1988.

3. Fenn, J.B., Mann, M., Meng, C.K., Wong, S.F. and Whitehouse, C.M., Science, **246**, 64, 1989.

4. Biemann, K., Annu. Rev. Biochem., **61**, 977, 1992.

5. Burlingame, A.L., Boyd, R.K. and Gaskell, S.J., Anal. Chem., **66**, 634R, 1994.

6. Buchanan, M.V. and Hettich, R.L., Anal. Chem., **65**, 245A, 1993.

7. Meng, C.K., McEwen, C.N. and Larsen, B.S., Rapid Commun. Mass Spectrom., **4**, 147, 1990.

8. Meng, C.K., McEwen, C.N. and Larsen, B.S., Rapid Commun. Mass Spectrom., **4**, 151, 1990.

9. Larsen, B.S. and McEwen, C.N., J. Am. Soc. Mass Spectrom., **2**, 205, 1991.

10. Chapman, J.R., Gallagher, R.T., Barton, E.C., Curtis, J.M. and Derrick, P.J., Org. Mass Spectrom., **27**, 195, 1992.

11. McEwen, C.N. and Larsen, B.S., Rapid Commun. Mass Spectrom., **6**, 173, 1992.

12. Cody, R.B., Tamura, J. and Musselman, B.D., Anal. Chem., **64**, 1561, 1992.

13. Loo, J.A., Ogorzalek Loo, R.R. and Andrews, P.C., Org. Mass Spectrom., **28**, 1640, 1993.

14. Starrett, A.M. and DiDonato, G.C., Rapid Commun. Mass Spectrom., **7**, 12, 1993.

15. Covey, T.R., Bonner, R.F., Shushan, B.I. and Henion, J., Rapid Commun. Mass Spectrom., **2**, 249, 1988.

16. Wong, W.Y., Irvin, R.T., Paranchych, W. and Hodges, R.S., Protein Science, **1**, 1308, 1992.

17. Sereda, T.J., Mant, C.T., Sonnichsen, F.D. and Hodges, R.S., J. Chromatogr. A, **676**, 139, 1994.

18. Zhou, N.E., Kay, C.M. and Hodges, R.S., J. Molecular Biology, **237**, 500, 1994.

19. Wishart, D.S., Kondejewski, L.H., Semchuk, P.D., Kay, C.M., Hodges, R.S. and Sykes, B.D., In: "Techniques in Protein Chemistry V", (J.W. Crabb, Ed.), American Press Inc. (in press).

20. Kohn, W.D., Kay, C.M. and Hodges, R.S., Protein Science, (in press).

Table I: Peptide Sample Molecular Weights

| Sample # | Theoretical Monoisotopic Mol. Wt. | Observed Monoisotopic Mol. Wt. Mean ± S.D. (n=3) | Difference Da (ppm) |
|---|---|---|---|
| 1 | 3708.95 | 3708.99 ± 0.02 | +0.04 (11) |
| 3 | 3708.95 | 3708.97 ± 0.07 | +0.02 (5) |
| 4 | 1639.95 | 1639.99 ± 0.02 | +0.04 (24) |
| 5 | 1682.00 | 1682.06 ± 0.01 | +0.06 (36) |
| 6 | 1682.00 | 1682.04 ± 0.01 | +0.04 (24) |
| 7 | 1439.87 | 1439.92 ± 0.01 | +0.05 (35) |
| 8 | 3708.95 | 3709.01 ± 0.03 | +0.06 (16) |
| 9 | 8353.29[a] | 8352.78 ± 0.15[a] | -0.51 (6) |
| 11 | 2280.37 | 2280.34 ± 0.04 | -0.03 (13) |
| 12 | 2266.35 | 2266.34 ± 0.03 | -0.01 (4) |
| 14 | 1767.72 | 1767.86 ± 0.02 | +0.14 (79) |
| 15 | 2255.28 | 2255.26 ± 0.04 | -0.02 (9) |
| 16 | 2315.34 | 2315.32 ± 0.04 | -0.02 (9) |
| 17 | 1970.95 | 1971.14 ± 0.01 | +0.19 (96) |
| 18 | 2338.35 | 2338.34 ± 0.03 | -0.01 (4) |
| 19 | 1881.77 | 1881.98 ± 0.03 | +0.21 (112) |
| 20 | 1168.74 | 1168.90 ± 0.01 | +0.16 (137) |
| 21 | 1186.75 | 1186.90 ± 0.01 | +0.15 (126) |
| 22 | 1824.71 | 1824.79 ± 0.02 | +0.08 (44) |
| 23 | 1851.76 | 1851.85 ± 0.02 | +0.09 (49) |
| 24 | 1855.75 | 1855.87 ± 0.02 | +0.12 (65) |
| 25 | 1838.76 | 1838.87 ± 0.02 | +0.11 (60) |
| 26 | 1781.00 | 1781.00 ± 0.02 | 0.00 (0.3) |

| Sample # | Theoretical Monoisotopic Mol. Wt. | Observed Monoisotopic Mol. Wt. Mean ± S.D. (n=3) | Difference Da (ppm) |
|---|---|---|---|
| 27 | 1196.55 | 1196.55 ± 0.02 | 0.00 (0.3) |
| 28 | 4162.24 | 4162.30 ± 0.02 | +0.06 (15) |
| 29 | 5883.22 | 5883.44 ± 0.12 | +0.22 (37) |
| 30 | 11327.00[a] | 11326.33 ± 0.15[a] | -0.67 (59) |
| 31 | 3704.21 | 3704.24 ± 0.03 | +0.03 (8) |
| 32 | 7368.17 | 7368.36 ± 0.08 | +0.19 (26) |
| 33 | 7379.98 | 7380.27 ± 0.07 | +0.29 (39) |
| 34 | 7379.98 | 7380.16 ± 0.22 | +0.18 (24) |
| 35 | 3692.97 | 3693.04 ± 0.03 | +0.07 (19) |
| 36 | 7372.11 | 7372.45 ± 0.05 | +0.34 (46) |
| 37 | 7376.04 | 7376.36 ± 0.13 | +0.32 (43) |
| 38 | 7372.11 | 7372.26 ± 0.08 | +0.15 (20) |
| 39 | 3676.00 | 3676.04 ± 0.01 | +0.04 (11) |
| 40 | 7349.98 | 7350.17 ± 0.06 | +0.19 (26) |

