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CAPILLARY ELECTROPHORESIS–ELECTROSPRAY IONIZATION-MASS SPECTROMETRY

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SUMMARY

New developments are described in the area of capillary electrophoresis combined with electrospray ionization-mass spectrometry (CE–ESI-MS), a promising new method for the separation and mass spectrometric characterization of labile, polar or ionic constituents of biological mixtures. The various experimental modes of capillary electrophoresis are reviewed. The ESI-MS technique is described and the significance of its combination with capillary electrophoresis is illustrated by MS analysis of large biomolecules. The design and performance optimization of a new ESI interface are discussed, including the influence of electrophoretic and MS operating parameters. Various examples from our laboratory illustrating the range of present application of this interface and direction of future development are presented. These include negative ion electrospray mass spectra of nucleotide co-enzymes, nucleotide mono-, di- and triphosphates and positive ion spectra of biologically important oligopeptides and proteins of $M_r > 75$ kilodaltons.

INTRODUCTION

The development of analytical methodology for the identification and characterization of biopolymers and their constituents is of broad significance in biochemistry, molecular biology and biotechnology. The important characteristics of high selectivity and high sensitivity offered by mass spectrometry (MS) are inherently advantageous in these areas of research. The electrospray ionization (ESI) method is applicable to broad classes of involatile and labile compounds. Coupled with capillary electrophoresis (CE), an ionic separation method of high efficiency, flexibility and speed, electrospray ionization-mass spectrometry (ESI-MS) is a potentially powerful tool for biomolecular analysis.

Electrophoresis, based on the separation of charged species in a background medium under the influence of an applied electric field, is probably the oldest differential migration technique, due originally to Wiedeman in 1856[1]. Since the introduction of discrete "band" separation of solutes by paper electrophoresis in 1937[2], the method has been extensively elaborated and applied, and is currently the classical method for the separation of biopolymers. Separation may be on the basis of molec-

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ular charge and/or size (when using an appropriate gel medium) and is especially useful for the analysis of complex protein mixtures and in DNA sequencing. Electrophoresis forms a family of related techniques including electrophoresis (polyacrylamide, agarose, etc.), isotachophoresis, gel electrofocusing (*i.e.*, isoelectric focusing) and free zone electrophoresis.

Analytical separation techniques based on these electrophoretic principles in the capillary format can be divided into four types: capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary isotachophoresis (CITP) and capillary isoelectric focusing (CIEF). Our present research efforts have focused on CZE and CITP development and application. In principle, all techniques may be carried out with the same type of electrophoretic equipment, providing considerable instrumental flexibility. Capillary electrophoresis methods present an opportunity for greatly enhanced separation speeds, improved automation and (potentially) greater sensitivity and compatibility with alternative detection methods.

CZE

In zone electrophoresis, a column is filled with a single electrolyte having a specific conductivity. The mixture of substances to be separated is applied as a narrow band to the head of a buffer filled column in a band whose width is much less than the length of the column and at a concentration ideally too low to affect the buffer conductivity. An electric field is then applied across the length of the column and the individual substances migrate and separate according to their net electrophoretic velocities. Zone electrophoresis carried out in small diameter (< 100 μm) fused-silica capillaries is a relatively new approach to the high-resolution separation of aqueous samples[3–5]. An important physical effect of the high electric field on any surface having a net charge (*e.g.*, fused silica) is the formation of an electric double layer[6]. Such a layer for the fused silica–water system results from exposed silanol groups, which acquire a net negative charge while the bulk solution remains neutral. The immediate neighborhood of the glass surface acquires a corresponding net positive charge which extends for a short distance into the fluid. Under the influence of an external electric field the ions of the diffusive part of the electrical double layer move toward the oppositely charged electrode and a flow originates near the wall. Viscous forces then drag the rest of the solution in the capillary creating a flat flow profile, in contrast to the parabolic flow profile of hydrodynamic flow[7]. The width of the double layer, dependent on ionic strength, is typically a few nanometers and steady state flow is reached almost immediately in a 100 μm I.D. (typical for CZE) capillary when the field is applied[3]. A substantial electric double layer (strong zeta potential at the silica surface) results in a strong electroosmotic flow and rapid analysis times (5–30 min) for typical capillary lengths (100 cm) and voltages (30 kV). The electroosmotic flow-rate is proportional to the applied voltage for the field gradients typically used. In addition to the bulk flow of the electrolyte, charged species in solution will migrate under the influence of the external field. This velocity is proportional to the applied field and separations are due to differences in electrophoretic mobility among species. Differences in the velocity of migration may be established, as in the case of electrokinetic chromatography, by secondary means utilizing partitioning with a counter-migrating charged micellar phase[8].

The most important contributions to band spread in CZE are molecular diffu-

sion (which is inherent and unavoidable), adsorption on capillary surfaces, and effects due to heating (*e.g.* convection driven by thermal gradients[9–13]). In CZE the effects of molecular diffusion can only be countered by minimizing the separation time (*i.e.*, using higher voltages). The effects due to Joule heating of the capillary are generally responsible for the greatest loss of separation efficiency and must be minimized. Small diameter columns reduce the resistance to heat transfer, minimize the radial temperature differential and have wall effects which act to suppress convection. Typically, molecular diffusion rates in liquids are quite small (10^{-6}–10^{-8} cm^2/s) and the ultimate efficiency of electrophoretic separations can be very high ($>10^6$ plates). Since maximum separation efficiencies are defined by molecular diffusion, increased molecular weights should, in principle, afford better separations.

