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Alternately Pulsed Nano-electrospray Ionization/Atmospheric Pressure Chemical Ionization for Ion/Ion Reactions in an Electrodynamic Ion Trap

Xiaorong Liang, Yu Xia, and Scott A. McLuckey*

Department of Chemistry, Purdue University, West Lafayette, IN 47907-2084

Abstract

The alternate operation of nano-electrospray ionization (nano-ESI) and atmospheric pressure chemical ionization (APCI), using a common atmosphere/vacuum interface and ion path, has been implemented to facilitate ion/ion reaction experiments in a linear ion trap-based tandem mass spectrometer. The ion sources are operated in opposite polarity modes whereby one of the ion sources is used to form analyte ions while the other is used to form reagent ions of opposite polarity. This combination of ion sources is well-suited to implementation of experiments involving multiply charged ions in reaction with singly charged ions of opposite polarity. Three analytically useful ion/ion reactions types are illustrated: the partial deprotonation of a multiply protonated protein, the partial protonation of a multiply deprotonated oligonucleotide, and electron transfer to a multiply protonated peptide. The approach described herein is attractive in that it enables both single proton transfer and single electron transfer ion/ion reaction experiments to be implemented without requiring major modifications to the tandem mass spectrometer hardware. Furthermore, a wide range of reactant ions can be formed with these ionization methods and the pulsed nature of operation appears to lead to no significant compromise in the performance of either ion source.

Keywords

Pulsed Atmospheric Pressure Chemical Ionization; Pulsed Nano-electrospray ionization; Ion/Ion reactions; Linear ion trap

INTRODUCTION

Gas-phase ion/ion reactions are being explored for their analytical utility in the analysis of biomolecules^{1,2}. This exploration has been enabled by the multiple charging phenomenon associated the various forms of spray ionization³ applied to relatively large biomolecules, such as peptides, proteins, and oligonucleotides. Most ion/ion reaction studies involving biomolecules have either mixed the oppositely charged reactant ions at or near atmospheric pressure prior to sampling ions into a mass spectrometer or the ions of oppositely polarity have been admitted into an electrodynamic ion trap for subsequent mutual storage and reaction. The former approaches have employed a spray ionization method as one of the ion sources and either a discharge^{4,5,6}, radioactive source^{7,8}, or another spray ionizer⁹ as the other ion source. In these approaches, the ion sources have been operated simultaneously and, in general, continuously. In the ion trap based approaches, the ion sources need not be operated simultaneously and, in general, ions have been admitted into the electrodynamic ion trap in

Address reprint requests to: Scott A. McLuckey, Department of Chemistry, Purdue University, West Lafayette, IN, USA 47907-2084. Phone: (765) 494-5270, Fax: (765) 494-0239, E-mail: mcluckey@purdue.edu.

serial fashion. The ion trap based approaches allow for the use of ion/ion reactions within the context of multi-stage mass spectrometry (MS^n) experiments. In such experiments, it is usually desirable to be able to control precisely the admission and removal of reactant ions in a sequenced fashion.

A variety of approaches have been employed to form and admit ions of opposite polarity into electrodynamic ion traps. In virtually all cases, the spray ionization source has been used to inject ions via an end-cap electrode, in the case of three-dimensional ion traps, or along the axial dimension of a linear ion trap. The ions of opposite polarity have been formed via electron ionization¹⁰, *in situ* chemical ionization¹¹, or laser desorption¹² within the ion trap or with one of a variety of external ion sources. Examples of external means of ionization have included atmospheric sampling glow discharge ionization^{13,14,15}, chemical ionization^{16,17}, and spray ionization^{18,19}. In most instances, distinct ion sources have been used with ion injection via the ring electrode, the opposite side of a linear ion trap, or via a turning quadrupole that directs ions into an ion trap alternately from distinct ion sources. We have recently described the use of two arrangements that allow for the admission of ions of opposite polarity via the same ion path. One is the use of sonic spray ionization²⁰, which is a spray ionization method that produces ions of opposite polarity simultaneously. This approach involves the continuous operation of the ion source with alternating potentials applied to the vacuum/atmosphere interface and ion path for admission of each reactant species into a linear ion trap²¹. Recently, we described a second approach that involves the use of alternate pulsing of two spray ionizers for formation and transmission of oppositely charged reagents²². The dual sprayer arrangement has the advantage over the sonic spray source of allowing for the independent optimization of solvent and ionization conditions for each reactant ion as well as greater ionization efficiency afforded by nano-electrospray. However, both of these approaches are restricted to forming ions via spray ionization. It is desirable to expand the range of ionic species available for ion/ion reaction studies. To this end, it is desirable to be able to access ionization methods with complementary capabilities in ion formation.

