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Corona Discharge in Charge Reduction Electrospray Mass Spectrometry

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Corona discharge is applied to charge reduction electrospray mass spectrometry for the analysis of complex mixtures of biological molecules. Recent work has described a method of charge reduction (reducing the charge states of analyte ions generated by the electrospray process) employing the radioactive isotope ^{210}Po to produce neutralizing species. A variation to this approach is presented, in which charge neutralization is mediated by ions produced in a corona discharge. Varying the corona discharge voltage controls the current and the degree of charge reduction, providing predominantly singly charged ions that are detected by a commercial electrospray time-of-flight mass spectrometer. This technique provides charge reduction for the simplification of ESI spectra, without need for any radioactive material.

Developments in ionization methods have revolutionized the field of mass spectrometry. Gas-phase ions of high molecular weight biopolymers can be produced by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)[1] and electrospray ionization mass spectrometry (ESI-MS).[2] In MALDI, analyte is mixed with a small organic compound (matrix) and dried on a sample plate; the matrix crystallizes while containing the analyte. These crystals are then irradiated with a short laser pulse at a wavelength close to a resonant absorption band of the matrix molecules, but not close to absorption bands of the analyte. The matrix undergoes a desorption process, producing gas-phase matrix ions along with desorbed and ionized analyte. In ESI, a small capillary is maintained at a high electrical potential, opposite a metal plate with a small orifice held near ground. Buffer containing the analyte is pumped through the capillary, producing a fine aerosol. The ions then are either ejected from the droplet[3] or are completely desolvated into multiply charged analyte ions.[4]

Despite the utility of these approaches, they possess several limitations. MALDI typically produces primarily singly charged ions, making it relatively well suited to the analysis of mixtures, as a single peak for each component of the mixture yields a simple and well-resolved mass spectrum. MALDI's disadvantages include variations in ion yield due to the chemical nature and size of the analyte and to the portion of the sample spot selected.[5] In some cases, MALDI desorption is also damaging to the analyte.[6,7] ESI is a gentler method and provides analyte in a continuous homogeneous stream in a repeatable fashion.[8] ESI can also be combined with on-line liquid-phase separation techniques such as HPLC and capillary electrophoresis,[8] while MALDI samples must be purified off-line. In contrast to MALDI, ESI produces a variety of charge states for each single analyte species present in the sample.[9−12] This difference makes ESI generally unsuitable for the analysis of mixtures because of excessive overlap in the spectral features from the various components. Computer algorithms have been used to deconvolute ESI spectra of simple mixtures,[13−18] but spectral resolution limits their utility as complexity increases.[19]

Two previous methods of charge reduction have either modified the solution conditions[20−22] or utilized gas-phase reactions within an ion trap spectrometer.[19,23,24] It has been shown that ESI can be made to produce mainly singly and doubly charged ions with the addition of organic acids or bases to the buffer. The ion trap approach separates the ionization and charge neutralization processes in order to independently control the degree of neutralization; this approach does not, however, allow predictable and controllable manipulation of the charge states of all of the components in a mixture.

Recently we described polonium-based charge reduction electrospray mass spectrometry (CREMS).[25,26] A ^{210}Po α particle source is used to generate bipolar (i.e., both positively and