Amino acids and small peptides are well-separated by CZE in untreated glass or fused-silica capillaries[3]. Capillary electrophoresis has been particularly effective in the analysis of fluorescently labelled amino acids[14–16]. Separation of fluorescently labelled tryptic peptides have also been reported[17,18]. Free zone separations of protein mixtures in the capillary format have been reported with high efficiencies[19–22]. Fluorescence detection of myoglobin with detection limits in the attomol (10^{-18} mol) range has been achieved[21] by CZE. Surface adsorption may be avoided by raising the pH of the buffer solution above the isoelectric point of the sample proteins, by dynamic modification of the buffer–silica wall interfacial double layer[20] or by covalent modification of the silica wall[22]. Nucleic acid constituents may be separated by CZE as nucleobases, nucleosides[23] and nucleotides[24,25]. The addition of detergent micelles with complexing metal ions allows improved separations of these analytes by differential complexation and good separations of small oligonucleotides are obtained.[26].

CITP

In isotachophoresis (sometimes called displacement electrophoresis) all analytes progress through the separating medium at the same final velocity[27]. Isotachophoresis, though utilizing similar equipment and principles as zone electrophoresis, can accommodate larger samples and may actually result in an *increase* in the concentration of the material being separated. In CITP the column is initially filled with an electrolyte (*i.e.*, leading electrolyte) which contains ions with a mobility higher than that of any ions in the sample mixture (and, ions of opposite polarity having a useful buffering capacity). The solutes are then loaded on the column as a second band. The head of the column is placed in an electrolyte solution which contains ions with an effective mobility lower than any in the sample mixture (*i.e.*, terminating electrolyte). On application of an electric field the lead electrolyte will attempt to "pull away" from the analyte ions. This results in a gap where the conductivity is dropping (the solvent having no significant conductivity) and the electric field is rising. The increased field will "pull" the analyte ions along until they catch up to the lead electrolyte, with the highest mobility analyte ion arriving first. This "pulling along" effect will continue as the analyte bands arrange in order of decreasing mobilities until finally the trailing electrolyte, which has the lowest mobility, is reached. Eventually a steady state will be reached in which each solute is isolated in its own band, and all bands are moving at the same velocity.

The steady state concentration of the analyte in CITP is largely determined by the leading electrolyte concentration. Accordingly, if the analyte is more dilute than

the leading ion concentration, the analyte will often be *concentrated* as it separates into its own band. In a fully developed separation the ionic concentration of each band is equivalent and the relative abundance of the analyte (and thus the sample concentration) is proportional to the length of the band. Thus, CITP offers the potential for higher sample loading than CZE (and increased *molar* sensitivity), and can provide (in many cases) actual concentration of separated sample bands. We have recently shown that the CITP–MS combination has advantages for certain trace analyses and is capable of providing high resolution separations[28,29].

Amino acids and small peptides can be easily separated by capillary isotachophoresis[27], usually as anions, provided suitable leading and terminating electrolytes are chosen. Recently, Stover has described the cationic CITP separations of proteins with average molecular weights of 13–35 kilodaltons[30].

ESI and CE–MS-ESI

Electrospray ionization, pioneered by the early work of Dole *et al.*[31], is a soft ionization method capable of producing gaseous ions of ionic molecules from highly charged evaporating liquid droplets. Fenn and co-workers[32–34] have further developed the technique as a means of ionizing charged, labile and involatile samples in solution for MS analysis, including nucleotide mono- and diphosphates. ESI generally provides production of multiply charged ions of large molecules and allows mass spectrometers with moderate mass/charge limit (*e.g.*, conventional quadrupole mass filters) to analyze macromolecules with dramatically higher molecular weights. For example, a net charge of 23+ (multiply sodiated) has been observed on a polyethylene glycol oligomer of nominal molecular weight 17 500 daltons[35]. Mass spectra have been obtained on peptides up to 40 000 daltons using a quadrupole mass spectrometer of m/z 1600 limit[36]. A nebulizer assisted variation of this technique is also described[37].

We have recently described the on-line combination of CZE based upon ESI-MS[38–40], the first direct combination of any electrophoretic separation technique in dynamic (*i.e.*, "on-line") combination with MS. This development was based upon the recognition that it is not necessary for both ends of the CZE capillary to be immersed in buffer reservoirs, as conventionally practiced. Thus, with an appropriately designed electrode MS detection is possible at the capillary terminus utilizing an ESI source. The interface allows compatibility with the low (approximately 0–1 μl/min) electroosmotic flow-rates of CE and provides for ion production at atmospheric pressure from the electrically induced nebulization process. Because CE relies on analyte charge in solution, and the ESI process appears to function most effectively for ionic species, the CE–ESI-MS combination is highly complementary. We have reported the analysis by CZE–ESI-MS of a mixture of quaternary ammonium compounds[38] obtaining a separation efficiency of over 330 000 theoretical plates, an order of magnitude better than obtainable by liquid chromatography in similar time. For scanning MS sample sizes in the femtomole range were required; however detection limits of *ca.* 10 attomol were obtainable using single ion detection. Thus, the CZE–ESI-MS approach offers previously unobtainable separation efficiencies (for the combination with MS) as well as significantly enhanced sensitivity. Further instrumentation development should provide even better detection limits.

CITP has also been evaluated as an alternative mode of electrophoretic sep-

aration with ESI-MS detection[28,29]. CITP is an attractive complement to CZE and is near ideally suited for combination with MS. Sample sizes which can be introduced in CITP are much greater (>100 fold) than CZE. Additionally, as discussed above, CITP may result in concentration of analyte bands, which is in contrast to the inherent dilution obtained in CZE. Samples elute in CITP ideally as broad, flat-topped bands, well suited to sensitive analysis with long integration times and the slow scan speed of the mass spectrometer, particularly for tandem MS detection. High-sensitivity and high-resolution separations for quaternary phosphonium and ammonium salts, amino acids and catacholamines have been demonstrated by CITP–ESI-MS[29]. Vinyltriphenyl and ethyltriphenyl phosphonium ions are well separated by this combined method, in contrast to previous CZE–ESI-MS results. CITP–ESI-MS is complementary to CZE–ESI-MS in many ways. The CZE column is easily overloaded, whereas CITP tolerates very high loading. Thus, larger injection volumes (0.1–1.0 μl) of extremely dilute sample solutions may be analyzed. Detection limits of approximately 10^{-11} M have been demonstrated for quaternary phosphonium salts[29] and substantial improvements appears feasible. CITP is well suited to low concentration samples where the amount of solution is relatively large whereas CZE is ideal for the analysis of minute quantities of solution.