In this note, we describe the use of pulsed nano-electrospray for the formation of multiply charged ions and pulsed corona discharge atmospheric pressure chemical ionization (APCI)²³ for formation of singly charged reactant ions. These ion sources share a common atmosphere/vacuum interface as well as a common ion path into a linear ion trap. This arrangement, therefore, does not require particularly specialized instrumentation but does require that the instrument control software allow for initiating each ion source and for applying the appropriate potentials to the ion path to admit the oppositely charged reactants during the course of a single ion/ion reaction experiment. Several of the key analytical experiments involving either single proton transfer or single electron transfer are demonstrated with this arrangement to illustrate its flexibility. This approach also offers significant advantages over a previous design that employed a separate glow discharge ionization source with ion injection via the side of the third quadrupole of the triple quadrupole instrument¹⁵. Trapping of ions injected via the side of the quadrupole (i.e., radially) is expected to be several orders of magnitude lower than trapping of ions injected axially. Furthermore, fragmentation of ions injected radially is commonly observed. Hence, the ability to admit axially both ion polarities into the linear ion trap has significant advantages over the previously reported approach.

EXPERIMENTAL SECTION

Materials

Methanol and glacial acetic acid were purchased from Mallinckrodt (Phillipsburg, NJ). Horse heart myoglobin and m-dinitrobenzene were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. The peptide KGAILKGAILR was synthesized by SynPep (Dublin, CA). The oligonucleotide, 5'-d(C)₆-3', was synthesized by Integrated DNA

Technologies (Coralville, IA) and used without further purification. Perfluoro(methyldecalin) (PMD) and the proton sponge (*N,N,N',N'*-tetramethyl-1,8-naphthalenediamine) were obtained from Aldrich (Milwaukee, WI). Solutions of peptides or proteins were dissolved to 5 μM in a 50/50 methanol/water solution with 1% acetic acid added for positive nano-electrospray ionization (nano-ESI). The oligonucleotide was dissolved to 10 μM in de-ionized water for negative nano-ESI.

Apparatus and Procedures

A home-built APCI and nano-ESI source was positioned directly in front of the atmosphere-vacuum interface of a modified Sciex 2000 Q TRAP mass spectrometer as shown in Figure 1.

The two ionization sources, typically a nano-ESI emitter for analyte ion formation and an APCI source for reagent ion formation, were clamped separately on a capillary holder. First, the position of the nano-ESI emitter was optimized with a distance 10–20 mm from the sampling orifice. The APCI Pt wire (0.1 mm O.D.) was then placed in a parallel fashion, with its position (3–10 mm from the nano-ESI emitter) manually adjusted to achieve the highest reagent ion signals. The nano-ESI solutions were loaded into nano-spray emitters with tip orifice diameters of 30 μm , which were pulled from borosilicate capillaries (1.55 mm O.D., 0.86 mm I.D.) using a P-87 Flaming/Brown micropipette puller (Sutter Instruments, Novato, CA). A stainless steel wire was inserted into the back of the capillary for electrical contact with the analyte solution. The reagent solids or solutions for APCI were placed in a 0.5 ml Eppendorf LoBind microcentrifuge tube which is attached directly under the sampling hole of the curtain plate. The vertical distance between the vial and the sampling hole is about 3 mm, see Figure 1. High voltages applied to either the APCI Pt wire or the nano-ESI emitter were optimized separately and were independently generated by a high voltage power supply from the instrument and a fast high voltage pulser (GRX-1.5K-E, Directed Energy Inc., Fort Collins, CO). The two power supplies were triggered from the software, so that the high voltages were alternately switched on (off) in each scan. The switching intervals for the oppositely charged high voltage can be as short as 10 ms; however, in the experiment it was set to 50 ms to allow injected ions to be collisionally cooled in the ion trap. The “off” voltages for each emitter were 0 V and the “on” voltages were between ± 1.2 –2 kV for the nano-ESI emitter, and ± 3 kV for APCI Pt wire. This pulsed source design enables stable ion production from each ion source and allows ions of opposite charge to be generated and injected into the mass spectrometer separately.