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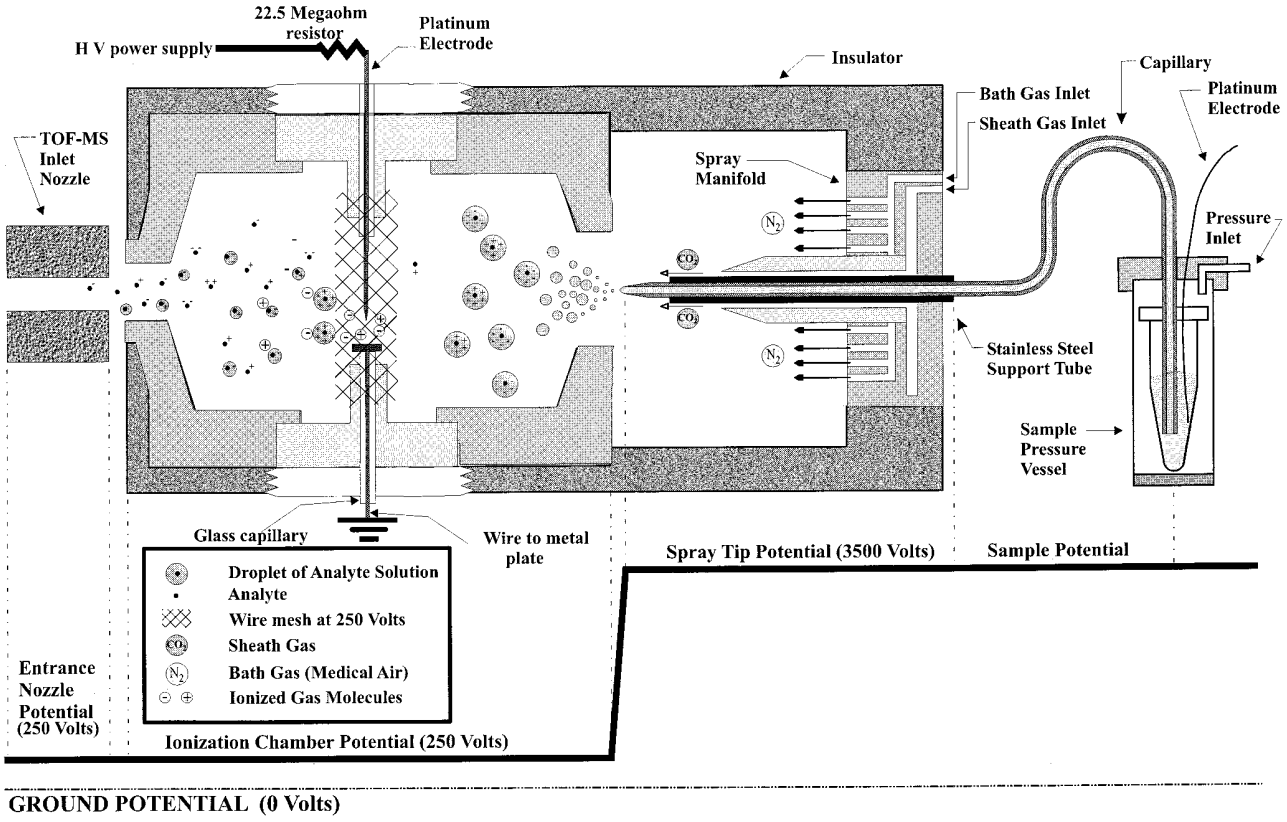

Figure 1. Schematic diagram of ESI source and corona discharge in the neutralization chamber.

negatively charged) ions in a "neutralization chamber" where they undergo reactions with electrospray-generated multiply charged analyte ions. The charge state of the analyte may be controlled to yield predominantly singly charged ions, which are subsequently drawn into the mass spectrometer at its inlet. CREMS successfully reduces charge in either positive or negative spray mode; this was demonstrated on mixtures of proteins and mixtures of DNA. A disadvantage of polonium-based CREMS is the need for a radioactive source for generation of charge-reducing species. In many settings, the use of radioactive materials is forbidden, and in others, proper licensing is difficult to obtain.

In this report, we describe a method of charge reduction based on corona discharge that obviates the need for a radioactive source, while maintaining the benefits of CREMS. A corona discharge creates ions from the bath gas that consists of medical air. The resultant ions react with analyte ions, reducing the charge states. Mixtures can be analyzed as anions or cations by merely changing the polarity of the corona discharge and the operating polarity of the instrument. The approach described here is readily implemented by a simple modification to an existing ESI source and is adaptable to virtually any ESI instrument, time-of-flight (TOF) being preferred because of its ability to analyze ions with large m/z values.