[a] average molecular weight

Table II: Sequence Determination for Peptide Sample #27

| Series | m/z | Amino Acid Residue | Theoretical Residue Mass | Observed Residue Mass | Difference Da |
|---|---|---|---|---|---|
| | | | | | |
| $b_8$ | 1052.4226 | Lys + $NH_3$<br>(Gln + $NH_3$) | 145.1215<br>(145.0851) | 145.1190<br>(145.1190) | -0.0025<br>(+0.0339) |
| $b_7$ | 955.3712 | Pro | 97.0528 | 97.0514 | -0.0014 |
| $b_6$ | 842.2960 | Ile (Leu) | 113.0805 | 113.0752 | -0.0089 |
| $b_5$ | 695.2364 | Phe | 147.0684 | 147.0596 | -0.0088 |
| $b_4$ | 567.1774 | Gln<br>(Lys) | 128.0586<br>(128.0950) | 128.0590<br>(128.0590) | +0.0004<br>(-0.0360) |
| $b_3$ | 438.1435 | Glu | 129.0426 | 129.0339 | -0.0087 |
| | | | | | |
| $y_9$ | 989.5035 | | | | |
| $y_8$ | 932.4641 | Gly | 57.0215 | 57.0394 | +0.0179 |
| $y_7$ | 875.4632 | Gly | 57.0215 | 57.0009 | -0.0206 |
| $y_6$ | 760.4436 | Asp | 115.0269 | 115.0196 | -0.0073 |
| $y_5$ | 631.4058 | Glu | 129.0426 | 129.0378 | -0.0048 |
| $y_4$ | 503.3389 | Gln<br>(Lys) | 128.0586<br>(128.0950) | 128.0669<br>(128.0669) | +0.0083<br>(-0.0281) |
| | | | | | |

Peptide Sequence: BB-Gly-Gly-Asp-Glu-Gln-Phe-Ile-Pro-Lys-$NH_2$
BB (Benzoyl Benzoyl crosslinking group)

Table III: Series Ions for Peptide Sample a) #5 and b) #6

| Series | m/z | Amino Acid Residue | Theoretical Residue Mass | Observed Residue Mass | Difference Da |
|---|---|---|---|---|---|
| a) #5 | | | | | |
| $y_{13}^{2+}$ | 756.5055 | Glu + $C_2H_2O$ | 85.5266 | 85.5316 | +0.0050 |
| $y_{12}^{2+}$ | 699.9693 | Ile (Leu) | 56.5420 | 56.5362 | -0.0058 |
| $y_{11}^{2+}$ | 635.4377 | Glu | 64.5213 | 64.5316 | +0.0103 |
| $y_{10}^{2+}$ | 599.9374 | Ala | 35.5186 | 35.5003 | -0.0183 |
| $y_{13}^{3+}$ | 504.6703 | Glu + $C_2H_2O$ | 57.0177 | 57.0256 | +0.0079 |
| $y_{12}^{3+}$ | 466.9676 | Ile (Leu) | 37.6947 | 37.7027 | +0.0080 |
| | | | | | |
| b) #6 | | | | | |
| $y_{13}^{2+}$ | 756.5055 | Glu + $C_2H_2O$ | 85.5266 | 85.5316 | +0.0050 |
| $y_{12}^{2+}$ | 699.9554 | Ile (Leu) | 56.5420 | 56.5501 | +0.0081 |
| $y_{11}^{2+}$ | 664.4480 | Ala | 35.5186 | 35.5074 | -0.0112 |
| $y_{10}^{2+}$ | 599.9254 | Glu | 64.5213 | 64.5226 | +0.0013 |
| $y_{13}^{3+}$ | 504.6703 | Glu + $C_2H_2O$ | 57.0177 | 57.0144 | -0.0033 |
| $y_{12}^{3+}$ | 466.9790 | Ile (Leu) | 37.6947 | 37.6913 | -0.0034 |
| | | | | | |

Peptide Sequence: #5 Ac-EIEALKKEIEALKK-$NH_2$
#6 Ac-EIAELKKEIAELKK-$NH_2$

Appendix I: Amino Acid Abbreviations and Residue Masses

| Amino Acid Name | Three Letter Code | One Letter Code | Monoisotopic Residue Mass | Average Residue Mass |
|---|---|---|---|---|
| | | | | |
| Alanine | Ala | A | 71.03711 | 71.0788 |
| Arginine | Arg | R | 156.10111 | 156.1876 |
| Asparagine | Asn | N | 114.04293 | 114.1039 |
| Aspartic Acid | Asp | D | 115.02694 | 115.0886 |
| Cysteine | Cys | C | 103.00919 | 103.1448 |
| Glutamic Acid | Glu | E | 129.04259 | 129.1155 |
| Glutamine | Gln | Q | 128.05858 | 128.1308 |
| Glycine | Gly | G | 57.02146 | 57.0520 |
| Histidine | His | H | 137.05891 | 137.1412 |
| Isoleucine | Ile | I | 113.08406 | 113.1595 |
| Leucine | Leu | L | 113.08406 | 113.1595 |
| Lysine | Lys | K | 128.09496 | 128.1742 |
| Methionine | Met | M | 131.04049 | 131.1986 |
| Phenylalanine | Phe | F | 147.06841 | 147.1766 |
| Proline | Pro | P | 97.05276 | 97.1167 |
| Serine | Ser | S | 87.03203 | 87.0782 |
| Threonine | Thr | T | 101.04768 | 101.1051 |
| Tryptophan | Trp | W | 186.07931 | 186.2133 |
| Tyrosine | Tyr | Y | 163.06333 | 163.1760 |
| Valine | Val | V | 99.06841 | 99.1326 |

Appendix II: Peptide Samples Received from University of Alberta

| Peptide Number | Primary Sequence | Monoisotopic Molecular Weight | Average Molecular Weight |
|---|---|---|---|
| | | | |
| 1 | Ac-KCEALEGKLGAVEEKLGAV EEKLGAVEEKLEALEG-$NH_2$ | 3708.9494 | 3711.2400 |
| 3 | Ac-KCEALEGKLEEVGAKLEEV GAKLEEVGAKLEALEG-$NH_2$ | 3708.9494 | 3711.2400 |
| 4 | Ac-EAEALKKEIEALKK-$NH_2$ | 1639.9508 | 1640.9410 |
| 5 | Ac-EIEALKKEIEALKK-$NH_2$ | 1681.9978 | 1683.0216 |
| 6 | Ac-EIAELKKEIAELKK-$NH_2$ | 1681.9978 | 1683.0216 |
| 7 | Ac-EALKKEIEALKK-$NH_2$ | 1439.8711 | 1440.7467 |
| 8 | Ac-KCGALEEKLGALEEKAGAL EEKLGALEEKLGALEE-$NH_2$ | 3708.9494 | 3711.2398 |
| 9 | (Ac-EIETLRAEYEALKHEVESLR DEIEFLKAEVEACRS-$NH_2$ $)_2$<br>Dimer with C-C bridge | 8348.1532 | 8353.2904 |
| 11 | Ac-ELEKLLKELEKLLKEKEK-$NH_2$ | 2280.3668 | 2281.7620 |
| 12 | Ac-ELEKLLKEQEKLLKELEK-$NH_2$ | 2266.3511 | 2267.7351 |
| 14 | Ac-SCATTVDAAFRPNGCTD<br>Internal C-C bridge | 1767.7193 | 1768.8906 |
| 15 | Ac-ELEKLLKECEKLLKELEK-$NH_2$ | 2255.2810 | 2256.7267 |
| 16 | Ac-ELEKLLKEYEKLLKELEK-$NH_2$ | 2315.3351 | 2316.7639 |
| 17 | Ac-EEAQAQAEAEAKAKAEEK-$NH_2$ | 1970.9545 | 1972.0963 |