This paper reports further results obtained by ESI-MS employing an atmospheric pressure interface adapted for combination with CZE for biomolecule analyses.

EXPERIMENTAL

The instrumentation developed at our laboratory has been elsewhere described in detail[38–40]. Fig. 1 shows the detailed construction of the interface. In earlier versions of the CZE–ESI interface the electrospray ionization was accomplished from an electrodeposited metal contact established at the end of the CZE capillary[38,39]. The most recent design employs a flowing liquid sheath electrode interface which allows the composition of the electrosprayed liquid to be controlled independently of the CZE buffer (which is desirable since high-percentage aqueous and high-ionic-strength buffers useful for CZE are not well tolerated by ESI)[40]. The electrical contact is through a conductive liquid sheath (typically methanol, acetonitrile, acetone or isopropanol). With this arrangement no significant additional mixing volume (< 10 nl) is produced and analyte contact with metal surfaces is avoided. This interface provides greatly improved performance and flexibility and is adaptable to other forms of CE. For direct ESI-MS experiments, syringe pumps control the flow of analyte solution and liquid sheath at 1 μl/min and 3 μl/min, respectively. CZE–ESI-MS experiments were conducted in untreated fused-silica capillaries using methods which have been described previously[28,38–40].

The ESI source consists of a 50 or 100 μm I.D. fused-silica capillary (which can be the CZE capillary) that protrudes 0.2–0.4 mm from a tubular stainless-steel electrode. High voltage, generally +5 kV for positive ions and −5 kV for negative ions, is applied to this electrode. The ESI source tip is mounted 1.5 cm from the ion sampling nozzle of the ion sampling orifice (nozzle) of the quadrupole mass spectrometer. A 3–6 l/min counter-current flow of warm (80°C) nitrogen gas is used between the nozzle and source to aid desolvation of the highly charged droplets and to mini-

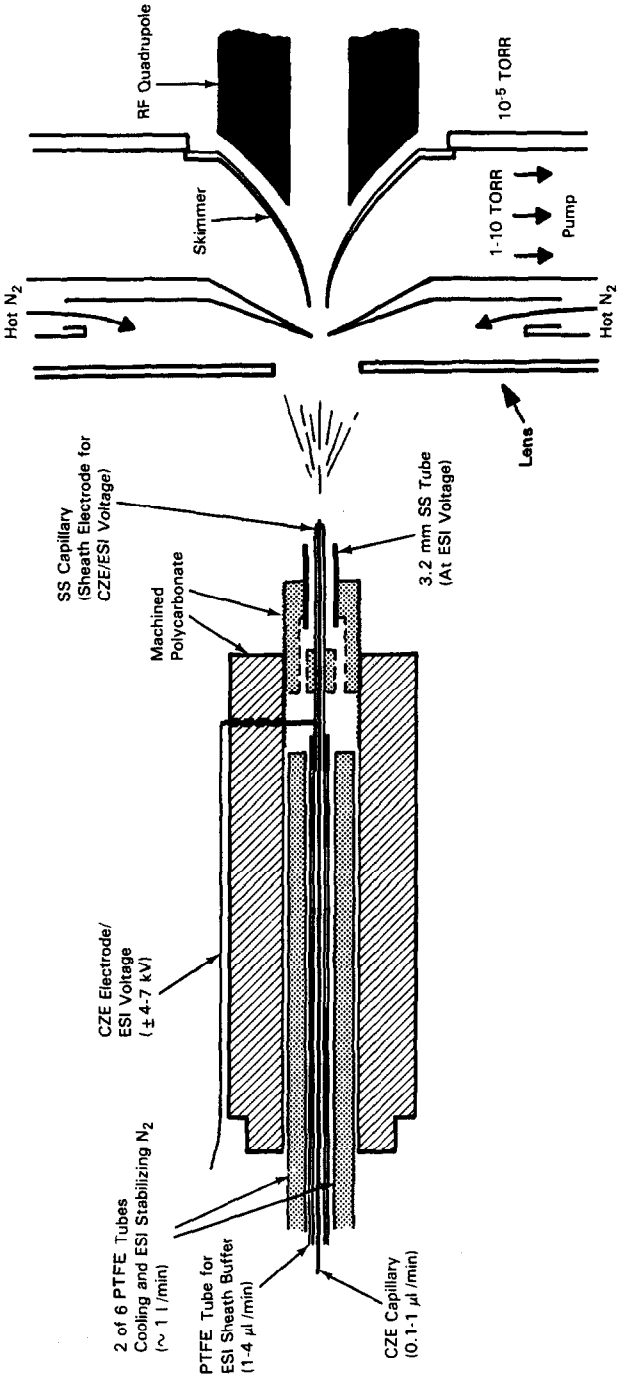

Fig. 1. Detailed schematic drawing of sheath electrode electrospray ionization interface and atmospheric pressure focusing and sampling region of the mass spectrometer entrance. SS = stainless steel.

mize solvent cluster formation during expansion into the vacuum chamber. Analyte clustering is further minimized by the mutual repulsion of highly charged ions and droplets (which, in contrast to the thermospray ionization, all have the same polarity). A lens placed in front of the sampling nozzle is used to help focus the ions or electrospray droplets to the point of ion sampling. Ions are sampled through the 1-mm diameter nozzle to a 2-mm skimmer directly in front of the radio frequency (RF) focusing quadrupole lens (Fig. 1). Typically, +350–500 V is applied to the focussing lens and +200 V to the nozzle (V_n), while the skimmer potential is at ground. A single-stage roots blower pumps the nozzle–skimmer region to 1–10 Torr. The cryopumped RF focusing region typically reaches pressures on the order of 10^{-6} Torr, while analysis quadrupole housing is maintained at 10^{-7} Torr with a turbomolecular pump (500 l/s). The analysis quadrupole (Extrel, Pittsburgh, PA, U.S.A.) has an upper m/z limit of 1700.