EXPERIMENTAL SECTION

An equimolar mixture of seven oligonucleotides (ranging from 15 to 51 nt in length) was diluted in a buffer of 1:2 H_2O/MeOH, 200 mM 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (adjusted to pH 7 with triethylamine)[27] to a concentration of 5 μM. All oligodeoxynucleotides were obtained reversed-phase HPLC purified from Integrated DNA Technologies, Inc. (Coralville, IA). The 51-mer mixed-base oligonucleotide sequence is as follows: 5′ (TGT AAA ACG ACG GCC AAG CTT GCA TGC CTG CAG GTC GAC TCT AGA) 3′. All other oligonucleotide sequences (15, 21, 27, 33, 39, and 45 mer) are identical to the 51-mer sequence, beginning at the 5′ end and ending at the indicated length. All protein samples were obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Protein samples were diluted in a buffer of 1:1 (v/v) H_2O/CH_3CN, 1% (v/v) acetic acid to a concentration of 5 μM.

The instrument (Figure 1) has a positive-pressure ESI source and a charge neutralization chamber, followed by an orthogonal TOF mass spectrometer. The ESI source consists of a 24-cm-long fused-silica polyimide-coated capillary (150 μm o.d., 25 μm i.d. No. 2001145, Polymicro Technologies Inc., Phoenix, AZ) with a conically ground spray end. A 0.5-mL polypropylene PCR tube contains the analyte solution that is forced through the opening of the capillary by a positive pressure of 10 psi (70 kPa) to produce a typical flow rate of 0.17 μL/min. The solution is maintained at a potential of 4.5 kV for positive ion mode and −3.5 kV for negative mode by means of a platinum electrode immersed in the sample. A stainless steel tube, (1.5 mm i.d.) concentric with the silica capillary, carries sheath gas (0.8 L/min CO_2) to stabilize the spray against corona discharge at the capillary tip.[28,29] Bath gas (3 L/min

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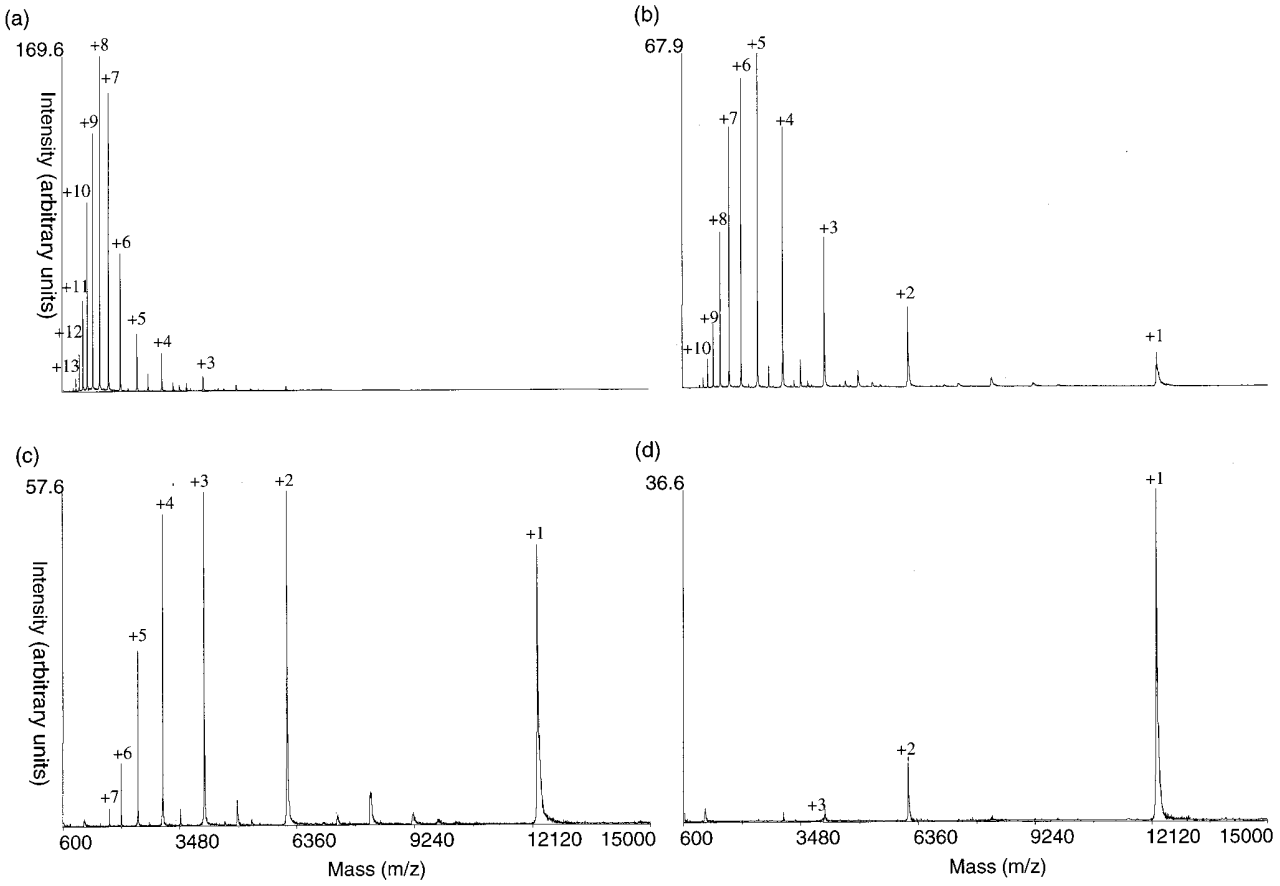