| Peptide Number | Primary Sequence | Monoisotopic Molecular Weight | Average Molecular Weight |
|---|---|---|---|
| 18 | Ac-ELEKLLKEWEKLLKELEK-$NH_2$ | 2338.3511 | 2339.8011 |
| 19 | Ac-ACKSTQDPMFTPKGCDN<br>Internal C-C bridge | 1881.7696 | 1883.0960 |
| 20 | FPVKLFPVKL (ring) | 1168.7372 | 1169.5188 |
| 21 | FPVKLFPVKL | 1186.7478 | 1187.5341 |
| 22 | Ac-ACASTQDPMFTPKGCDN<br>Internal C-C bridge | 1824.7117 | 1826.0007 |
| 23 | Ac-ACKSTQDPMFAPKGCDN<br>Internal C-C bridge | 1851.7590 | 1853.0697 |
| 24 | Ac-ACKSTQDPMFTAKGCDN<br>Internal C-C bridge | 1855.7539 | 1857.0581 |
| 25 | Ac-ACKSTQDPMFTPKGCDA<br>Internal C-C bridge | 1838.7638 | 1840.0710 |
| 26 | ATKKEVPLGVAADANKLG | 1781.0046 | 1782.0703 |
| 27 | BB-GGDEQFIPK-$NH_2$ | 1196.5502 | 1197.3131 |
| 28 | Ac-EIEALKAEIEALKAGGDEQFIPKGGEIEALKAEIEALKA-$NH_2$ | 4162.2412 | 4164.7632 |
| 29 | ATKKEVPLGVAADANKLGEIEALKAEIEALKAGGDEQFIPKGGEIEALKAEIEALKA-$NH_2$ | 5883.2247 | 5886.7809 |
| 30 | (Ac-EIEALKAEIEALKAEIEALKAGGDEQFIPKGGEIEALKAEIEALKAEIEACKA-$NH_2$)$_2$<br>Dimer with C-C bridge | 11320.0070 | 11326.9950 |
| 31 | Ac-KCGALEKKLGALEKKAGALEKKLGALEKKLGALEK-$NH_2$ | 3704.2113 | 3706.5328 |
| 32 | (Ac-QCGALQKQVGALQKQVGALQKQVGALQKQVGALQK-$NH_2$)$_2$<br>Dimer with C-C bridge | 7368.1718 | 7372.7146 |

| Peptide Number | Primary Sequence | Monoisotopic Molecular Weight | Average Molecular Weight |
|---|---|---|---|
| 33 | (Ac-ECGALQKQVGALEKEVGA LQKQVGALEKEVGALEK-$NH_2$)$_2$ Dimer with C-C bridge | 7379.9800 | 7384.5322 |
| 34 | (Ac-ECGALEKEVGALEKEVGA LEKQVGALQKQVGALQK-$NH_2$ )$_2$ Dimer with C-C bridge | 7379.9800 | 7384.5322 |
| 35 | Ac-ECGALEKEVGALEKEVGAL EKEVGALEKQVGALQK-$NH_2$ | 3692.9658 | 3695.2436 |
| 36 | (Ac-ECGALEKQVGALQKQVGA LQKQVGALQKQVGALQK-$NH_2$)$_2$ Dimer with C-C bridge | 7372.1078 | 7376.6538 |
| 37 | (Ac-ECGALEKEVGALEKQVGA LQKQVGALQKQVGALQK-$NH_2$)$_2$ Dimer with C-C bridge | 7376.0438 | 7380.5930 |
| 38 | (Ac-QCGALQKQVGALQKEVGA LEKQVGALQKQVGALQK-$NH_2$ )$_2$ Dimer with C-C bridge | 7372.1078 | 7376.6538 |
| 39 | Ac-QVGALKEKVGALQEQVGA LKEKVGALQEQVGACKE-$NH_2$ | 3675.9981 | 3678.2624 |
| 40 | (Ac-QVGALEKEVGALQKQVGA LEKEVGALQKQVGACEK-$NH_2$)$_2$ Dimer with C-C bridge | 7349.9806 | 7354.5090 |