All experiments were performed using a prototype version of a Q TRAP mass spectrometer²⁴ (Applied Biosystems/MDS SCIEX, Concord, Ontario, Canada) that has been modified for ion/ion reactions²⁵. All experiments were controlled by MS Expo 1.7.8 software, a research version of software provided by MDS SCIEX. The Q TRAP electronics were modified to allow superposition of an auxiliary RF signal to the containment lenses of the third quadrupole array of the instrument. The auxiliary RF signal applied to the containment plates was optimized for frequency at fixed 100 V_{0-p} for each ion/ion reaction experiment. For a typical ion/ion reaction experiment, the high voltage power supply connected to the nano-ESI wire was pulsed on to generate one polarity of ions. Analyte ions were isolated by Q1 in the RF/DC mode and injected axially into the Q3 quadrupole array with nitrogen as buffer gas at a pressure of 3.5×10^{-5} Torr. The ions were cooled in Q3 for 50 ms, during which time the high voltage on this emitter was turned off. The ions were sometimes subjected to a second isolation by using Q3 in the RF/DC mode in cases in which there were signals due to the fragmentation of the ions arising from passage through the high pressure Q2 cell (6–8 mTorr). After the cooling step, the power supply connected to the APCI wire, which was operated in polarity opposite to that for the nano-ESI emitter, was triggered on while the DC potentials applied to the ion path before Q3 were adjusted to allow ions of the other polarity to enter Q3 at relatively low kinetic energies (Q3 DC offset was about 10 V attractive relative to the Q0 DC offset).

During this period, the DC potentials on the Q3 containment lenses, as well as on the Q3 rods themselves, were adjusted to a common value while an auxiliary RF voltage was applied to exit containment lens. In the subsequent mutual ion polarity storage step, the potential applied to the APCI emitter was turned off and the auxiliary RF signals were applied to both containment lenses to store ions axially. After a defined period of mutual storage, the residual reagent ions were ejected from Q3 by applying attractive DC potentials to the Q3 containment lenses while the auxiliary RF signals were terminated. The product ions of interest were stored in Q3 for 50 ms before they were subjected to mass selective axial ejection (MSAE)²⁶ using a supplementary RF signal at frequency 380 kHz, or at a frequency selected to give the desired mass range extension. The spectra shown here were typically the averages of 20–100 individual scans. Note that the ion/ion reactions could also be effected in the higher pressure Q2 array where ion/ion reaction rates are at least an order of magnitude higher than those generally observed in Q3²², presumably due to better spatial overlap of the oppositely charged ions. However, the instrument can currently be configured for mutual ion polarity storage in either Q2 or in Q3 and conversion between the two requires removal of the triple quadrupole array from the vacuum system. The instrument was configured for mutual ion storage in Q3 to accommodate unrelated studies being conducted in parallel with this work.

RESULTS AND DISCUSSION

The pulse-to-pulse reproducibility for the alternating pulsed ionization approach described in the experimental section is reflected by the traces in Figure 2, which shows signals from cationic and anionic reagents upon repeated pulsing.