Biochemical samples were purchased from Sigma (St. Louis, MO, U.S.A.) except bovine apotransferin (Calbiochem, San Diego, CA, U.S.A.) and were used without further purification. Sample solutions were prepared in distilled water with varying ratios of acetonitrile —100% water to water–acetonitrile (20:80)]. For protein solutions, 1–5% glacial acetic acid was added.

RESULTS AND DISCUSSION

Preliminary studies with ESI have shown it to be a useful technique for ionization of nucleotides[34], especially in the negative ion mode. In the experiments which follow sample solutions were delivered directly to the ESI interface. Spectra are recorded over 1–2 min intervals requiring approximately 1–100 pmol of sample. Fig. 2 shows an ESI negative ion mass spectrum from an equimolar mixture of adenosine mono-, di- and triphosphate (sodium salt). We observe decreasing abundance of molecular ions with increasing phosphorylation. The molecular anion $(M-H)^-$ and

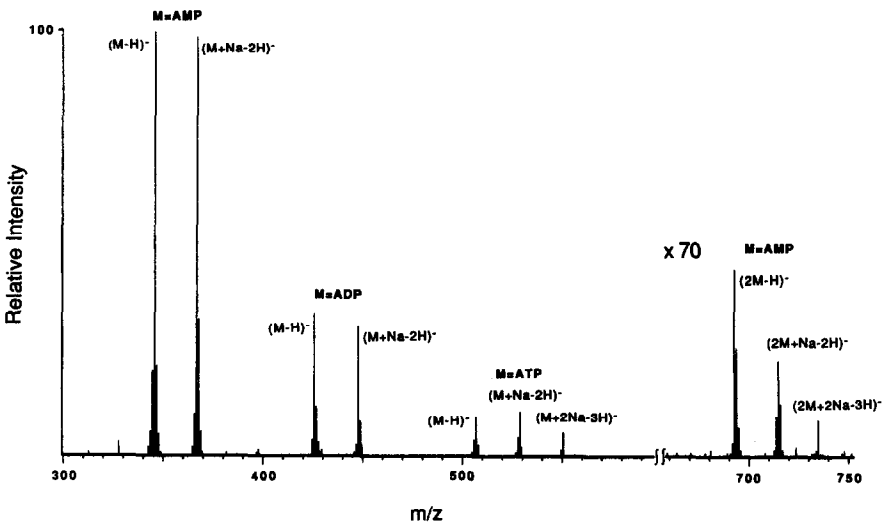

Fig. 2. Negative ion ESI mass spectrum of an equimolar mixture of AMP, ADP and ATP.

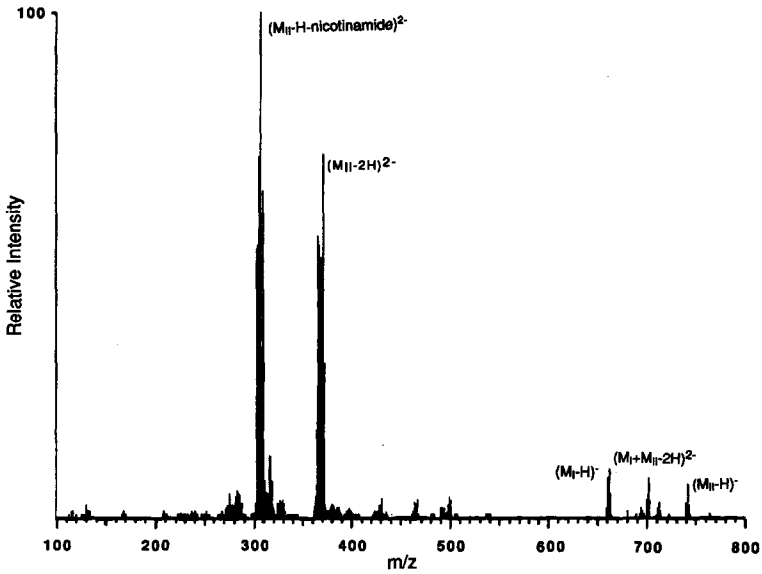

Fig. 3. Negative ion ESI mass spectrum of an equimolar mixture of β-NAD (I) and β-NADP (II).

the sodiated molecular anion $(M+Na-2H)^-$ are of approximately equal relative abundance for AMP, ADP and ATP. In the case of ATP a disodiated molecular anion is also abundant. Some solute clustering is evident in the spectrum, as demonstrated by the appearance of the AMP dimeric species $(2M-H)^-$, $(2M+Na-2H)^-$ and $(2M+2Na-3H)^-$ at approximately 1% relative abundance compared to the molecular anion. No ions of higher m/z were observed. We tentatively attribute such dimer species to self association in solution, since recombination after desorption is unlikely. For dinucleotides, such as β-nicotinamide-adenine dinucleotide and its phosphate analogue (β-NAD and βNADP), as shown in Fig. 3, we observe large signals for the negative ion molecular species for β-NAD and β-NADP, as well as the doubly charged molecular anion for β-NADP. A fragment ion, tentatively the loss of the nictinamide moiety from β-NADP, forms the base peak of the spectrum. A mixed dimeric dianion is also observed which implies contributions of the unmixed dianion dimers. No ions of higher m/z were observed. Multiple charging is also demonstrated in ESI mass spectra of larger oligonucleotides[41].

