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A Dual-Trap Design and Its Applications in Electrospray Ionization FTICR Mass Spectrometry

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A new arrangement consisting of two separate Fourier transform ion cyclotron resonance (FTICR) ion traps was used to develop methods for the manipulation of the ions produced by an electrospray ionization source (ESI). A first, "accumulation" trap, is generally maintained at a higher pressure than the second, high-performance "analyzer" trap. The manipulations developed and demonstrated include the following: (1) mass-selective ion transfers between the traps; (2) mass-selective step-wise accumulation of low-abundance ions of different mass-to-charge ratios transferred from the first trap to the analyzer trap; (3) simultaneous detection of ions in the analyzer trap and ion accumulation in the source trap; (4) simultaneous ion detection in the accumulation trap and ion storage in the analyzer trap; (5) sequential multiple transfers of the ions into the analyzer trap from the same ion population stored in the accumulation trap; (6) collision-induced dissociation of ions stored in the accumulation trap followed by mass-selective transfer of the product ions into the analyzer trap; (7) sequential transfer of the ions of different mass-to-charge ratios into the analyzer trap from the same ion population stored in the accumulation trap followed by the collision-induced dissociation of transferred ions in the analyzer trap. These ion manipulations benefit multistage studies and are projected to be useful in many biochemical applications of ESI-FTICR, including structural determination of biopolymers and study of noncovalent complexes.

Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS)[1,2] and its combination with an electrospray ionization ion source (ESI) holds the promise of significant utility for the characterization of large biopolymers and their noncovalent interactions.[3−6] One of the possible approaches for enhancing the biochemical applications of FTICR involves the use of dual-trap configurations,[7] a version of which has been implemented in commercial FTICR mass spectrometers. In this design, the FTICR trap is effectively divided into two sections (typically, cylindrical or rectangular) separated by a small conductance limit (i.e., a hole), so that the pressure in either trap is substantially independent of the other (the commercial implementation allows an ∼2 order of magnitude pressure difference to be maintained). Ions are generated in, or introduced to, the higher pressure "source" trap and subsequently isolated and transferred into the "analyzer" trap, which allows higher resolution detection. The introduction of azimuthal quadrupolar excitation (QE) in the presence of collision gas for ion "cooling"[8−10] enhances the efficiency of ion trapping and storage in the source trap as well as ion transfer into the analyzer trap. However, in the commonly used dual-trap configuration, the trapping plate containing the conductance-limiting aperture necessarily serves as the trapping plate for both traps. This design limits the capabilities of dual trap for ion manipulations. For example, broad-band quadrupolar excitation in the source trap will capacitively induce an rf signal in the detection circuit of the analyzer trap, thus prohibiting simultaneous ion detection in one trap and excitation in the other trap. A major disadvantage of this is that, in the presence of gas phase collisions, ions will be rapidly lost from the trap due to the growth of magnetron motion unless QE is applied simultaneously. In addition, high-performance detection in the analyzer trap often requires the use of low trapping potentials, while efficient accumulation and storage of externally generated ions in the source trap are generally more effective at higher trapping potentials. Thus, optimal conditions cannot readily coexist for dual trap systems that utilize a common trapping plate.

We are developing an alternative approach aimed at improving ion manipulation processes, which involves the use of individual traps with different size, geometry, and functional capabilities.[11] The use of multiple integrated FTICR traps provides extended flexibility and selectivity for ion storage and manipulations in many applications. For example, the ions of different mass-to-charge ratios (m/z) can be stored in one trap and sequentially transferred to another trap for analysis. Multistage MS^n experiments can be significantly improved by the accumulation of certain daughter ions in an analyzer trap after multiple collision-induced dissociation (CID) events in a high-pressure "accumulation" trap and mass-selective transfer followed by another MS stage (e.g., involving CID) in the analyzer trap. This option may be especially useful, since the population of any selected product ions from the first stage of MS^n could potentially be built up in the "analyzer" trap through a series of transfer steps. It is also generally desirable to maintain optimal and constant higher buffer gas pressure