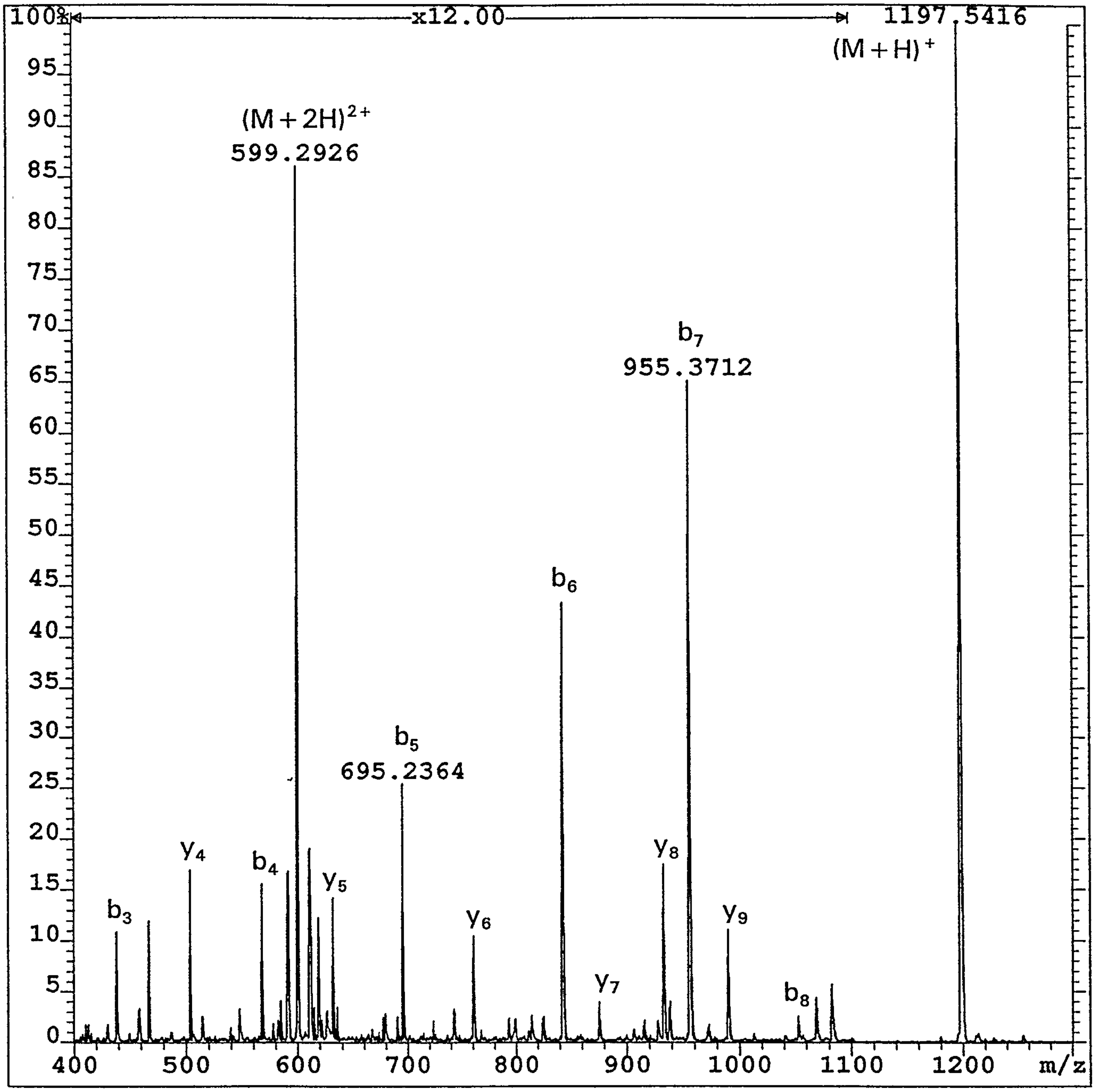

Figure 1: Electrospray mass spectrum of peptide sample #27 at 2500 resolution. Interpretation of the $b_n$- and $y_n$-series ions enabled complete sequencing of this unknown (Refer to Table II).

Observed Monoisotopic Mol. Wt.: 1196.55±0.02,
Sequence: BB-GGDEQFIPK-$NH_2$

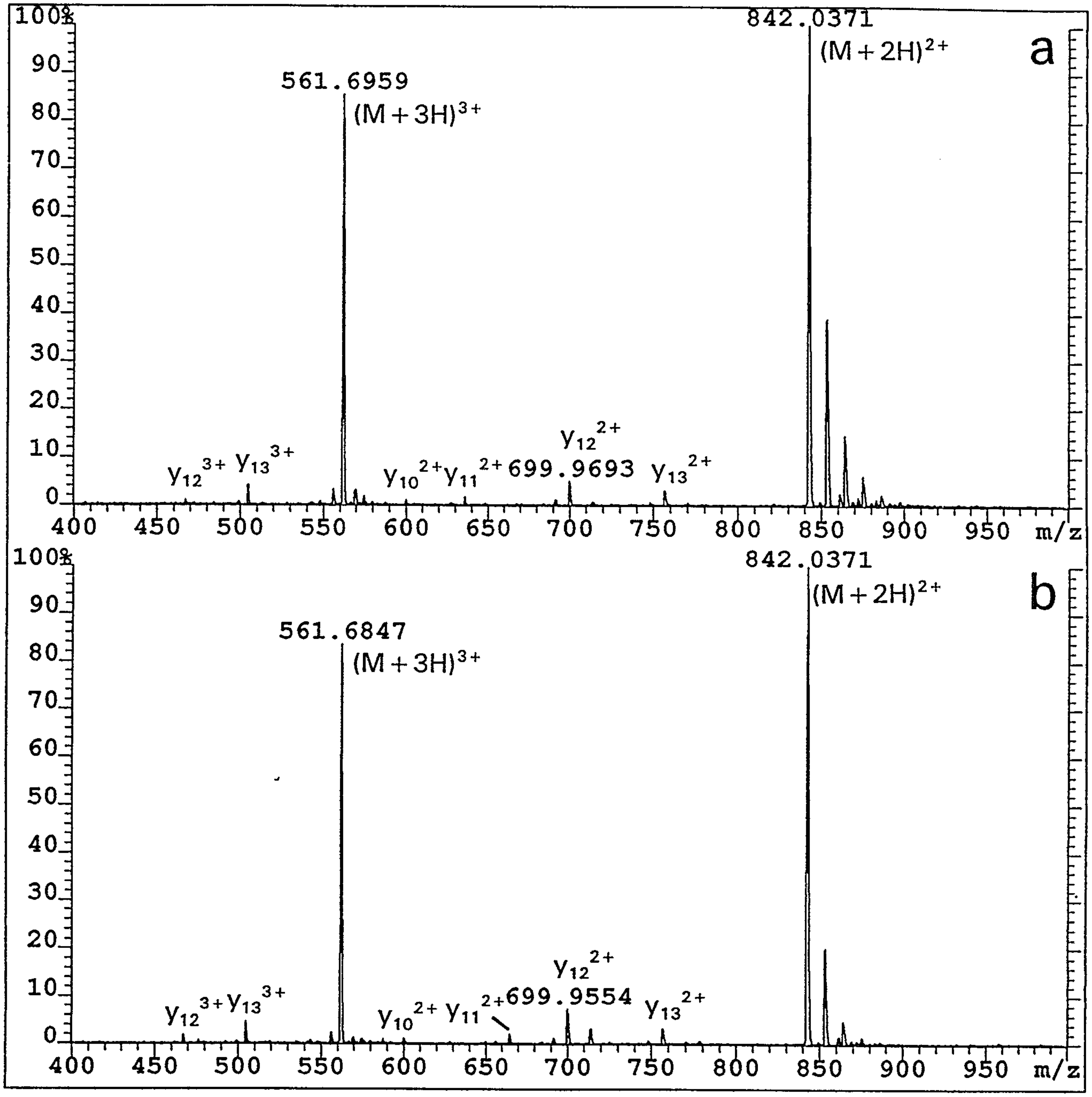

Figure 2: Electrospray mass spectra of peptide samples a) #5 and b) #6 at 2500 resolution. Interpretation of the $y_n^{n+}$-series ions enabled differentiation of the two peptides with identical monoisotopic molecular weights (Refer to Table III).