The positive ion ESI mass spectra of small peptides are dominated by molecular ions (*i.e.*, protonated or cation adducts) which can be either singly or multiply charged. The extent of multiple charging increases as the number of basic amino acids (*i.e.*, arginine, lysine, histidine, etc.) present in the peptide sequence increases. As demonstrated in Fig. 4 for bradykinin ($M_r = 1060$), ESI is a soft ionization technique with little fragmentation evident. The triply protonated molecule is observed, arising from ionization of two basic arginine residues and the N-terminus. The doubly protonated molecular ion constitutes the base peak of the spectrum. A similar spectrum is generated for gramicidin S (Fig. 5), a cyclic decapeptide ($M_r = 1141$) containing two basic ornithine residues.

The extent of multiple charging by ESI can be influenced by the composition of

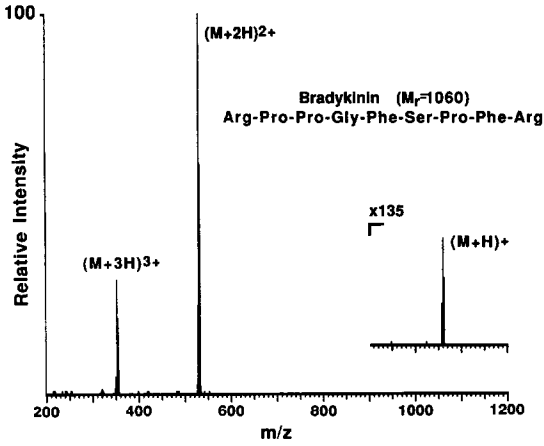

Fig. 4. Positive ion ESI mass spectrum of bradykinin.

the liquid sheath electrode[42] (*i.e.*, the charge distribution is shifted toward lower m/z values with solvents of greater surface tension and/or dielectric constant) as illustrated in a study on gramacidin S with various sheath liquids shown in Fig. 6. For example, the $(M+2H)^{2+}$ to $(M+H)^{+}$ ratio increases by a factor greater than 5 by changing the sheath liquid from isopropanol to methanol. Up to a factor of ten loss in sensitivity, in addition to an increase in the amount of analyte–solvent clustering, results with the use of an isopropanol sheath. The nature of the solvent association

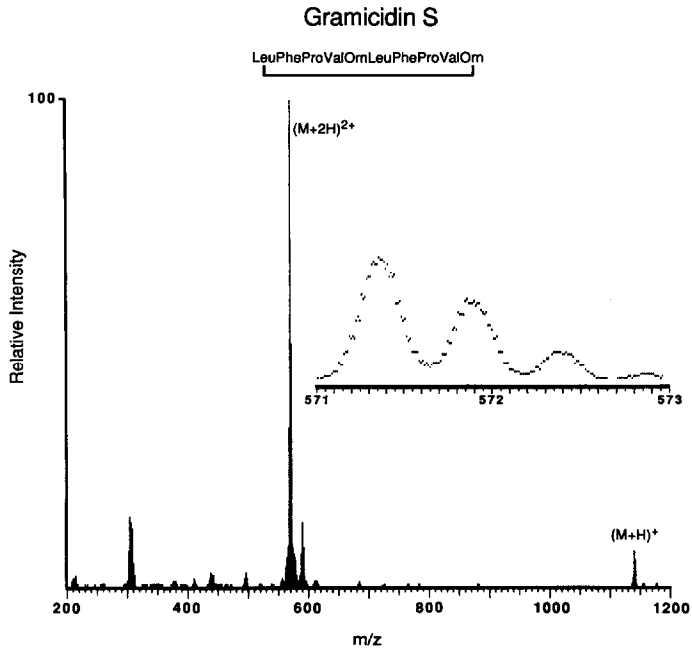

Fig. 5. Positive ion ESI mass spectrum of gramicidin S. Inset shows profile on the $(M+2H)^{2+}$ ion.