(1) Comisarow, M. B.; Marshall, A. G. *Chem. Phys. Lett.* **1974**, *25*, 282−283.
(2) Comisarow, M. B.; Marshall, A. G. *Chem. Phys. Lett.* **1974**, *26*, 489−490.
(3) Henry, K. D.; Williams, E. R.; Wang, B. H.; McLafferty, F. W.; Shabanowitz, J.; Hunt, D. F. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9075−9078.
(4) Henry, K. D.; Quinn, J. P.; McLafferty, F. W. *J. Am. Chem. Soc.* **1991**, *113*, 5447−5449.
(5) Hofstadler, S. A.; Laude, D. A. *Anal. Chem.* **1992**, *64*, 569−572.
(6) Winger, B. E.; Hofstadler, S. A.; Bruce, J. E.; Udseth, H. R.; Smith, R. D. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 566−577.
(7) Littlejohn, D. P.; Ghaderi, S. U.S. Patent No. 4,581,533, issued 8 April 1986.
(8) Bollen, G.; Moore, R. B.; Savard, G.; Stolzenberg, H. *Appl. Phys.* **1990**, *68*, 4355−4374.
(9) Schweikhard, L.; Guan, S. H.; Marshall, A. G. *Int. J. Mass Spectrom. Ion Processes* **1992**, *120*, 71−83.
(10) Guan, S.; Marshall, A. G. *J. Chem. Phys.* **1993**, *98*, 4486−4493.
(11) Chen, R.; Bruce, J. E.; Cheng, X.; Hofstadler, S. A.; Anderson, G. A.; Rockwood, A. L.; Udseth, H. R.; Smith, R. D. In *Proceedings, 43rd ASMS Conference on Mass Spectrometry and Allied Topics*; Atlanta, GA, 1995; p 801.

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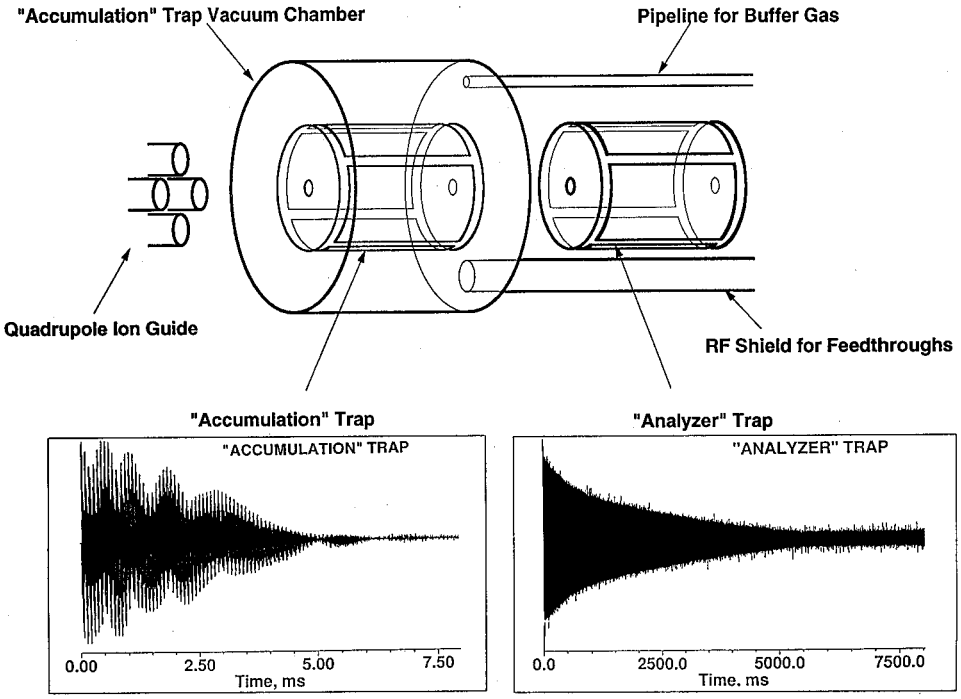

Figure 1. Schematic illustration of the dual FTICR trap configuration developed in this work and used for multiple and sequential ion transfers. Both traps are located in a vacuum system capable of very high pumping speed.[6] Enclosure of the accumulation trap inside the sealed chamber allows relatively high pressure to be maintained in this trap, and the electrical isolation allows FTICR signal detection in the analyzer trap simultaneously with azimuthal quadrupolar excitation/axialization in the accumulation trap. The different pressure conditions in the two traps are reflected by the much different time domain signals (bottom).

conditions in one trap to improve trapping efficiency and/or to facilitate more effective CID of large biomolecules produced with external ion sources.[6,12] In all such experiments, it would also be highly desirable to have the traps electrically insulated and well shielded from each other to allow the simultaneous application of quadrupolar excitation for ion trapping and/or extended storing in one trap and ion detection in the other trap. Note that some of these capabilities can also be obtained by placing the ion accumulation region in one of the differential pumping stages[13,14] or using an additional "ion cage" in the main vacuum region but placed far away from the FTICR trap.[15] These approaches are limited by differential pumping requirements or limit the capabilities for ion manipulations and detection in the accumulation region.

