Rapid Communications in Mass Spectrometry 4, 1990, 81.

An Electrospray-ionization Mass Spectrometer with New Features

Swapan K. Chowdhury, Viswanatham Katta and Brian T. Chait[†]
The Rockefeller University, New York 10021, USA

SPONSOR REFEREE: R. D. Smith, Battelle, Pacific Northwwest Laboratories, Richland, Washington, USA

The construction and performance of an electrospray-ionization mass spectrometer with new features are described. The mass spectrometer consists of a newly designed electrospray ion-source that is plugged directly into a modified commercial quadrupole mass spectrometer with the ions entering the mass analyzer through a long metal capillary tube and three stages of differential pumping. The present ion source differs from previous designs in the *combination* of techniques employed in the transportation and desolvation of solvated biomolecule ions, prior to mass analysis. Transport of ionized entities between atmospheric pressure and vacuum is carried out through a 203 mm long stainless steel capillary tube with a 0.5 mm bore. Desolvation is effected by the use of controlled heat transfer through the long capillary tube and collisional activation in a region of reduced pressure between the capillary tube exit and the skimmer. Desolvation with this system is convenient and effective and does not involve the strong countercurrent flows of gases that have been used by all previous workers. The effects on the spectra of peptides of capillary tube temperature and desolvation collision energy are investigated. Electrospray-ionization mass spectrometric results are described for thirteen proteins with molecular masses ranging from 5000 to 77 000 Da. The performance of the present instrument, with respect to mass accuracy and sensitivity, is comparable with previously reported systems. The effect of protein concentration in solution on the electrospray mass spectrometric response and charge-state distribution is discussed.

Some twenty years ago, Dole and co-workers carried out a series of experiments that suggested the feasibility of producing isolated gas-phase ions from high molecular weight polymers in solution.[1] These macromolecule ions were produced by electrospraying a polymer solution into a bath gas at atmospheric pressure. Since conventional mass analyzers available at the time could not accommodate ions of such high mass, Dole had to resort to a low-accuracy, indirect determination of the mass-to-charge ratio of the ionized macromolecules by measuring the retarding potential required to stop them from reaching a Faraday cage. Recently, Fenn and co-workers[2,3] overcame the difficulties encountered by Dole by interfacing an atmospheric pressure electrospray-ionization source to a quadrupole mass analyzer. They discovered that the electrospray-ionization process exhibits a strong propensity for producing very highly charged ions from organic polymers. A practical consequence of the efficient production of these highly charged ion species is that the mass range of the quadrupole analyzer is effectively increased by a factor equal to the number of charges on the polymer ions. More recently, Covey *et al.*[4] and Loo *et al.*[5] used electrospray ionization with quadrupole mass analyzers to obtain accurate molecular masses of a variety of proteins that were not previously measurable by mass spectrometry. The latter have obtained the electrospray mass spectra of proteins with molecular masses ranging to 133 000 Da.[5]

Since electrospray ionization occurs directly from solution at atmospheric pressure, the ions formed in this process tend to be strongly solvated. To carry out meaningful mass measurements, it is necessary that any solvent molecules attached to the ions be removed efficiently. In the instruments mentioned above,[2–5] desolvation is achieved by interacting the droplets and solvated ions with a strong countercurrent flow (6–9 L/min) of a heated gas, before the ions enter into the vacuum of the mass spectrometer. To further enhance the desolvation process, Loo *et al.*[5,6] also used collisional activation in a region of reduced pressure between the sampling orifice of the mass spectrometer and the skimmer.

Although high speed pumping is incorporated in all the above instruments to allow for the direct sampling of electrosprayed ions into the mass analyzer, the detailed method of ion transport from atmospheric pressure to vacuum is different in each case. Thus, ion transport has been achieved through a 0.2 mm bore 60 mm long glass capillary tube and skimmer;[2,3] a simple 100 μm diameter sampling orifice with no skimmer;[4] and a 1.0 mm diameter sampling orifice and skimmer.[5,6]

In this Communication we report the design, construction and performance of an electrospray-ionization mass spectrometer that differs, in several respects, from all the previously reported mass spectrometers[2–6] both in the procedure adopted for desolvation and ion transport. This new desolvation and ion-transport system was successfully coupled to a modified commercial thermospray-ionization quadrupole mass analyzer.

EXPERIMENTAL

A schematic representation of the electrospray-ionization mass spectrometer constructed, that was used in the present investigation, is shown in Fig. 1. The apparatus consists of three major components: (a)

[†] Author to whom correspondence should be addressed.