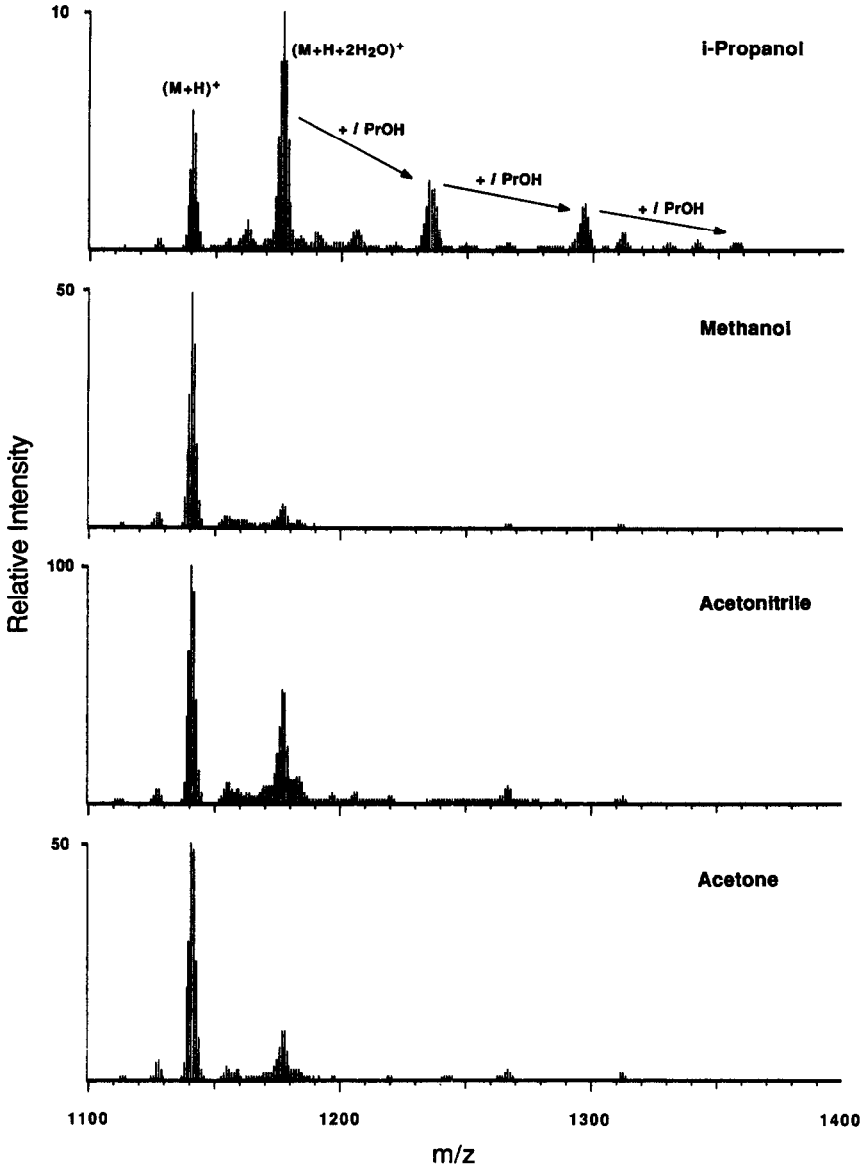

Fig. 6. Positive ion ESI $(M+H)^+$ ion region for gramicidin S with liquid sheath compositions as indicated. For this experiment the mass spectrometer was tuned at substantially less than unit mass resolution.

remains uncertain, but a number of isopropanol ions clearly can become somewhat more strongly associated with the molecular species than generally observed for other solvents.

As molecular weight increases with increasing numbers of basic amino acid residues, the ESI mass spectrum remains within the m/z 1700 range of our quadrupole mass spectrometer. Typical positive ion mass spectra for picomole amounts of pep-

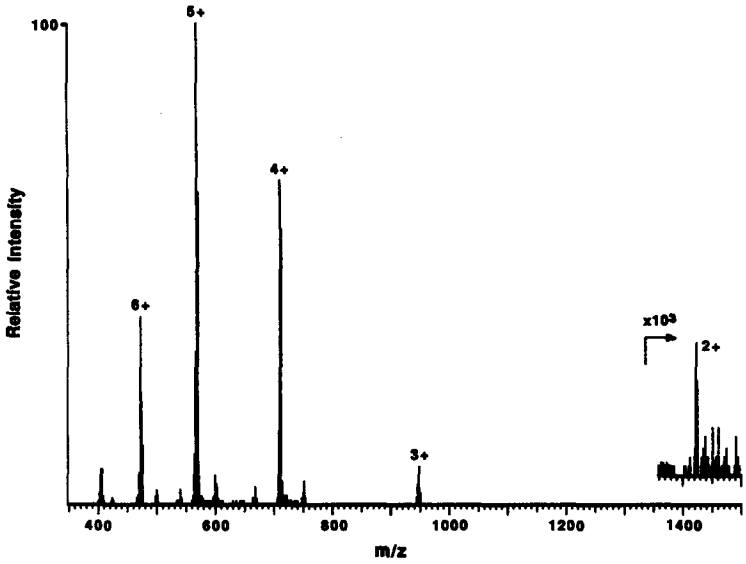

Fig. 7. Positive ion ESI mass spectrum of melittin ($M_r = 2845$).

tides are shown in Figs. 7 and 8 for melittin, a water-soluble 26-residue polypeptide ($M_r = 2845$), and an equimolar mixture of bovine ($M_r = 5734$) and porcine ($M_r = 5778$) insulin, respectively.

For larger protein molecules, a bell-shaped distribution of multiply charged molecular ions dominates the ESI mass spectrum with the distribution maxima gener-

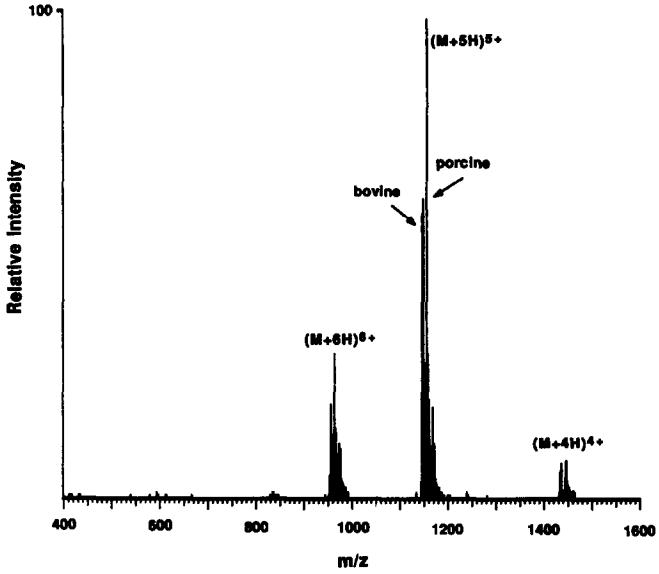

Fig. 8. Positive ion ESI mass spectrum of an equimolar mixture of bovine ($M_r = 5734$) and porcine insulin ($M_r = 5778$).