In this work, we have developed and evaluated a new dual-trap design that is derived from an earlier dual-trap design that has not been described in detail[11] but that generally illustrated the potential of our approach. The preliminary results showed that the ion population in the analyzer trap could be built up in sequential multistage mass-selective ion transfer from the first trap. In the present work, we report a detailed evaluation of this approach using a new design based on two separate cylindrical FTICR traps. To better reflect the functions performed by the traps, we refer to the first, higher pressure trap as the accumulation trap, and the low-pressure trap as analyzer trap. The results demonstrate new capabilities for ion manipulation and detection that should greatly facilitate a wide variety of demanding biological applications.

EXPERIMENTAL SECTION

All experiments were performed with an ESI source coupled to a 7 T FTICR mass spectrometer described elsewhere.[6] Ions were transferred from the ESI source to the accumulation trap using two sets of quadrupole ion guides operated in the rf-only mode (2 MHz, ~800 V_{p-p}). The schematic illustration of dual-trap assembly is shown in Figure 1. Ions were accumulated in the first trap by collisional loss of *z*-axis translational energy (due to the introduction of dry N_2 as a buffer gas) in conjunction with azimuthal quadrupolar excitation.[16] The signal in the accumulation trap was detected using a standard FTICR experimental sequence: frequency sweep excitation followed by broad-band detection and magnitude-mode Fourier transform, controlled by an Odyssey data station (Finnigan FT/MS, Madison, WI). Ion axialization was accomplished using the additional azimuthal broad-band or single-frequency quadrupolar excitation (depending on the type of experiment). Ions of selected mass-to-charge ratios were transferred to the analyzer trap by simultaneously lowering the trapping potential of the rear accumulation and front analyzer trapping plates. This transfer potential was 0.0 V for maximum transfer efficiency, or 1.0 V for transfer of the ions of selected m/z while the rest of the ions were retained in the accumulation trap for the subsequent experiments. The signal in the analyzer trap was detected by using the same standard FTICR experimental sequence: frequency sweep excitation followed by broad-band

(12) Beu, S. C.; Senko, M. W.; Quinn, J. P.; Wampler, F. M.; McLafferty, F. W. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 557−565.
(13) Hofstadler, S. A.; Laude, D. A. *Anal. Chem.* **1991**, *63*, 2001−2007.
(14) Senko, M. W.; Hendrickson, C. L.; White, F. M.; Quinn, J. P.; Marto, J. A.; Pasa-Tolic, L.; Guan, S.; Marshall, A. G. In *Proceedings, 44th American Soc. Mass Spectrometry Annual Conference on Mass Spectrometry and Allied Topics*; Portland, OR, 1996; p 485.
(15) Kofel, P.; Allemann, M.; Kellerhals, H.; Wanczek, K.-P. In *Proceedings, 35th ASMS Conference on Mass Spectrometry and Allied Topics*; Denver, CO, 1987; pp 1130−1131.
(16) Bruce, J. E.; Anderson, G. A.; Hofstadler, S. A.; Van Orden, S. L.; Sherman, M. S.; Rockwood, A. L.; Smith, R. D. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 914−919.

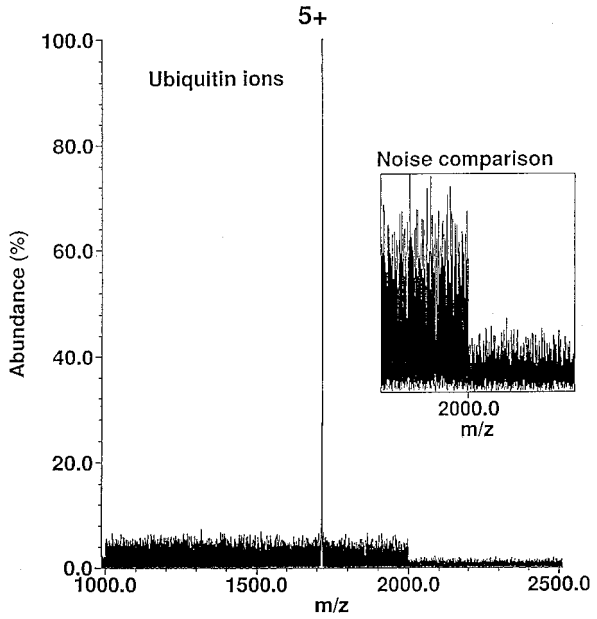

Figure 2. Mass spectrum of $(M + 5H)^{5+}$ ubiquitin ions detected in the analyzer trap simultaneously with azimuthal tailored noise quadrupolar excitation of 20 V_{p-p} amplitude applied in the accumulation trap in the mass range of m/z 1000−2000. The noise associated with capacitive coupling between the traps is 2−3 times greater than the noise level when quadrupolar excitation is not applied. Simultaneous detection would not be possible without rf shielding of the traps from each other.