CCC-0951-4198/90/0081-0087 $3.50

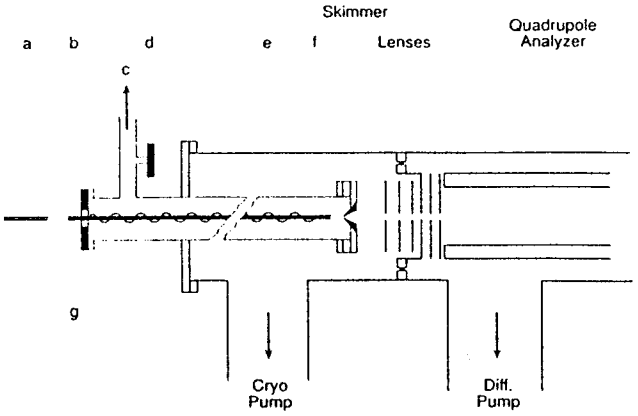

Figure 1. A schematic diagram of the electrospray-ionization mass spectrometer (not drawn to scale). a, 152 μm ID syringe needle at 5.0 kV; b, 0.5 mm ID, 203 mm-long stainless steel capillary tube at 100–300 V; c, to rotary pump; d, Pirani gauge; e, heating tape; f, teflon insulating plate; g, Swagelock teflon fitting. The skimmer (0.5 mm ID orifice), lens elements and quadrupole analyzer are from a Vestec 201 thermospray mass spectrometer.

the electrospray system; (b) the ion desolvation and transport system; and (c) the mass analyzer.

The analyte solution is electrosprayed from a syringe through a stainless steel needle (0.15 mm ID, point not angled) that is maintained at 4–6 kV relative to a metal capillary tube through which droplets, ions, and gases enter the mass spectrometer. A syringe pump (Sage Instrument Incorp., Cambridge, Massachusetts, USA, model 341B) maintains a constant rate of flow through the needle (0.5–2 μL/min). The distance between the electrospray needle tip and the capillary tube is typically 1 cm. The quality of the mass spectrum is strongly dependent on the quality of the spray emitting from the needle. In the present design, the spray can rapidly be optimized by direct observation from outside the vacuum housing and by monitoring the current emitted from the needle.

Electrospraying of the analyte solution produces fine, highly charged droplets. These droplets attempt to follow the electric field lines and migrate towards the metal capillary tube (1.59 mm OD, 0.50 mm ID, 203 mm length) that projects into the first vacuum-stage of the mass spectrometer. The first vacuum region is evacuated by a rotary pump (Edwards, Crawley, West Sussex, England, ISC 900, pumping speed = 1100 L/min) that maintains a pressure of 1.2 Torr at the position of the Pirani gauge shown in Fig. 1. A fraction of the migrating droplets enter the long stainless steel capillary tube assisted by the strong flow of gas that results from the large pressure difference between the extremities of the tube. Droplets entering the tube tend to be focused towards its axis by this strong gas flow[7] and are thus transported through the tube. The temperature of the tube is elevated to ca 85 °C by a resistively-heated coil so that ionized droplets and solvated ions undergo continuous desolvation as they pass through it. A fraction of the material that emerges from the capillary tube passes into a second vacuum chamber through a coaxial, 0.5 mm diameter orifice in a skimmer situated 3.3 mm from the tube end. The tube and skimmer are electrically isolated to allow for the application of an electric field in the region between them. En route to the skimmer, remaining solvent molecules that adhere to the biomolecule ions of interest are removed by collisional activation[5,6] induced by this electrostatic field. The second vacuum chamber is differentially pumped by a helium-cryogenic pump (Air Products, Allentown, PA, USA, model AP-6; pumping speed = 680 L/s for N_2) to give a pressure of 4×10^{-4} Torr. The ions that emerge from the skimmer are focused by a set of lenses into the mass-analysing chamber through a 2.4 mm diameter hole in a baffle that separates this intermediate-pressure region from the mass analyzer. Beyond the baffle, the ions pass through another set of lenses and enter a quadrupole analyzer where their mass-to-charge ratios (m/z) are determined. The pressure in the analyzer chamber is held at 2×10^{-5} Torr by an oil diffusion pump (Edwards, West Sussex, England, Diffstak-63M; pumping speed = 155 L/s). Ions are post-accelerated by a potential of between −2200 and −3000 V and are detected by an off-axis electron multiplier.