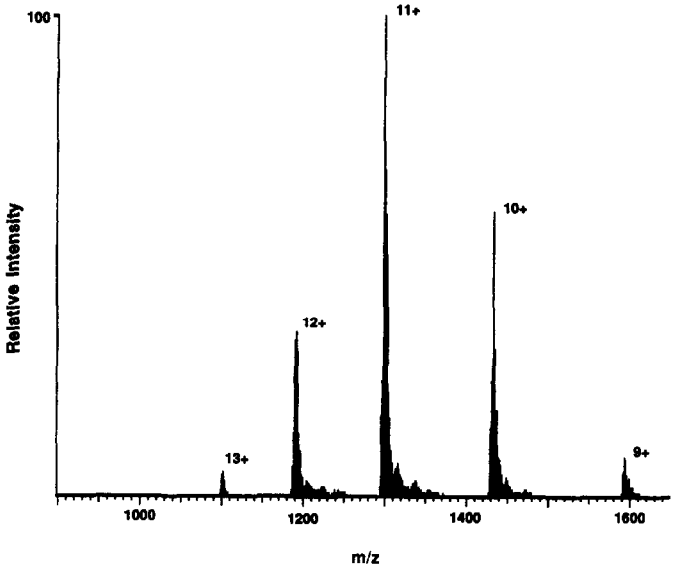

Fig. 9. Positive ion ESI mass spectrum of hen egg lysozyme ($M_r = 14\,300$).

ally observed between *m/z* 500 and 1500, as evident in the mass spectra for hen egg lysozyme ($M_r \approx 14\,300$) and equine heart myoglobin ($M_r \approx 17\,600$), shown in Figs. 9 and 10, respectively. With increasing molecular weight and extent of multiple charging the broadening due to the unresolved isotopic envelope is balanced by the charge

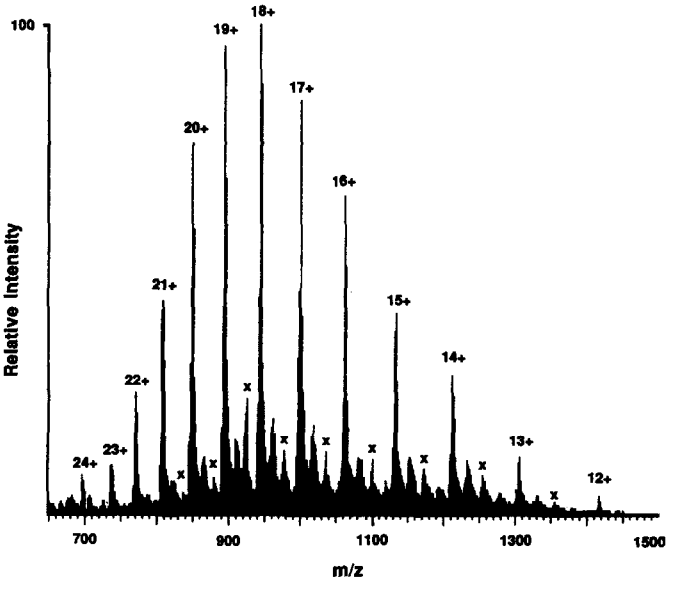

Fig. 10. Positive ion ESI mass spectrum of horse heart myoglobin ($M_r = 17\,600$). Principal ions represent the protein portion ($M_r = 16\,950$) of the molecule with loss of the heme moiety. Ions arising from the intact molecule were marked (X).

state (at a given m/z) to maintain similar peak widths for members of the distribution. Additional small contributions are often evident arising from cationization and solvent association. The latter effects may be minimized by adjustment of temperature and flow-rate of drying nitrogen gas in the ESI interface and increased collison energy (warming) in the interface provided by the nozzle–skimmer bias voltage. While such steps may not always be fully effective, as observed in the electrospray mass spectra of hen egg lysozyme (Fig. 9), in general we operate under conditions such that only strongly associated adducts and actual chemical heterogeneity contribute to the spectra[40]. Myoglobin is composed of a single 153 amino acid polypeptide chain (globin) bound to an iron–heme unit. Multiply charged ions are indicated for both the globin portion and the intact myoglobin molecule in Fig. 10. Over 65+ charges are detected by ESI-MS for a sample of bovine apotransferrin (Fig. 11). The spectrum shows a series of doublets indicating two species present with average molecular weights approximately 76 750 and 77 000 daltons. Elsewhere we have presented ESI mass spectra for proteins exceeding 130 000 daltons[43].

The charge distributions for large molecules are also sensitive to the nozzle–skimmer bias voltage[44], indicating that highly efficient collisionally activated dissociation processes may be occurring for these highly charged molecules in a manner which discriminates on the basis of the charge state of the molecule. More highly charged species (at lower m/z values) are more susceptible to collisional activation, as evident by their apparent dissociation at lower collision energy. For example, melittin yields multiply charged ions up to the 6+ species with the nozzle potential (V_n) below +200 V (Fig. 7) but disappears above this potential. Similarly the $(M+5H)^{5+}$ abundance drops below background at nozzle voltages greater than +370 V. Additional evidence of this effect is seen in the ESI mass spectrum of bovine hemoglobin ($M_r \approx 65\,000$) (Fig. 12). At $V_n = +200$ V the spectrum is dominated by multiply charged ions up to the $(M+25H)^{25+}$ species due to the α- and β-polypeptide chains. Increasing V_n to +300 V causes the multiply charged ions with charges 21+ to 25+ to disappear, shifting the distribution to lower charge state (higher m/z and revealing

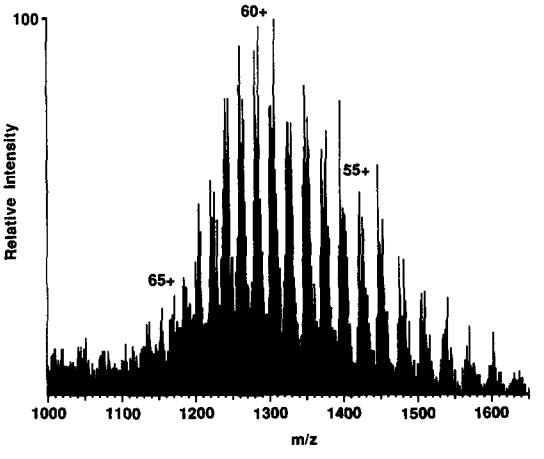

Fig. 11. Positive ion ESI mass spectrum of bovine apotransferrin. Two closely spaced series of multiply charged ions are consistent with a binary mixture of $M_r = 77\,000$ and $M_r = 76\,750$ proteins.