In the case of nano-ESI, it was noted that ions continued to evolve from the nano-ESI source for roughly 50 ms after the voltage applied to the wire was reduced, reflecting a time delay before the spray process completely terminated. To avoid the continued injection of nano-ESI ions after the nominal injection period, a stopping potential was applied to an aperture plate at the entrance of the mass resolving quadrupole immediately after the nano-ESI ion accumulation period. The data in Figure 2 reflect well the reproducibility noted in these experiments. The performance of the pulsed nano-ESI source is expected to be a function of the quality of the emitter. In general, the pulse-to-pulse reproducibility for nano-ESI is similar to that shown in Figure 2 in our experience. Pulsed operation of APCI is not uncommon, particularly in conjunction with ion mobility spectrometry²⁷, and has been found to show good pulse-to-pulse reproducibility in our hands as well. While it is possible that species admitted for APCI of sufficiently high vapor pressures and with relevant chemical characteristics (e.g., high basicities, high acidities, high surface activities, etc.) might give rise to a matrix effect in the nano-ESI of the analyte of interest, no evidence of the presence of the compounds used in this study was noted in the spectra acquired from nano-ESI. During operation of the corona discharge, however, strong signals arising from one or more background species were noted, particularly at high nitrogen curtain gas flow rates. It was determined that these background species originated from the curtain gas and are likely to be plasticizers evolving from the plastic line used to feed the curtain gas into the interface region. Efforts were not made to identify the ions from these compounds, although we determined that they reacted via proton transfer in both the positive and negative ionization modes. The abundances of these species could be minimized by reducing the curtain gas flow rates and, under such conditions, did not prevent formation of the reagent ions of interest.

APCI is an appropriate ionization method for forming singly charged reagent (or analyte) ions and is clearly compatible with the hardware used to transfer ions formed via electrospray. We illustrate here the utility of the nano-ESI/APCI combination with examples of a few key types of ion/ion experiments. Proton transfer ion/ion reactions, for example, have been demonstrated

both for $n+1-$ and $n-1+$ combinations. Figure 3 illustrates the charge state reduction of multiply-protonated horse heart myoglobin via reaction with anions derived from PMD.

Figure 3a shows the positive ion mass spectrum of myoglobin and Figure 3b shows the negative ion mass spectrum derived from PMD. Figure 3c shows the post-ion/ion reaction positive ion mass spectrum collected after the $[M+17H]^{17+}$ ion of myoglobin was subjected to reaction with the PMD anions for 1.3 s. PMD has been used previously for deprotonation of peptide and protein cations and is particularly well-suited to this application due to its relatively high mass (F.W. = 512), which is desirable for ion/ion reactions involving high mass molecules, and its low propensity for attachment to cations. As a rule, anions derived from perfluorocarbons have proven to be good proton abstraction reagents. The ability to form readily PMD anions with APCI enables all of the key single proton transfer reaction experiments developed for protein identification and characterization. These include, for example, the application of proton transfer reactions to ions derived from electrospray of protein mixtures²⁸, charge state reduction of product ions following dissociation for simplification of spectral interpretation²⁹, ion parking³⁰ and parallel ion parking³¹. PMD anions have been used successfully in all of these applications.

Charge state manipulation of multiply-charged anions derived from bio-molecules has been effected both via electron transfer³² and proton transfer³³. The protonated form of the proton sponge (i.e., *N,N,N',N'*-tetramethyl-1,8-naphthalenediamine) is among the most useful proton transfer reagents that we have studied for reducing charge states of multiply-charged anions³⁴. This ion is readily formed via APCI and an example of its application is demonstrated in Figure 4. This figure illustrates the charge state reduction of the $[M-4H]^{4-}$ ion derived from 5'-d(CCCCCC)-3'. Figure 4a shows the isolated oligonucleotide cation prior to ion/ion reaction, Figure 4b shows the positive ion mass spectrum of the cations derived from the proton sponge and Figure 4c shows the post-ion/ion reaction negative ion spectrum after the ions of Figure 4a and 4b were allowed to react in Q3.

There is a noticeable degree of fragmentation associated with the ion/ion reactions that give rise to the data of Figure 4c, which is not uncommon for oligonucleotide anions³³. The fact that these reactions were conducted in Q3, where the bath gas pressures are relatively low, also likely contributes to the observation of fragmentation because the collisional cooling rates of the bath gas are expected to be directly related to bath gas pressure³⁵. The protonated proton sponge is among the softest reagents thus far identified for the manipulation of the charges of multiply charged anions and it is significant that it is readily formed with the pulsed APCI source.