The quadrupole mass analyzer, vacuum housing, detector, and all lens elements beyond the skimmer are components of a standard model 201 thermospray mass spectrometer (Vestec Corp., Houston, TX, USA). The m/z range of the quadrupole system was extended to 2000 by reduction of the radiofrequency applied to the rods. Typical operating voltages are: syringe needle (+5 kV), metal capillary tube (+250 V), skimmer (+18 V), and baffle (0 V). All external flanges and the vacuum housing are at 0 V. The spectra were acquired using a commercial data system (Vector-1, Teknivent, St. Louis, MO, USA) on an IBM compatible computer (Dell-310, 80386/20 MHz) by scanning through the m/z range of interest (typically 1000) over periods of 1–4 min. In some cases, multiple scans were added together and averaged to obtain higher signal-to-noise ratios. It should be noted, however, that this data-system records ion abundances at integral m/z values and thus produces data of limited accuracy. In order to obtain accurate m/z values, the centroids of the peaks of interest are determined by scanning the mass spectrometer through a narrow range of m/z values (typically 2–20) in the so-called "calibration mode". This latter procedure normally requires approximately 30 s for each peak. The mass spectrometer was calibrated with the intense series of multiply charged ions generated from equine apomyoglobin, ranging from m/z 848.53 for the $[M+20H]^{20+}$ ion to m/z 1304.88 for the $[M+13H]^{13+}$ ion, and the doubly protonated molecule of bradykinin at m/z 531.10. The stability of the mass spectrometer is sufficiently high that recalibration has not been required over the first two months of operation.

All the peptides and proteins were obtained from Sigma Chemical Co., St. Louis, MO, USA, with the exception of human apolipoprotein AI, which was provided by Drs. J. Breslow, E. Brinton and Y. Ito (Rockefeller University). The proteins, their origin and the catalog number are, respectively: albumin (bovine serum, A-6793), bradykinin (B-3259), carbonic anhydrase II (bovine erythrocyte, C-6403), conalbumin (turkey egg, C-3890), cytochrome *c* (horse heart, C-3256), insulin (bovine pancreas, I-5500), β-lactoglobulin (bovine milk, L-5137), lysozyme (chicken egg, L-6876), myoglobin (equine skeletal muscle, M-9267), ribonuclease A (bovine pancreas, R-4875), subtilisin BPN′ (*Bacillus amyloliquefaciens*,

P-5255), and trypsin inhibitor (soybean, T-1021). The proteins were dissolved in a mixture of water + methanol + acetic acid (47:47:6, v/v).

RESULTS AND DISCUSSION

Desolvation of ions

Charged droplets generated at the electrospray needle pass through a 1 cm space, filled with the ambient atmosphere in the laboratory, en route to the sampling capillary tube (Fig. 1). Solvent continuously evaporates from the droplets during this transit. However, unlike previous workers,[2-6] we do not use a countercurrent of gas to assist desolvation in this region. Instead, the whole length of the 203 mm-long metal capillary tube is heated to effect a controlled evaporation of solvent from the droplets. The remaining solvent molecules bound to the ions of interest are then removed by collisional activation in the space between the point of exit from the capillary tube and the entrance to the skimmer, as a result of an electrostatic field applied to this region of reduced pressure.

The process of collision-induced desolvation of ions is demonstrated in Fig. 2. The skimmer is operated at

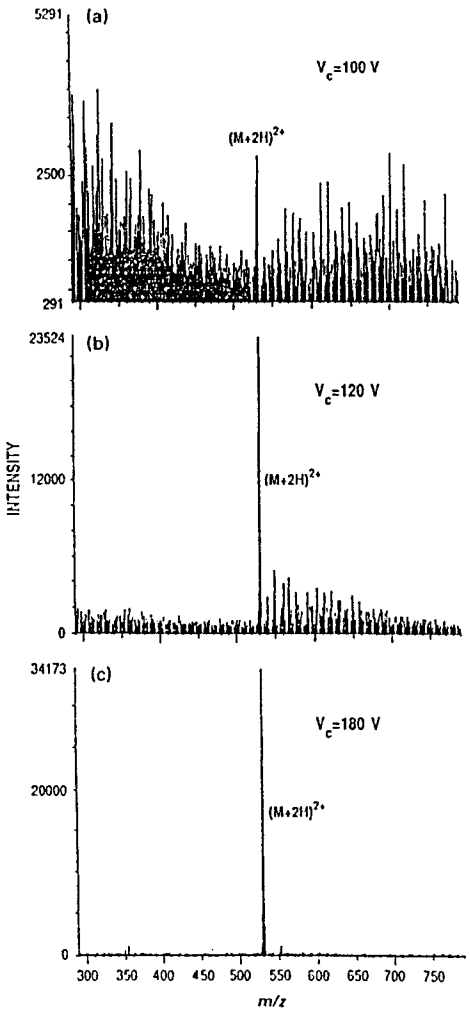

Figure 2. Electrospray-ionization mass spectra of bradykinin measured at different voltages (V_c) applied to the capillary tube. The temperature of the tube is 85 °C and $V_{skimmer}$ is 17.5 V. (a) V_c, 100 V; multiplier voltage, −3000 V; (b) V_c, 120 V; multiplier voltage, −3000 V; and (c) V_c, 180 V; multiplier voltage, −2400 V.