detection and magnitude-mode Fourier transform. The pressure difference between the accumulation and analyzer traps was estimated from the decay constants for the signals measured in these traps. Typically, the time domain transient in the analyzer trap was $\sim 10^3$ times longer than in the accumulation trap when buffer gas was introduced to enhance trapping efficiency, implying a similar difference in pressure. Buffer gas (dry N_2) was injected directly into the accumulation and/or analyzer region via two piezoelectric pulse valves (Lasertechniques, Inc., Albuquerque, NM), controlled independently using TTL pulses generated by the data system. Frequency wave forms for azimuthal quadrupolar excitation were generated using a PC board (PCIP-AWFG, 5 MHz, 12 bit, Keithley MetraByte Co., CA) and applied separately to both accumulation and analyzer traps through a home-built "dipole/quadrupole" switch, based upon relays having a 200 ms retarding time and controlled by the Odyssey data station.

Both traps were of 2 in. diameter cylindrical design made from gold-plated aluminum. To provide rf electric field insulation, the silver leads between the UHV feedthroughs and the trap electrodes were placed inside separate grounded aluminum tubes for both the accumulation and analyzer traps. Figure 2 shows the results of an experiment in which a 20 V_{p-p} "tailored noise" quadrupolar excitation wave form[17] in the frequency range of 50−100 kHz, corresponding approximately to the m/z range of 1000−2000, was applied to the "accumulation" trap's excitation/detection electrodes during the simultaneous detection of the 5+ charge state of ubiquitin in the analyzer trap. This experiment was not possible without the noise reduction. With the present arrangement, the observed noise level was only 2−3 times greater than the inherent noise level of the preamplifier. We expect that further improvements in rf shielding should serve to largely eliminate induced "cross-talking" between the traps, and such efforts are now in progress.

In CID experiments, a single-frequency azimuthal dipolar off-resonance excitation (SORI) of low to medium amplitude (typically, 20 V_{p-p}) at the frequency of 1000−1500 Hz above the reduced cyclotron frequency of the ions selected for dissociation was applied for periods of 0.5−1 s. During irradiation, nitrogen collision gas was introduced to the trap at a pressure of $\sim 10^{-5}$ Torr.

The standard samples used (purchased from Sigma, St. Louis, MO) were dissolved in 5% HOAc (0.5 mg/mL). The solutions were infused into an ESI source (modified Analytica of Branford, Branford, CT), which includes a heated stainless steel "desolvation" inlet capillary.[6] Typically, the infusion rates were 0.2−0.3 μL/min, controlled using a syringe pump (Harvard Apparatus, South Natick, MA).

RESULTS AND DISCUSSION

1. Multiple Ion Transfer Experiments and Efficiency of Ion Transfer between the Traps. In their early work, Giancaspro and Verdun studied ion transfer behavior in a dual FTICR trap.[18] For axialized ions, this behavior is defined primarily by the ion population's axial oscillations between the traps. This ion motion is damped primarily by Coulomb interactions and ion−neutral collisions on the time scale of milliseconds. The oscillation frequency, ν, depends on ion mass m and charge q for a given trapping potential and trap geometry as

$$\nu \sim (q/m)^{1/2} \qquad (1)$$

By studying the transfer efficiency as a function of time (see Figure 3) we defined the mass range in which the ions can be effectively transferred for a given period. For example, the transfer time of 0.36 ms was optimal for $(M + 7H)^{7+}$ ubiquitin ions at m/z 1225 and all ions in the mass range of m/z 1225 ± 300 can be effectively transferred (although, with some degree of m/z discrimination). Once characterized, the optimal transfer period can be estimated for ions of any particular m/z using eq 1.

In this work, we also demonstrated the capability for multiple ion transfers between the traps. This has potential utility in many applications such as high-sensitivity multistage tandem mass spectrometry with specific mass selection at each stage (i.e., MS^n, where $n > 2$). The efficiency of ion transfer between the traps, α, is the most important factor in these experiments. Figure 4 shows the mass spectra of $(M + 6H)^{6+}$ and $(M + 5H)^{5+}$ ubiquitin ions for the following: (a) single-ion transfer from the accumulation trap to the anaylzer (Figure 4a), accumulation trap → analyzer; (b) the same ions from an experimental sequence involving three ion transfers between the traps (Figure 4b), i.e., accumulation trap → analyzer → accumulation trap → analyzer; (c) transfer of the same ions five times between the traps (Figure 4c), i.e., accumulation trap → analyzer → accumulation trap → analyzer → accumulation trap → analyzer. Assuming that the transfer efficiency α is the same for both accumulation trap → analyzer and analyzer → accumulation trap transfer steps and does not depend on number of ions, one can estimate this efficiency from the results presented in Figure 4 to be ~0.7. Note, that this experiment

(17) Bruce, J. E.; Anderson, G. A.; Smith, R. D. *Anal. Chem.* **1996**, *68*, 534−541.