Figure 2. Modulation of charge-state reduction of cytochrome *c* (5 μM in 1:1 H_2O/CH_3CN, 1% acetic acid) as a function of corona discharge voltage: (a) 0 (control), (b) −1, (c) −1.25, and (d) −1.75 kV.

medical air) carries the spray into the neutralization chamber, which consists of an aluminum cylinder with a diameter of 19 mm and a length of 4.3 cm that is insulated from the spray tip with a Teflon coating. There are 31-mm-diameter holes cut into the top and bottom of the cylinder and casing, into which aluminum disks that hold the corona discharge electrodes are inserted. The corona discharge is created in the "point to plane" geometry,[30] using a platinum wire (0.5 mm diameter) ground to a 10-μm radius point[31] and a flat stainless steel disk connected to ground. The platinum electrode is connected to a high-voltage power supply through a 22.5 -MΩ current limiting resistor. A positive or negative voltage can be put on the wire, depending on the desired corona mode. The gap length can be adjusted by sliding the platinum wire inside the glass capillary; 3 mm was used in obtaining the reported spectra. A transmission screen forms a cylinder around the corona area and is connected to the neutralization chamber potential. This screen forms a Faraday cage that contains the electric field generated by the high-potential platinum wire, so that the neutralization chamber is kept field free. The mesh does allow partial transmission of neutralizing ions, allowing analyte and reducing species to react outside of the screen.

The analyte exits the neutralization chamber through a 3-mm-diameter outlet, and a portion is drawn into the entrance nozzle of the mass spectrometer held equipotential to the neutralization chamber. The orthogonal TOF mass spectrometer (PerSeptive Biosystems Mariner Biospectrometry Workstation) provides an m/z range up to 25 000 atomic mass units (amu). The ESI source and neutralization chamber described above was put in place of the instrument's original electrospray chamber.

RESULTS AND DISCUSSION

The protein cytochrome *c* (12 360 amu) was analyzed in positive ion mode after varying degrees of neutralization by corona discharge (Figure 2). Tuning of the neutralization was achieved by adjusting the voltage applied to the point to plane corona source. With no voltage applied, a typical ESI spectrum was observed, with charge states +3 to +13. As the voltage increased, the spectra exhibited lower and fewer charge states, until at 1750 V, predominantly singly charged species are seen. As reported previously, signal intensity decreases with increased charge reduction; beyond 1750 V spectral simplification is negligible compared to the signal drop. Analysis of the molecular weights of each charge state showed that charge reduction occurs as a result of proton-transfer reactions; similar results were demonstrated in negative ion mode.[26]