17.5 V, while the voltage V_c on the capillary tube is varied from 100 V to 300 V. Shown in Fig. 2(a) is the electrospray-ionization mass spectrum of bradykinin, obtained at $V_c = 100$ V. The intensity of the doubly protonated molecule $[M+2H]^{2+}$ is small, and the presence of a large number of cluster ions is observed. These cluster species are mainly of composition $[M+nH_2O+2H]^{2+}$ with n extending to greater than 28. When V_c is increased to 120 V (Fig. 2(b)), the $[M+2H]^{2+}$ ion intensity increases by a factor of 4.5 and the maximum of the cluster-ion intensity shifts downwards to $n = 3$ or 4. At $V_c = 180$ V (Fig. 2(c)), almost all the cluster ions have disappeared and a further enhancement of the $[M+2H]^{2+}$ ion is obtained. The enhancement in intensity is such that the multiplier voltage had to be reduced from −3000 to −2400 V to prevent saturation of the electronics. The present findings concerning collision-induced desolvation are in agreement with the earlier observations of Loo *et al.*[6] who, however, also used a strong countercurrent of hot gas to enhance desolvation. As mentioned above, no such gas flow was used in the present investigation. Instead, regulation of the temperature of the 203 mm long capillary tube provides a fine control of the desolvation of the droplets passing through it. The intensity of the peptide ions of interest is found to maximize at a capillary tube temperature of 85 °C. We assume that, at this temperature, the rate of solvent evaporation from the charged droplets is such as to produce entities large enough for relatively efficient transport through the long tube and at the same time, the droplets are desolvated sufficiently upon leaving the tube to allow the remaining solvent molecules to be removed completely by collisional activation (see above). Below 80 °C, the intensity of peptide ions decreases rapidly. We ascribe this decrease to insufficient desolvation of the ions. Above 90 °C, the intensity also decreases, but relatively slowly. We ascribe this latter decrease to relatively less-efficient transport of the resulting smaller ionized entities through the long tube.

For all thirteen of the proteins investigated, the capillary tube was maintained at a constant temperature (85 °C). It proved necessary, however, to adjust the voltage, V_c, on the capillary tube in order to maximize the response from each different protein. For the majority of the proteins, the optimum value of V_c was found to be 250 ± 10 V. The optimum value of V_c was outside this range for β-lactoglobulin ($V_c = 272$ V); carbonic anhydrase II ($V_c = 160$ V); ribonuclease A ($V_c = 300$ V); and myoglobin ($V_c = 201$ V). We ascribe these different values of V_c to the different energies required for complete desolvation of these protein ions.

Mass spectra of proteins

We have used the instrument described above to investigate thirteen different proteins with molecular masses ranging from 5000 to 77 000 Da. The performance of the instrument is illustrated by the spectra shown in Figs 3–6 and the data given in Table 1. Figure 3 shows the electrospray-ionization mass spectrum of horse heart cytochrome *c* (molecular mass (MM) = 12360.9 Da) between *m/z* 400 and 1400. The spectrum is the result of a single scan acquired in 125 s from a solution of cytochrome *c* (1.6 pmol/μL) dissolved in water +

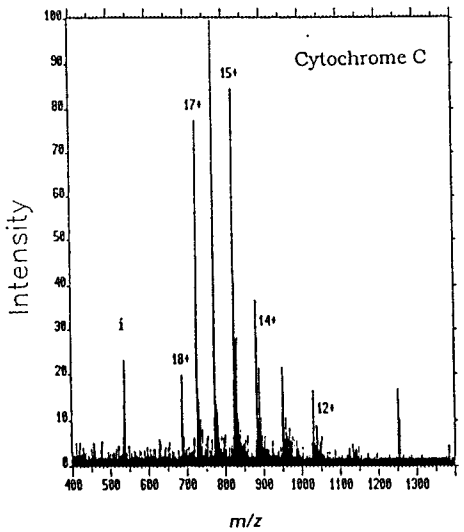

Figure 3. Electrospray-ionization mass spectrum of cytochrome *c* obtained from a solution of methanol + water + acetic acid (47:47:6 v/v): concentration, 1.6 pmol/μL; flow rate, 0.5 μL/min. The single scan spectrum was acquired in 125 s. Thus, 1.6 pmol of cytochrome *c* was consumed. V_c, 242 V; $V_{skimmer}$, 19 V. The peak labelled i arises from an unidentified impurity.