#5 Observed Monoisotopic Mol. Wt.: 1682.06±0.01, Sequence: Ac-EIEALKKEIEALKK-$NH_2$
#6 Observed Monoisotopic Mol. Wt.: 1682.04±0.01, Sequence: Ac-EIAELKKEIAELKK-$NH_2$

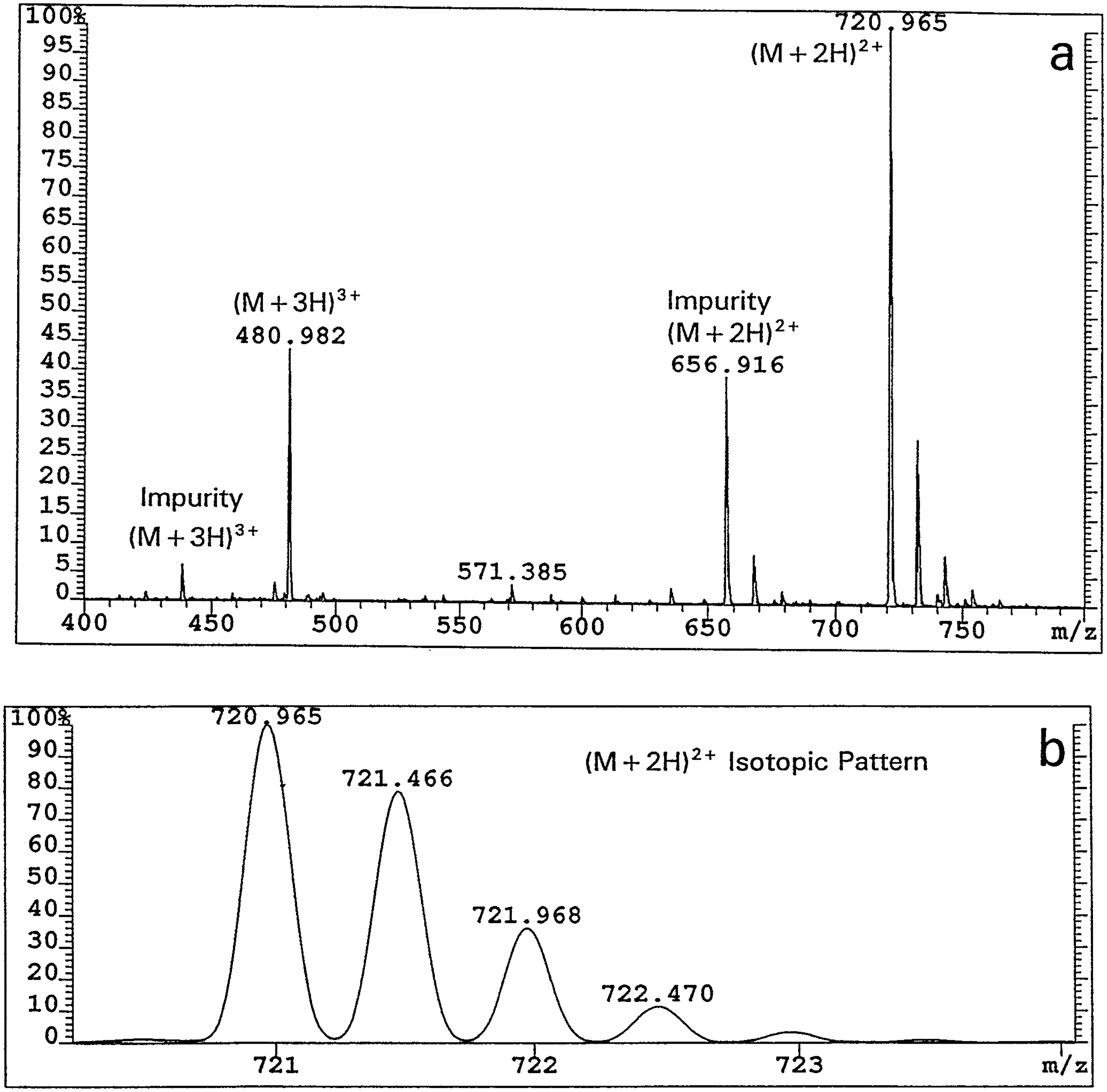

Figure 3: a) Electrospray mass spectrum of peptide sample #7 at 2500 resolution (note impurity at m/z 656.916). b) (M+2H)$^{2+}$ Isotopic pattern illustrating resolution of ions differing by 1/2 u.