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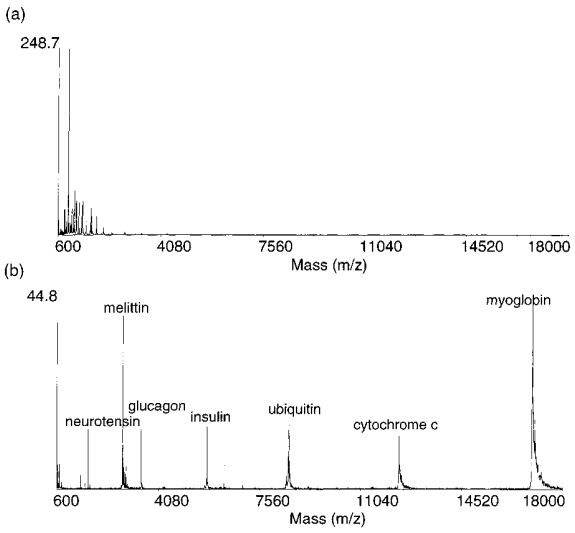

Figure 3. Mass spectra of equimolar seven-component protein mixture (a) without charge reduction and (b) with charge reduction (−1.75 kV).

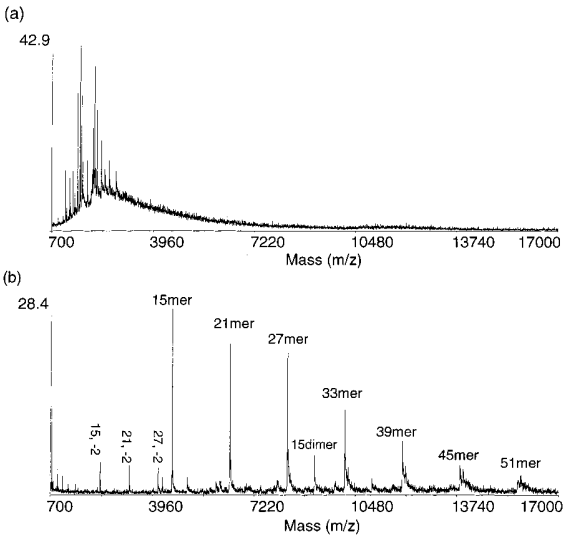

Figure 4. Mass spectra of equimolar seven-component DNA mixture (a) without charge reduction and (b) with charge reduction (+1.75 kV).

Each of the spectra presented in this paper was collected over a 250-s time period at a spectral acquisition rate of 10 kHz; they were then smoothed by a convolution with a Gaussian function included in the software supplied with the spectrometer. Each run consumed 0.71 μL of sample at a concentration of 5 μM for each component.

The effect of corona discharge charge reduction was demonstrated on a mixture of proteins (Figure 3). An equimolar mixture of seven proteins (neurotensin, 1672.9 amu; melittin, 2847.5 amu; glucagon, 3482.8 amu; bovine insulin, 5736.6 amu; bovine ubiquitin, 8564.8 amu; equine cytochrome *c*, 12 360 amu; apomyoglobin, 16 951 amu) was prepared and analyzed in positive ion mode. The mass spectrum obtained with no charge reduction (Figure 3a) corresponds to a typical ESI spectrum for such a mixture; it is complex, with many peaks in a small m/z window. A negative corona discharge (negative potential applied to the platinum wire) was used to produce a less convoluted spectrum, exhibiting predominantly singly charged peaks (Figure 3b).

An equimolar mixture of seven oligonucleotides (15 mer, 4586.1 amu; 21 mer, 6440.3 amu; 27 mer, 8293.5 amu; 33 mer, 10 147 amu; 39 mer, 12 002 amu; 45 mer, 13 856 amu; 51 mer, 15 709 amu) was prepared and analyzed in negative ion mode (Figure 4). A positive corona discharge was used to reduce the spectrum, giving a much clearer picture of the components in the mixture (Figure 4b), albeit characterized by the same falloff in signal with increasing m/z as reported previously.[26]

CONCLUSIONS

Corona discharge charge reduction requires no radioactive source, is gentle, is easily coupled with on-line purification, and is also suitable for mixture analysis as is MALDI. While methods to improve the process are being explored, corona discharge charge reduction is already a simple and useful electrospray technique. The elimination of a need for radioactive material from the CREMS process should facilitate adoption of charge reduction for the simplification of ESI mass spectra.

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