methanol + acetic acid (47:47:6, v/v), and electrosprayed at a rate of 0.5 μL/min. Thus, 1.6 pmol of the sample was consumed in acquiring this spectrum. The spectrum exhibits the gaussian distribution of multiply charged ion peaks characteristic of electrospray ionization,[2-6] resulting from the attachment to cytochrome *c* of 11–18 protons. Each of these ionic species provides an independent determination of the molecular mass of the protein. The maximum number of charges (Z_{max}) acquired by cytochrome *c* is observed to be 18 (Fig. 3), despite the fact that the total number of basic sites (sum of the number of Arg, Lys, and His residues plus the amino terminus) present in the protein is 25. The observed Z_{max} for the majority of the other proteins is also lower than the total number of basic sites present in the molecule. This finding, which

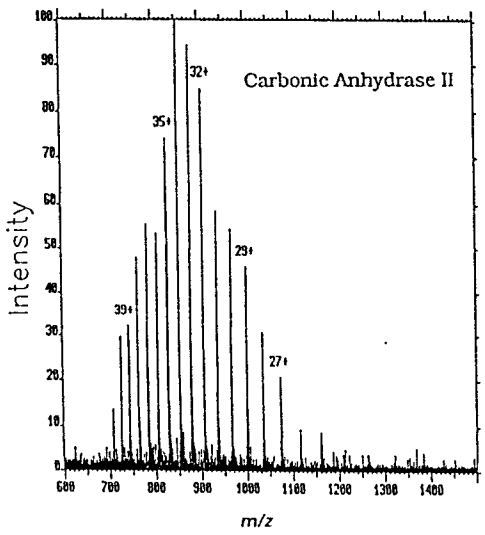

Figure 4. Electrospray-ionization mass spectrum of bovine carbonic anhydrase II dissolved in a mixture of water + methanol + acetic acid (47:47:6 v/v): concentration, 10.0 pmol/μL; flow rate, 0.6 μL/min. The single-scan spectrum was acquired in 3.5 min. Amount of sample consumed, 21 pmol; V_c, 180 V; $V_{skimmer}$, 17 V.

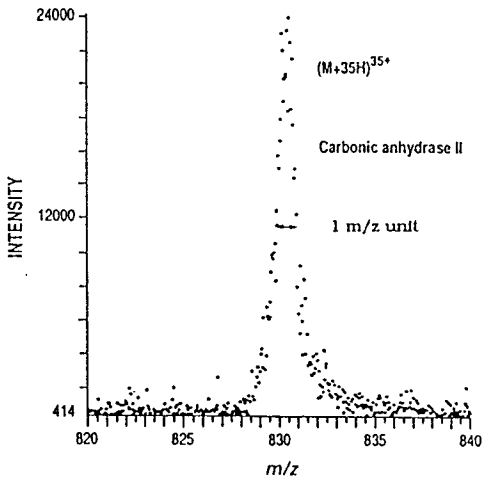

Figure 5. Detailed mass spectrum of bovine carbonic anhydrase II in the vicinity of the $[M+35H]^{35+}$ ion.

has previously been noted by others,[3,5,8] is especially evident in proteins containing intact disulfide bonds[8] and/or a large number of basic residues that occur in groups. An exception to the above general observation, is illustrated in Fig. 4, which shows the mass spectrum of bovine carbonic anhydrase II (MM = 29021.3) between *m/z* 600 and 1500. In this case, $Z_{max}(=41)$ is greater than the total number of basic sites present in the molecule, i.e. 39. The high value of Z_{max} is probably the consequence of the absence of disulfide linkages, presence of relatively few clusters of basic amino-acid residues, and the use of a low desolvation potential ($V_c = 160$ V).