(18) Giancaspro, C.; Verdun, F. R. *Anal. Chem.* **1986**, *58*, 2097−2099.

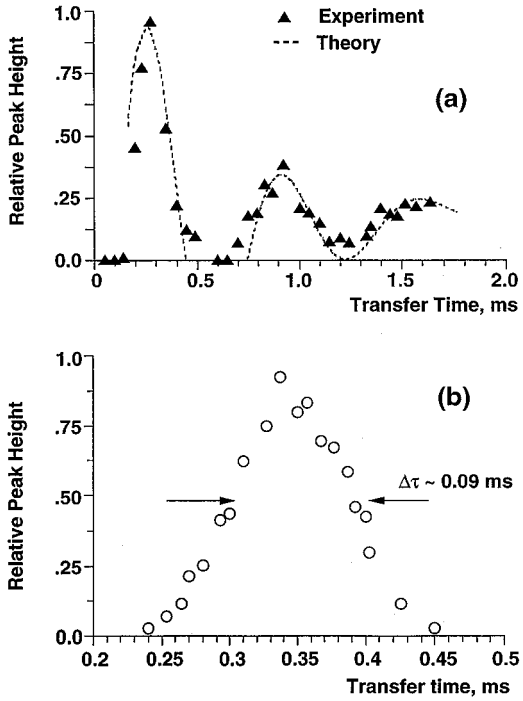

Figure 3. Experimental dependence of the ion population in the analyzer trap on theperiod of ion transfer time. (a) The ion oscillations between the traps are rapidly damped by Coulomb interactions and ion−neutral collisions. (b) The width of this peak defines the *m*/*z* range of the ions that can be effectively transferred between the traps.

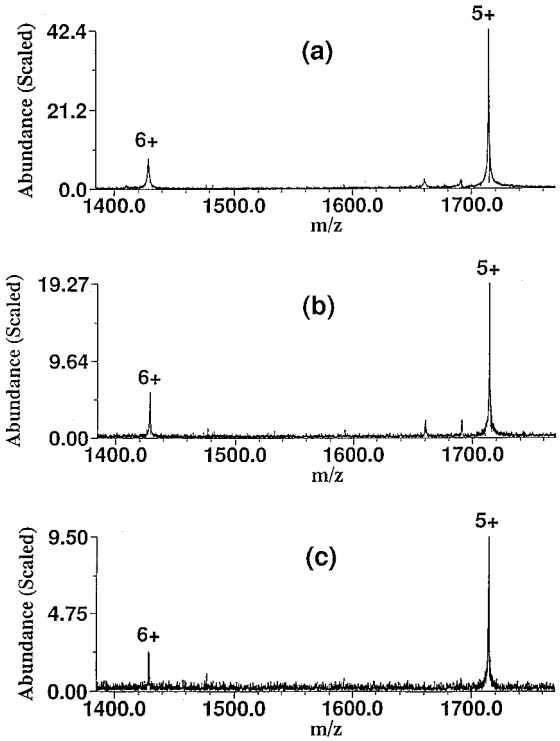

Figure 4. ESI-FTICR mass spectra of ubiquitin ions subjected to multiple ion transfer between the accumulation and the analyzer traps: (a) single-transfer accumulation → analyzer; (b) three transfers accumulation → analyzer → accumulation → analyzer; (c) five transfers accumulation → analyzer → accumulation → analyzer → accumulation → analyzer. The ions were axialized before each transfer to the other trap using broad-band QE. Each trap was also "quenched" to remove any remaining ions after the transfer to another trap. The overall estimated efficiency of ion transfer was 70%.

differs from the simple observation of ion oscillations between the traps. After each transfer, the ions were kept in respective trap for periods of 5−20 s, thus, allowing time for additional ion manipulations (e.g., detection, QE axialization).

2. Sequential and Selective Ion Transfers between the Traps. The selectivity of ion transfer between the traps can be increased using the azimuthal quadrupolar excitation technique. The use of two traps provides the opportunity to select a particular *m*/*z* range from the initial population stored in the accumulation trap and to transfer these ions into the analyzer for high-resolution detection while keeping the rest of the ions in the accumulation trap. After completion of this first step, other ions from the *same* initial population (i.e., without injection of additional ions from the source) can be selected and transferred into the analyzer, etc.