Observed Monoisotopic Mol. Wt.: 1439.92±0.01,
Sequence: Ac-EALKKEIEALKK-$NH_2$

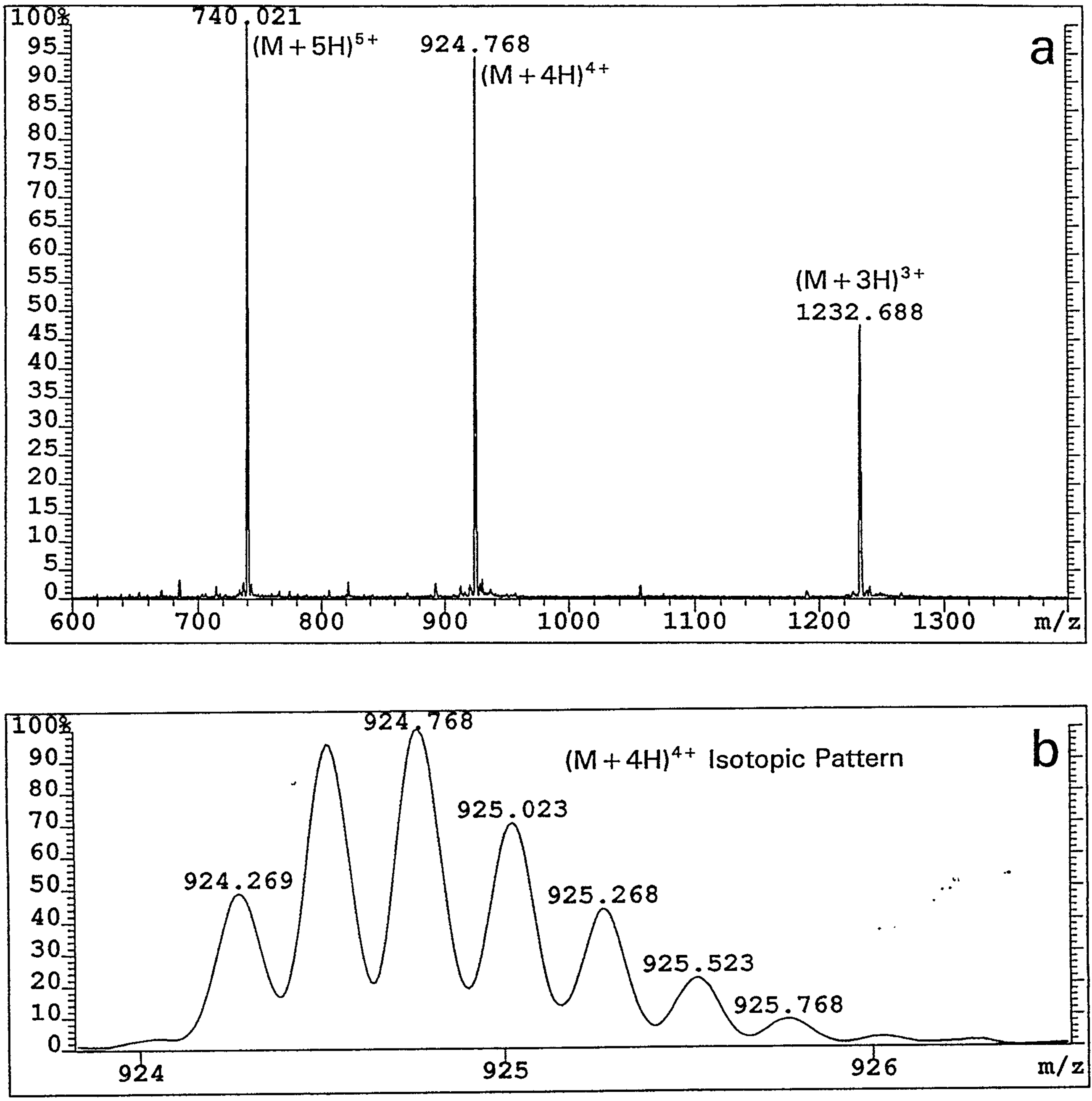

Figure 4: a) Electrospray mass spectrum of peptide sample #35 at 4500 resolution. b) $(M+4H)^{4+}$ Isotopic pattern illustrating resolution of ions differing by 1/4 u.

<u>Observed Monoisotopic Mol. Wt.:</u> 3693.04±0.03,
<u>Sequence:</u> Ac-ECGALEKEVGALEKEVGALEKEVGALEKQVGALQK-$NH_2$

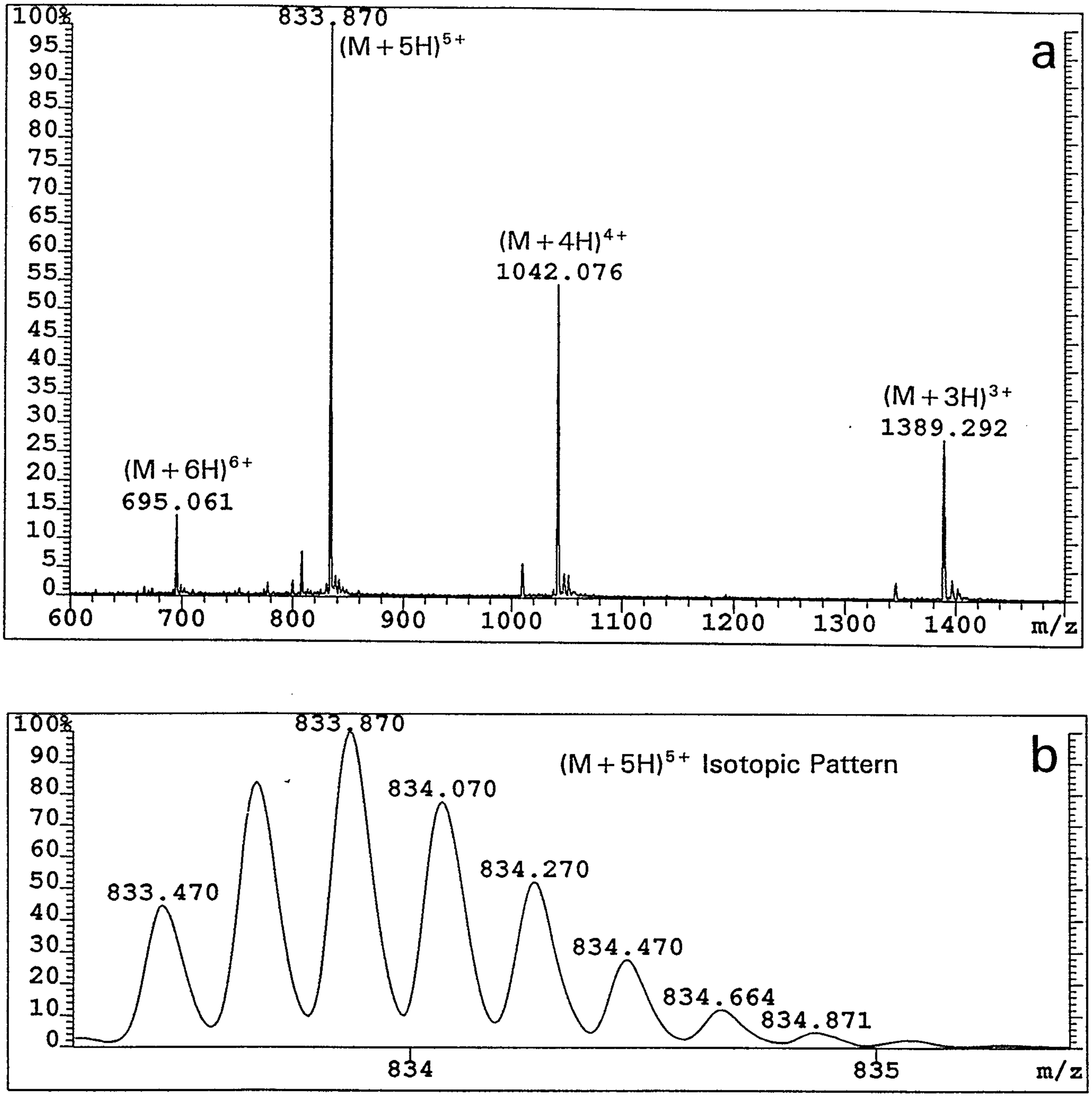

Figure 5: a) Electrospray mass spectrum of peptide sample #28 at 6000 resolution. b) $(M+5H)^{5+}$ Isotopic pattern illustrating resolution of ions differing by 1/5 u.