The quality of the data obtained with the present instrument can be assessed by inspection of an expanded portion of the mass spectrum of carbonic anhydrase II. Figure 5 shows the region of the mass spectrum between *m/z* 820 and 840 containing the $[M+35H]^{35+}$ ion. The observed peak is quite symmetrical and has a peak width at half maximum of 1 *m/z*, which is typical of the resolution used, except in those cases where the mass spectral response is weak. The

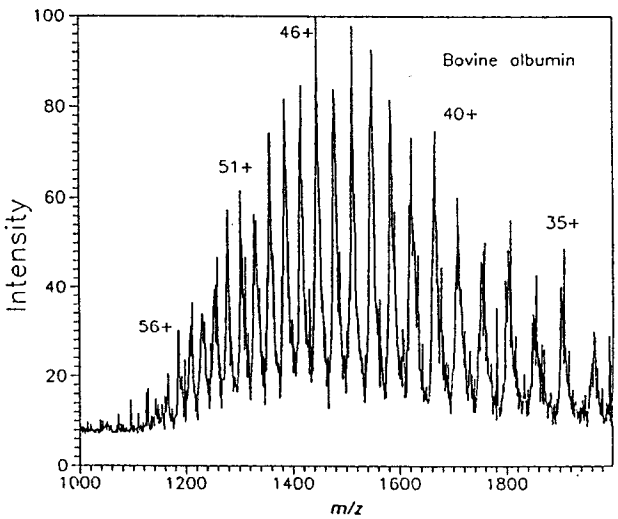

Figure 6. Electrospray-ionization mass spectrum of bovine serum albumin. The spectrum is an average of 7 scans (130 s/scan). Other experimental parameters were: V_c, 258 V; $V_{skimmer}$, 40 V; concentration, 10 pmol/μL; and flow rate, 0.5 μL/min. Sample consumed, 76 pmol.

Table 1. Comparison of experimentally observed and calculated molecular masses (MM) of the thirteen proteins investigated

Protein	Observed MM	Calculated[a] MM	Δ[b] (ppm)	Sensitivity[c]
Insulin (bovine)	5734.2 ± 0.9[d]	5733.6	+105	high
Cytochrome *c* (horse heart)	12 359.1 ± 1.7	12 360.9	−145	high
Ribonuclease A (bovine pancreas)	13 678.0 ± 2.8	13 682.3	−314	low
	13 776.0 ± 1.6[e]			low
	13 876.6 ± 0.9[e]			low
Lysozyme (chicken egg)	14 308.2 ± 4.2	14 305.2	+210	medium
Apomyoglobin (equine skeletal muscle)	calibrant	16 950.5	—	high
β-Lactoglobulin A (bovine)	18 364.7 ± 1.4	18 363.1	+87	medium
Trypsin inhibitor (soybean)	20 090 ± 7	20 091.1	−50	low
	19 978.6 ± 0.5[f]			medium
Trypsinogen (bovine pancreas)	23 981.6 ± 2.0	23 981.1	+21	medium
Subtilisin BPN' (*Bacillus amyloliquefaciens*)	27 327 ± 7	27 534.0	−7600	low
Apolipoprotein A1 (human)	28 078.1 ± 0.8	28 078.6	−18	high
Carbonic anhydrase II (bovine)	29 021.8 ± 1.3	29 021.3	+17	high
Albumin (bovine)	66 509 ± 23	66 267	+3650	low
Conalbumin (turkey egg)	77 563 ± 23	[g]	—	low

[a] Molecular masses are calculated using the sequences compiled in the Dayhoff Protein Sequence Database[9] and the currently accepted IUPAC values for the isotopically averaged atomic masses.
[b] Difference between the observed (column 2) and the calculated molecular mass (column 3).
[c] The sensitivity scale is: high, 0.5–10 pmol/experiment; medium, 10–50 pmol/experiment; low, weak intensity even when a higher sample amount was used.
[d] The error given is the standard deviation of the multiple determinations of the molecular mass.
[e] Ion species of unknown origin related to ribonuclease A (see text).
[f] Two components were observed in the mass spectrum of trypsin inhibitor (see text).
[g] Sequence is not available.

mass spectrum of bovine albumin shown in Fig. 6 represents an example of a protein exhibiting a very weak mass spectrometric response. Under identical operating conditions, the signal-to-noise ratio was observed to be considerably lower than that for cytochrome *c* or carbonic anhydrase II. In order to increase the ion intensity, the acceleration potential was therefore increased from ca 17 V to 40 V resulting in a decrease in mass resolution. The observed weak response can be attributed to: (i) the formation of a very wide distribution of charge-states resulting in a decreased intensity in any given charge-state; (ii) the lower transmission efficiency and detection efficiency for the higher *m/z* ions; and (iii) other less well understood factors such as sample heterogeneity and incomplete desolvation.