To accomplish this "sequential ion transfer", several experimental approaches were used. First, after initial broad-band QE for the axialization of all ions in the accumulation trap, an additional single-frequency QE was applied for axialization of ions having the selected *m*/*z*. During this event, other ions in the trap experience magnetron expansion, separating them radially from the ions axialized by single-frequency QE. This allows only the preselected ions to be transferred to the analyzer trap. Immediately after the transfer to the analyzer trap, broad-band QE was applied in the accumulation trap to axialize the remaining ions for subsequent manipulations. Note that the selected quadrupolar excitation/axialization parameters depend strongly on the design of the FTICR trap, the nature of collision gas, its pressure, and the magnetic field. Indeed, for the oscillatory driving term *k*, which couples the cyclotron and magnetron motions,[8] we have

$$k \approx -\frac{q}{m}\frac{2\beta V_{xy}^{(0)}}{d^2(\omega_+ - \omega_-)} \qquad (2)$$

in which β is a geometry factor in the first order of approximation for the azimuthal quadrupolar excitation field (which to first approximation can be defined in cyclotron plane as $E_x \sim 2\beta V_{xy}^{(0)}(y/d^2)$, $E_y \sim -2\beta V_{xy}^{(0)}(x/d^2)$; d is the diameter (for a cylindrical trap); $V_{xy}^{(0)}$ is the amplitude of quadrupolar excitation field (using the notation suggested in ref 19); ω_+ and ω_- are the reduced cyclotron and the magnetron frequencies, respectively. For effective collisional damping, this oscillatory driving term should be of the same order of magnitude as the collisional frequency γ, e.g.

$$k \sim \gamma \qquad (3)$$

Thus, assuming that $\omega_- \ll \omega_+ \approx \omega_c$, we have for the optimal amplitude of azimuthal QE field from eqs 2 and 3

$$V_{xy}^{(0)} \sim \frac{m}{q}\frac{d^2\gamma\omega_c}{2\beta} = \frac{d^2\gamma B}{2\beta} \qquad (4)$$

where B is the magnetic field strength. Equation 4 is in good agreement with the equation for QE amplitude, corresponding to

(19) Guan, S. H.; Kim, H. S.; Marshall, A. G.; Wahl, M. C.; Wood, T. D.; Xiang, X. Z. *Chem. Rev.* **1994**, *94*, 2161−2182.

the maximal rate of ion axialization in an FTICR trap reported earlier.[19] For the experiments described here, we found that the optimal QE amplitude for ion axialization in the accumulation trap was 5 V_{p-p} for single-frequency excitation and at least 20 V_{p-p} for tailored noise broad-band excitation.

Second, a nonzero transfer potential was used so that after axialization of the selected portion of the mass spectrum in the accumulation trap, these ions could be transferred into the analyzer by lowering the potentials on rear accumulation and front analyzer trap electrodes (defined as transfer potential). To retain the remaining ions in the accumulation trap, this potential should exceed their axial kinetic energy. Too high of a potential will limit the efficiency of the transfer of selected ions, while too low of a potential will lead to the loss for the "nontransferred" ions.

After ion transfer, we performed simultaneous detection of transferred ions in the analyzer trap and broad-band ion axialization in the accumulation trap. To prevent ion losses due to magnetron expansion in high-pressure accumulation trap, the broad-band azimuthal QE was applied during the entire period of ion manipulations and detection in the analyzer trap, a period that lasted up to several minutes. This was enabled by careful rf shielding of the two traps from each other described above.

In a typical experimental sequence for sequential ion transfer, the ions of different m/z were first trapped and stored in the accumulation trap. Then, the first selected ions, or a group of ions, from a particular m/z region were further axialized using a QE event that was long enough to allow magnetron expansion of all the other ions to radii greater than the 3 mm conductance orifice in the trapping plates. The rate of this expansion depends on experimental conditions in the trap such as pressure, as well as magnetron ω_m and cyclotron ω_c frequencies according to the following expression:

$$\rho_m(t) \sim \exp\left\{+\gamma\left(\omega_m/\omega_c\right)t\right\} \qquad (5)$$

in which ρ_m is the magnetron radius of the ions. Axialized ions were then transferred into the analyzer followed by their detection. During this period, broad-band QE was used to prevent further magnetron expansion of ions stored in the accumulation trap. In the next step of the sequence, the ions of a second chosen m/z were axialized in the accumulation trap and transferred into the analyzer by repetition of the procedures described above.

Figure 5 shows mass spectra for different charge states of ubiquitin sequentially transferred for high-resolution detection into the analyzer trap from the *same* ion population initially trapped and stored in the accumulation trap. In this experiment, a transfer potential of 1 V was applied to the rear accumulation and front analyzer trap electrodes. At this potential, the nontransferred ions were effectively retained in the accumulation trap due to their larger magnetron radii. The transfer efficiency for the selected ions was estimated at ~50%, based upon comparison with magnitude-mode FT peak heights of the same ions transferred with zero transfer potential (for which the experimentally determined transfer efficiency was ~70%). Higher transfer efficiency can potentially be obtained by incorporating a small-diameter central auxiliary trapping electrode into the cell design. During ion transfer, lower potential is applied to this electrode, thus allowing the transfer of all axialized ions and leaving all other ions nearly unperturbed.[20]