Observed Monoisotopic Mol. Wt.: 4162.30±0.02,
Sequence: Ac-EIEALKAEIEALKAGGDEQFIPKGGEIEALKAEIEALKA-$NH_2$

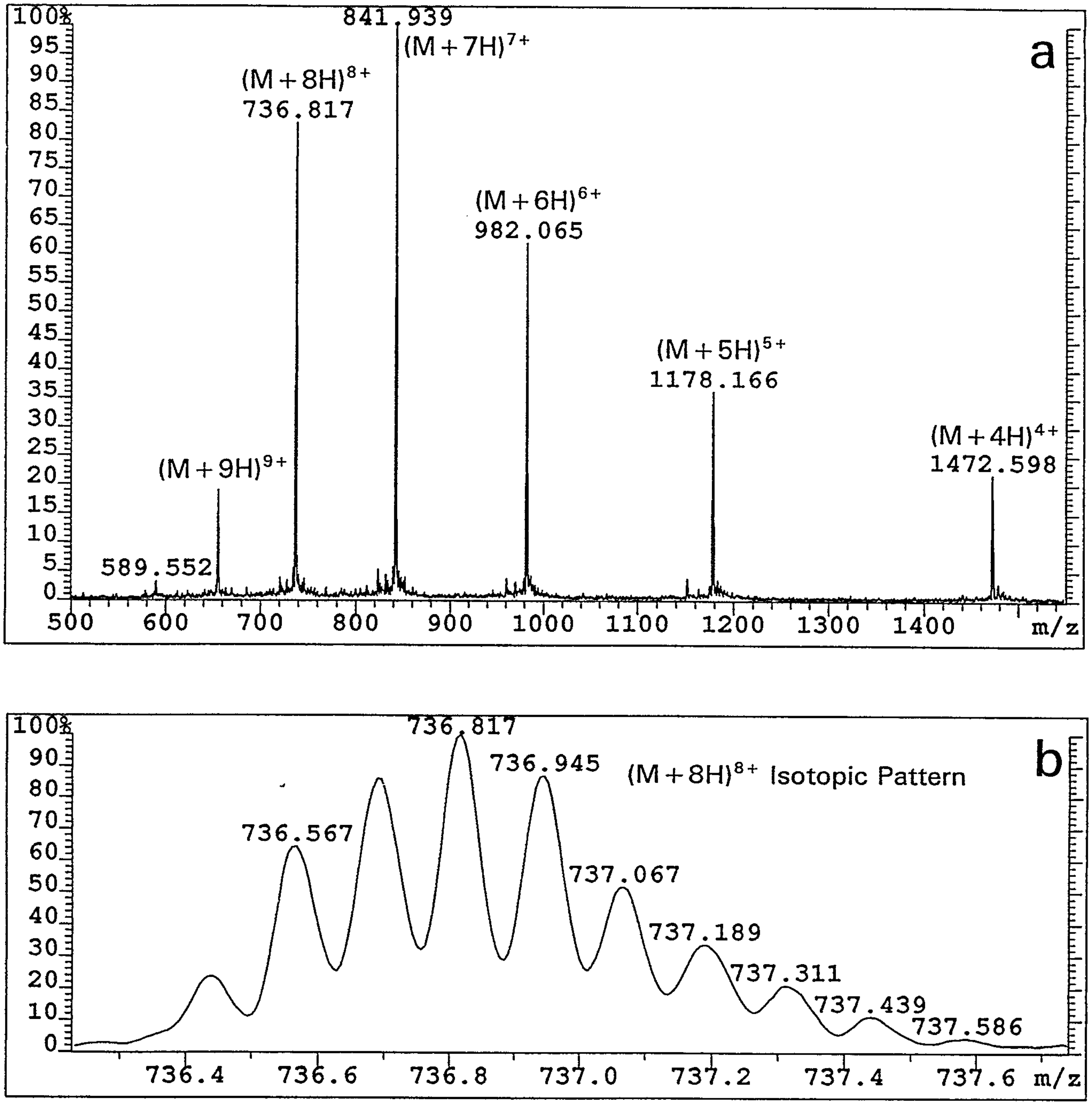

Figure 6: Electrospray mass spectrum of peptide sample #29 at 6000 resolution. b) $(M+8H)^{8+}$ Isotopic pattern illustrating resolution of ions differing by 1/8 u.

Observed Monoisotopic Mol. Wt.: 5883.44±0.12,
Sequence: ATKKEVPLGVAADANKLGEIEALKAEIEALKAGGDEQFIPKGGEIE-ALKAEIEALKA-$NH_2$