Molecular-mass determination

The calculation of molecular masses of proteins from the measured *m/z* values of multiply charged ions observed in electrospray-ionization spectra has been described previously.[2–6] The experimentally determined molecular masses of the proteins studied are given in Table 1 together with the corresponding calculated values, the difference between the observed and calculated masses, and the relative sensitivities. The calculated molecular masses were obtained using the sequences compiled in the Dayhoff Protein Sequence Database[9] and currently accepted IUPAC values for the isotopically averaged atomic masses.

An illustration of the accuracy and precision obtained from a protein exhibiting a good response is provided in Table 2, which gives the molecular masses derived from the experimentally observed *m/z* values of the nine most intense multiply protonated ions of human apolipoprotein AI. The precision of these nine separate determinations is very high as evident from the observed standard deviation of 0.8 mass unit. The accuracy is also high; the mean measured molecular mass of 28078.1 Da is in close agreement with the calculated value of 28078.6 Da. The measured molecular masses of most of the other proteins studied also agree, with the calculated values to within ca 200 ppm. (Table 1).

Table 2. Experimentally observed molecular masses from apolipoprotein AI ions having different number Z of attached protons

Z	Observed m/z	Molecular Mass	Δ[a]
36	780.95	28 077.9	−0.7
35	803.25	28 078.5	−0.1
34	826.85	28 078.6	+0.0
33	851.87	28 078.5	−0.1
32	878.45	28 078.2	−0.4
31	906.80	28 079.6	+1.0
30	936.90	28 076.8	−1.8
29	969.18	28 077.0	−1.6
28	1003.8	28 078.2	−0.4
		Mean = 28 078.1 ± 0.8	

[a] Difference between the observed (column 3) and the calculated average molecular mass (28 078.6 Da) of apolipoprotein A1.

Two notable exceptions are the masses obtained for subtilisin BPN′ from *Bacillus amyloliquefaciens* and bovine albumin. The sources of these discrepancies have not yet been elucidated. The mass spectrum of the sample of soybean trypsin inhibitor showed the presence of two components. The observed molecular mass (19 978.6) of the component having the higher intensity does not correspond to the calculated molecular mass of any of the three known types of trypsin inhibitor.[10] However, the weaker of the two components has a mass that corresponds to trypsin inhibitor (Table 1). The sample of bovine ribonuclease A yielded a spectrum showing the presence of two components in addition to the expected molecule. These components appear to arise from single or multiple attachment of a moiety with mass 98 ± 2 mass units to the ribonuclease A molecule. We are currently conducting experiments aimed at elucidating the identity of this moiety, which was also observed to be attached to several other proteins.

The different proteins studied were found to give widely different mass spectrometric responses. The resulting sensitivies are compared in the fifth column of Table 1. In general, proteins containing internal disulfide linkages yielded lower responses than those without crosslinks.

We have also investigated the mass spectrometric response as a function of protein concentration in the electrospray solution. Figure 7 shows a plot of the sum of the intensities of the four most-intense ions in the mass spectrum of equine apomyoglobin as a function of the electrospray-solution concentration. The response increases, approximately linearly as a function of the concentration, between 0.1 pmol/μL and 20 pmol/μL, where the intensity is at a maximum. Above 20 pmol/μL, the response drops rapidly with further increase in concentration. The decrease in intensity may be a consequence of an increase in competition for the limited charge available on the droplets at these higher protein concentrations. This hypothesis is supported by the observed decrease of the relative intensities of ions containing a higher number of charges with an increase in concentration (data not shown).

CONCLUSION

We have constructed and tested the performance of an electrospray-ionization mass spectrometer with new features. The mass spectrometer consists of a newly designed electrospray ion-source that is plugged directly into a modified commercial quadrupole mass spectrometer with the ions entering the mass analyzer through a long capillary tube and three stages of differential pumping. The present ion source differs from previous designs[2-6] in the *combination* of techniques employed in the transportation and desolvation of solvated biomolecule ions, prior to mass analysis. Specific differences are: (i) The use of a 203 mm-long metal capillary tube (0.5 mm ID) to transport ionized species from atmospheric pressure to a region of reduced pressure (1–10 Torr). The long tube allows (a) convenient injection of ions into the commercial mass spectrometer system; (b) efficient pumping of the region between the capillary-tube exit and the skimmer; (c) ready observation of the electrosprayed droplets as they are emitted from the needle; and (d) efficient and controlled heat transfer to the droplets. This transport system differs from that previously described by Fenn and co-workers[2,3] who employed dielectric capillary tubes of shorter length. The use of metal in the present design, reduces charging problems that are sometimes encountered with glass capillary tubes. We believe that this transport system can be used with a wide range of capillary tube lengths, allowing ready coupling to mass analyzers with different geometries. (ii) The use of a combination of controlled heat transfer to the charged droplets during transport through the long capillary tube and collisional activation in a region of reduced pressure to bring about the removal of solvent molecules adhering to the biomolecule ions. Desolvation with this system is convenient and effective and does not involve the strong countercurrent flow of gas that has been employed by all previous workers.[2-6]