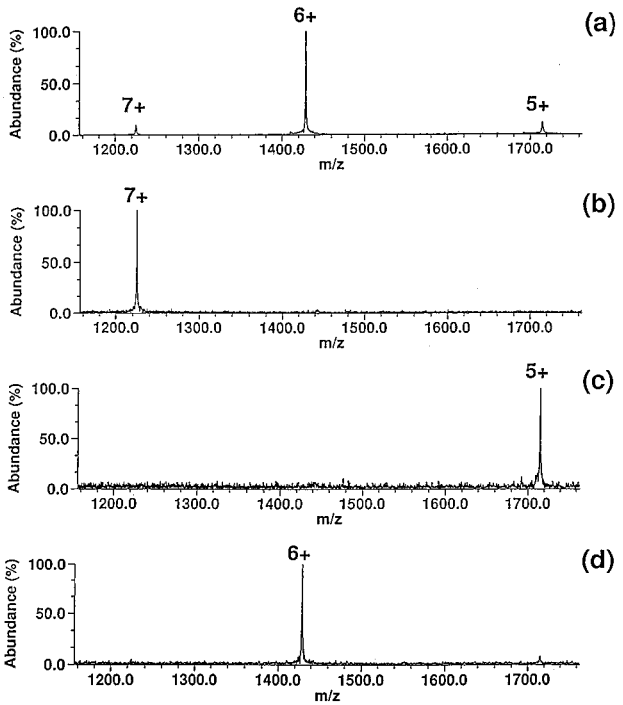

Figure 5. ESI-FTICR mass spectra of ubiquitin ions subjected to sequential ion transfer between the accumulation and the analyzer traps. (a) The original mass spectrum of the ions trapped in the accumulation. (b) $(M + 7H)^{7+}$ ions were axialized in the accumulation trap and transferred to the analyzer. (c) $(M + 5H)^{5+}$ ions from the same initial population were axialized in the accumulation trap and transferred into the analyzer. (d) $(M + 6H)^{6+}$ ions from original population were axialized in the accumulation trap and transferred to the analyzer.

3. Mass-Selective Ion Accumulation in the Analyzer Trap Using the Sequential Ion Transfers. The methods described above for "multiple ion transfer" and sequential ion transfer can be successfully used for the stepwise accumulation of low-abundance ions or product ions from an MS^n experiment in the analyzer trap. This accumulation may be necessary in some cases such as more effective or extended multistage mass spectrometry of ions for obtaining higher signal-to-noise ratios or for increasing dynamic range. The sequence used in these experiments was similar to the sequential ion transfer, with additional ion injection into the accumulation trap after each mass-selective ion transfer into the analyzer trap. Because ion trapping and axialization in the accumulation trap can last as long as tens of seconds (depending upon experimental details), we also studied the ion storage efficiency in the analyzer trap. Figure 6 shows the mass spectrum of $(M + 6H)^{6+}$ ubiquitin ions at different time delays after their transfer into the analyzer trap. We found no substantial ion losses during the period of up to 10 min even without applying QE. This observation implies that the growth of magnetron radius in the analyzer trap is very slow and demonstrates the large pressure gradient between the two traps, as well as small initial magnetron radius for transferred ions.

Stepwise mass-selective ion accumulation in the analyzer trap is shown in Figure 7. The $(M + 5H)^{5+}$ low-abundance ubiquitin ions were axialized from the initial trapped ion population by using single-frequency QE. These ions were then transferred to the analyzer trap and stored there using broad-band QE during which

(20) Nikolaev, E. N.; Mordehai, A. V.; Tal'rose, V. L. *Rapid Commun. Mass Spectrom.* **1990**, *4*, 64−66.

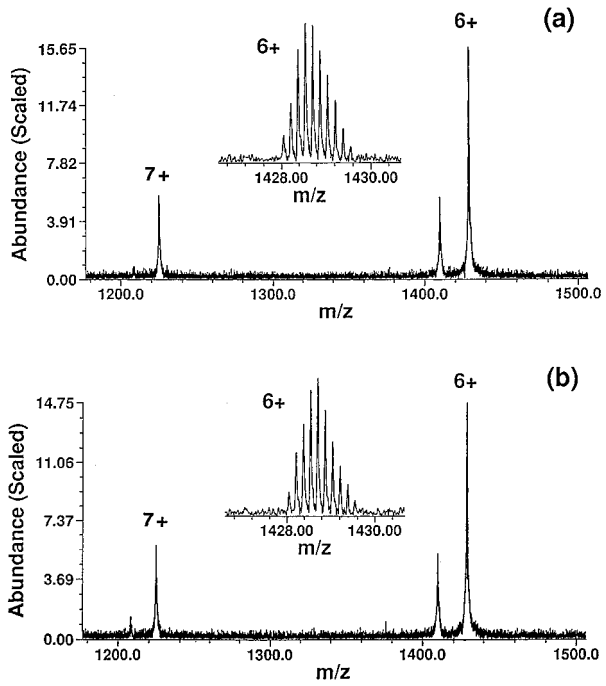

Figure 6. ESI-FTICR mass spectrum of ubiquitin ions detected in the analyzer 60 (a) and 180 s (b) after transfer of ions. No ion axialization was used during delay period.