A major motivation for the use of the pulsed APCI/nano-ESI arrangement is the facility with which it can be used to access ion/ion electron transfer reactions. Negative ion APCI is expected to be able to form essentially all of the anions that have been identified to date as having a tendency to transfer electrons to multiply-charged peptide and protein cations^{16,17,36}. Figure 5 shows the post-ion/ion reaction positive ion data collected for the reaction of the $[M+3H]^{3+}$ ion of KGAILKGAILR with the molecular anion of 1,3-dinitrobenzene.

The fragment ions arise from dissociation following electron transfer to the polypeptide ion in a process referred to as electron transfer dissociation (ETD)¹⁶. The identities and relative abundances of the ETD fragments observed in Figure 5 are very similar to those noted in a three-dimensional quadrupole ion trap for this and other reagent anions³⁶. The APCI source has also been shown to be capable of forming abundant anions from several other reagents already known to give rise to ETD, such as nitrobenzene and diazobenzene (data not shown). Hence, this approach will allow for a high degree of flexibility in the selection of reagent anions for ETD.

CONCLUSIONS

The alternately pulsed ESI/APCI approach is a straightforward and robust means for forming ions for either $n+/1-$ or $n-/1+$ ion/ion reactions. The ability to use a common vacuum/atmosphere interface and ion path is particularly attractive because this requires no major modifications to the instrument hardware. However, the instrument control software must be able to support the coordination of the ion source pulsing and the establishment of appropriate conditions for transmitting sequentially the ions of opposite polarity. A particularly important capability of the ESI/APCI approach is the facility with which electron transfer ion/ion reactions can be implemented. The previously described alternately pulsed ESI emitters that share a common vacuum/atmosphere interface can facilitate implementation of single and multiple proton transfer ion/ion reactions but no electron transfer reagents readily formed via electrospray have yet been identified. The dual ESI and ESI/APCI sources are therefore complementary. Either can be used for single proton transfer studies. Charge inversion reactions, which involve multiple proton transfers, are most readily implemented with the dual ESI approach whereas ETD is more readily implemented with the ESI/APCI approach. However, the use of pulsed ion sources for the generation of analyte and reagent ions is not necessarily restricted to two ion sources. Provided the necessary power supplies can be gated via the instrument software, it should be possible to incorporate additional emitters. For example, the ability to pulse two distinct ESI sources and an APCI source would enable a wider range of ion/ion experiments for a given analyte species. Future work will examine this possibility.

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Figure 1. Schematic of an APCI and nano-ESI source couple with the modified Q TRAP mass spectrometer (only the vacuum/atmosphere interface of the Q TRAP instrument, which was unmodified, is represented in the schematic diagram).

Figure 2.

Signals derived from triply protonated KGAILKGAILR, formed via positive nano-ESI (15 ms +HV pulse followed by a 100 ms cooling step), and the radical anion of m-dinitrobenzene, derived via negative APCI (30 ms -HV pulse followed by a 100 ms cooling step). In each case, the mass resolving quadrupole was set to pass the reactant ion of interest.

Figure 3. Mass spectra derived from the pulsed APCI/nano-ESI source: (a) positive nano-ESI/MS of 5 μ M horse heart myoglobin and (b) negative APCI/MS of PMD. (c) Spectrum acquired (28 kHz ejection frequency) after reaction of myoglobin $[M+17H]^{17+}$ with the anions of (b) for 1300 ms.

Figure 4.

Mass spectra derived from the pulsed APCI and nano-ESI source: (a) negative nano-ESI/MS of 10 μ M 5'-d(C)₆-3' (isolated $[M-4H]^{4-}$) and (b) positive APCI/MS of proton sponge. (c) Post-ion/ion spectrum was acquired at 300 kHz ejection frequency after 3000 ms reaction of ions shown in (a) and (b).

Figure 5. Product spectrum from the reaction of KGAILKGAILR $[M + 3H]^{3+}$ with m-dinitrobenzene anions (1200 ms reaction time).