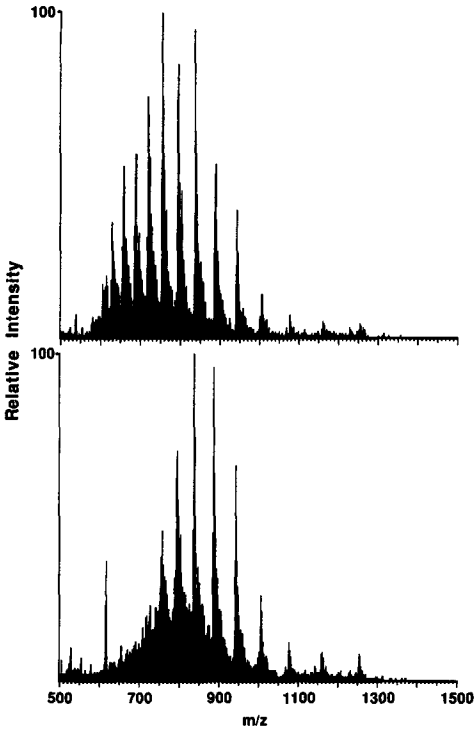

Fig. 12. ESI–MS of bovine hemoglobin ($M_r = 65\,000$) with nozzle potential (top) +200 V and (bottom) +300 V. α = 15 000; β = 15 970.

an ion which may be ascribed to the iron–heme unit at m/z 617. For small peptides such as angiotensin I ($M_r = 1296$) and melittin, singly and multiply charged fragment ions (generally A, B and Y type sequence ions) are clearly evident with V_n above +200 V[44]. The potential for tandem mass spectrometry (MS–MS)[45] with collisionally activated dissociation of multiply charged molecular ions from large proteins is an exciting prospect for structural elucidation. Initial results on the MS–MS of multiply charged ions produced by ESI are presented elsewhere[46].

An example of a preliminary attempt at CZE–MS of peptides and proteins is shown in Fig. 13. Previous studies of CZE of proteins in untreated fused silica capillaries (with UV detection) have resulted in broad, tailing peaks due to protein adsorption on active sites of the negatively charged silica wall[3]. However, as demonstrated by Lauer and McManigill[20], using buffered solutions with the pH above the isoelectric point of the peptides and proteins allows both the sample and the capillary wall to be negatively charged and mutually repulsive. The 2 pmol per component separation shown in Fig. 13 was performed with a 0.01 *M* sodium phosphate–sodium hydroxide buffer (pH 11). CZE–MS separations of proteins as large as myoglobin have been demonstrated in our laboratory[43]. Although improved sensitivity and resolution are desired, these CZE–MS results are encouraging initial steps for the electrophoretic separation and mass spectrometric detection of large biomolecules.

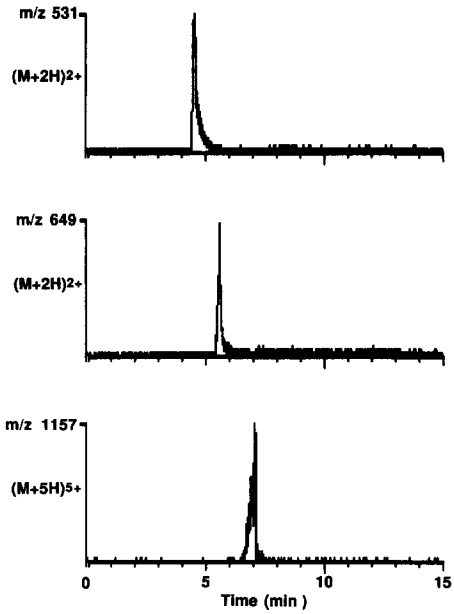

Fig. 13. Single ion electropherograms for a CZE–MS separation of a synthetic mixture of bradykinin ($M_r = 1060$), angiotensin I ($M_r = 1296$) and porcine insulin ($M_r = 5778$) with use of a 0.6 m × 100 μm I.D. fused-silica capillary at 15 kV.

CONCLUSIONS

The ability to ionize molecules of high molecular weight has opened new applications of MS in the biological sciences. The production of multiply charged molecular ions by ESI increases the detectable mass range by a factor equal to the number of charges. The effect of this is to bring into the mass range (mass/charge) of conventional mass spectrometers macromolecules whose relative mass approaches or exceeds the limits of any current high MS technique while solving the major problem associated with such applications by providing efficient ionization, detection, and (possibly) collisionally activated dissociation.

In many cases, molecular weight information on large molecules provided by MS may be sufficient to solve a problem[47]. Alternatively, however, collisionally activated dissociation of large multiply charged ions is an exciting approach that may lead to more efficient fragmentation and provide a route to obtaining structural information. Preliminary results show that the high charge state of ESI produced ions allows the extension of MS–MS methods to much larger compounds than previously tractable[48].

The speed and reliability of capillary electrophoretic methods have allowed peptide and protein separation and characterization to be nearly routine. Although CZE–MS is a promising methodology, the related technique of CE–MS should also be practical and provides an attractive combination for biomolecule analysis. High-resolution CGE[49] and CIEF[50] with ESI-MS should also be viable and feasible in the near future.

As stated in a recent review article[51], "mass spectrometry is on the verge of becoming a primary research tool in the biological sciences". Coupled with high-resolution separation techniques, the ability of CE–MS and CE–MS–MS to provide structure determination of larger and larger biomolecules is expected to become of premier importance to the biological science community.

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