The performance of the present system, with respect to the appearance of the mass spectra, sensitivity, and mass accuracy was found to be comparable to that of the systems previously reported.[2-6]

Acknowledgements

We thank Mrs. Gladys McMilleon for typing the manuscript and Mr. Rudolf K. Franz for skilled assistance in constructing the ion source. This work was supported in part by grants RR00862 and RR07065, Division of Research Resources and GM38274, National Institutes of General Medical Sciences, National Institute of Health.

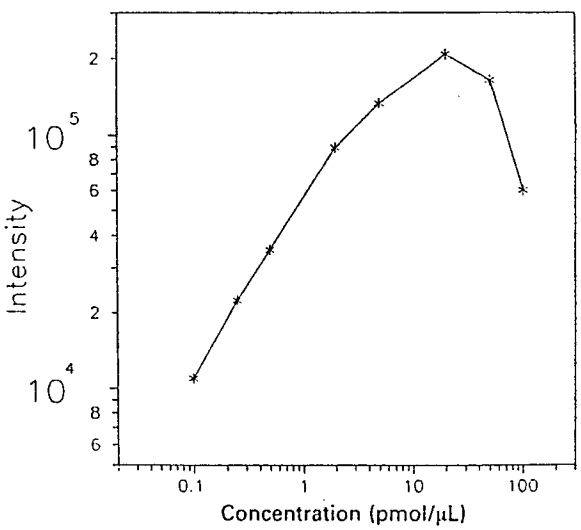

Figure 7. A plot of the sum of the intensities of the four most intense ions in the mass spectrum of equine apomyoglobin {$[M+17H]^{17+}$, $[M+18H]^{18+}$, $[M+19H]^{19+}$, and $[M+20H]^{20+}$} as a function of the electrospray-solution concentration.

REFERENCES

1. (a) M. Dole, L. L. Mach, R. L. Hines, R. C. Mobley, L. P. Ferguson and M. B. Alice, *J. Chem. Phys.* **49**, 2240 (1968); (b) L. L. Mach, P. Kralik, A. Rheude and M. Dole, *ibid.* **52**, 4977 (1970).
2. (a) C. M. Whitehouse, R. N. Dreyer, M. Yamashita and J. B. Fenn, *Anal. Chem.* **57**, 675 (1985); (b) C. K. Meng, M. Mann and J. B. Fenn, *Z. Phys. D* **10**, 361 (1988); (c) S. F. Wong, C. K. Meng and J. B. Fenn, *J. Phys. Chem.* **92**, 546 (1988).
3. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Science* **246**, 64 (1989).
4. T. R. Covey, R. F. Bonner, B. I. Shushan and J. D. Henion, *Rapid Commun. Mass Spectrom.* **2**, 249 (1988).
5. J. A. Loo, H. R. Udseth and R. D. Smith, *Anal. Biochem.* **179**, 404 (1989).
6. J. A. Loo, H. R. Udseth and R. D. Smith, *Rapid Commun. Mass Spectrom.* **2**, 207 (1988).
7. (a) J. B. Anderson, R. P. Andres and J. B. Fenn, *Adv. Chem. Phys.* **10**, 275 (1966); (b) R. D. Macfarlane, R. A. Gough, N. S. Oakey and D. F. Torgerson, *Nucl. Instr. Methods* **73**, 185 (1969); (c) D. M. Moltz, R. A. Gough, M. S. Zisman, D. J. Vieira, H. C. Evans and J. Cerny, *Nucl. Instr. Methods* **172**, 507 (1980).
8. (a) R. D. Smith, H. R. Udseth, J. A. Loo, C. G. Edmonds and C. J. Barinaga, *Proceedings of 37th Conference on Mass Spectrometry and Allied Topics*, Miami Beach, FL, p. 21, ASMS East Lansing (1989). (b) J. A. Loo, C. J. Edmonds, H. R. Udseth and R. D. Smith, *Anal. Chem.* (In press).
9. *Protein Identification Resource (PIR), National Biomedical Research Foundation (NBRF) Release* 19.0 (3187 December 1988).
10. S.-H. Kim, S. Hara, S. Hase, T. Ikenaka, H. Toda, K. Kitamura and N. Kaizuma, *J. Biochem.* **98**, 435 (1985).

Received 4 January 1990; accepted 5 January 1990.