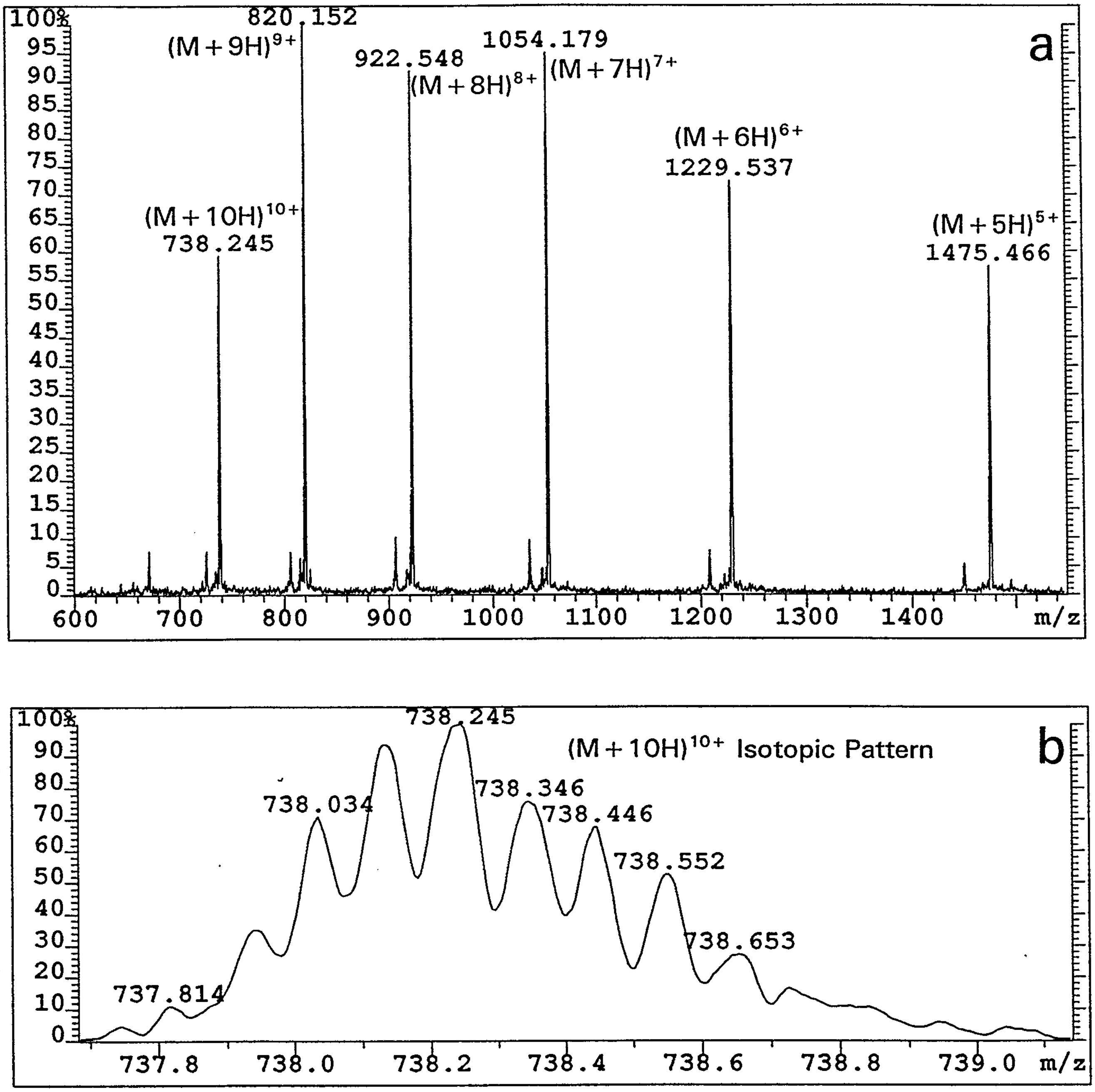

Figure 7: a) Electrospray mass spectrum of peptide sample #32 at 8000 resolution. b) (M+10H)$^{10+}$ Isotopic pattern illustrating resolution of ions differing by 1/10 u.

Observed Monoisotopic Mol. Wt.: 7368.36±0.08,
Sequence: (Ac-QCGALQKQVGALQKQVGALQKQVGALQKQVGALQK-NH$_2$)$_2$,
Dimer with C-C Bridge.

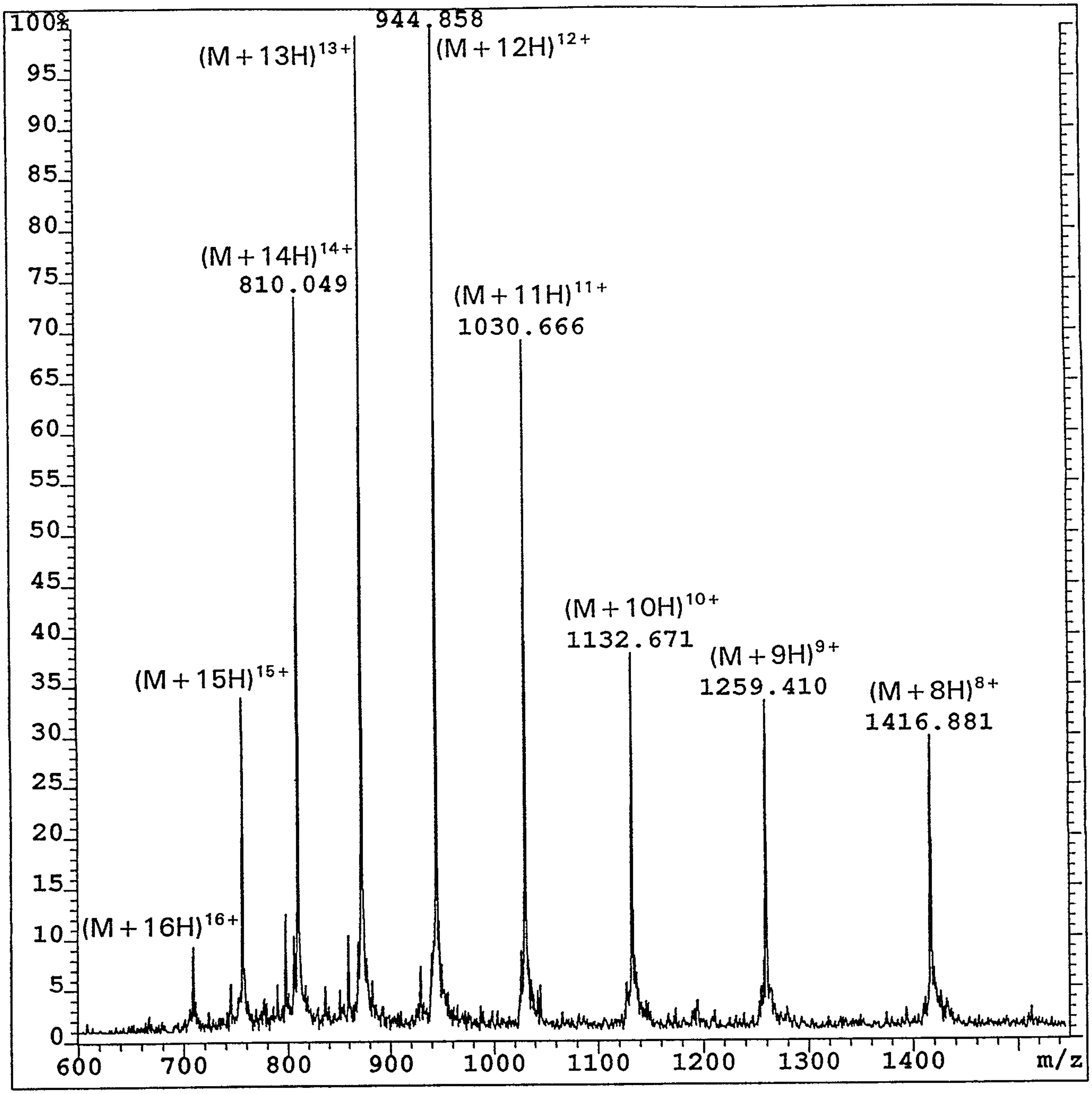

Figure 8: a) Electrospray mass spectrum of peptide sample #30 at 9000 resolution.

Observed Average Mol. Wt.: 11326.33±0.15,
Sequence: (Ac-EIEALKAEIEALKAEIEALKAGGDEQFIPKGGEIEALKAEIEA-LKAEIEACKA-NH$_2$)$_2$, Dimer with C-C Bridge.

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The accurate molecular weights for a series of thirty-eight unknown synthetic peptides used in research studies involving synthetic vaccines, antibacterial peptides or the de novo design of helical petides and proteins, were determined with a magnetic sector instrument. All data were obtained with external calibration over a wide mass range during magnetic scanning. Errors between observed and theoretical monoisotopic molecular weights were typically in the 5 to 60 ppm range for the unknowns at sector resolutions between 2500 and 9000 (10% valley). Isotopic clusters for charge states up to +10 were resolved through the use of high sector resolution. Collisional activated dissociation in the electrospray interface afforded production of product ions that enabled either full or partial sequencing of most unknown peptides below 2000 Da. The complete primary sequence for one peptide was determined and the importance of high resolution was demonstrated by the differentiation of Lysine from Glutamine, two amino acids differing in residue mass by only 0.0364 Da. Two other peptides, with identical monoisotopic masses, but different primary sequences, were differentiated based on their CAD/MS data.

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