time additional ions from ESI source were injected and accumulated in the first trap. Then, the new $(M + 5H)^{5+}$ ions in the accumulation trap were axialized, transferred into the analyzer and added to the ions stored there from the first transfer event. There are two important aspects of this procedure: (i) gated trapping was used to trap ions transferred into the analyzer, and the transfer time was chosen so that the ions were trapped in the center of the analyzer region with minimal amplitude of axial oscillations; (ii) the transfer potential on the rear accumulation and front analyzer trapping plates was 1 V. This allowed for higher energy ions from the accumulation trap to penetrate this potential and be transferred to the analyzer trap while prohibiting the lower axial energy ions from escaping the analyzer.

Figure 7d shows the efficiency of ion accumulation in the analyzer trap as a function of number of ion transfer steps. The relative peak height of the FTICR signal is proportional to the number of transfers, until the analyzer trap is effecitively "filled". This ion population limitation arises due to the repulsive "space charge" effect upon the trapping potential well and depends on the trapping potential used as well as trap dimensions (we are currently exploring the use of larger traps in this regard). Another likely effect due to Coulomb ion−ion interactions is that the larger ion population in the analyzer trap, the greater ion loss from the analyzer during the transfer period.

4. Multistage MS in Combination with Mass-Selective Sequential Ion Transfer between the Traps. Tandem and multistage mass spectrometry methods (MS^n) are used primarily in conjunction with ion dissociation methods for structural studies of gas phase ions.[21] Multiply charged biomolecules of very large size can be effectively fragmented by multiple collisions with neutrals in the FTICR trap after excitation of their cyclotron motion. The high m/z selectivity of the excitation process makes it possible to analyze components of peptide or protein mixtures without the need to separate the individual components prior to mass spectrometric analysis. The use of two FTICR traps provides many opportunities for making MS^n process more effective and for its extension to additional steps. In one approach, all ions over a wide m/z range are accumulated and detected in a first trap. Then, the ions of selected mass-to-charge ratio are transferred into a second trap for MS^n analysis. This process can be repeated using sequential ion transfer to obtain MS^2 spectra of different primary ions or could be further extended using multiple ion transfer to move product ions selected in the second trap back into the first trap for the next step in the MS^n process.

In a typical sequence for MS^2 experiments, the ions are first trapped and detected in the accumulation trap followed by their transfer into the analyzer for high-resolution analysis. After detection, single-frequency azimuthal QE is applied to collisionally axialize the chosen ions. Dipolar off-resonance excitation (i.e., SORI) at a frequency above (or below) the reduced cyclotron frequency is used to induce dissociation of selected species. The resulting product ions are then axialized and transferred into the analyzer for further analysis. The lower pressure and the higher magnetic field homogeneity in the analyzer trap facilitate high-resolution measurements.

The analyzer trap can also be used in MS^n experiments. In combination with the sequential ion transfer, this option allows the opportunity for higher sensitivity and more efficient sequencing of biopolymers through the increased efficiency of multistage MS^n experiments or study of the gas phase binding properties in noncovalent complexes. A typical experimental sequence for the experiments of this kind is shown in Figure 8. First, the ions are trapped and detected in the accumulation trap. Then, the single-frequency azimuthal quadrupolar excitation is used to axialize selected ions, and they are then transferred to the analyzer trap. Azimuthal dipolar off-resonant excitation at the frequency above or below the cyclotron frequency (e.g., SORI) is applied to induce dissociation of these ions in the analyzer trap. For more efficient collision-induced dissociation, buffer gas from a second pulsed valve is injected into the analyzer trap region, and product ions are axialized during and/or after the SORI-CID event. During this period, the rest of the ions are kept in the accumulation trap using broad-band azimuthal QE. After the product ions are detected, the analyzer trap is quenched and new parent ions from the accumulation trap can be introduced. Again, single-frequency azimuthal QE was used to axialize the next selected ions in the accumulation trap. These ions are then transferred into the analyzer trap for MS^2 analysis, etc. An interesting option not yet implemented is retention of selected products in the analyzer trap. This could allow particular product ions to be accumulated over several experimental cycles. This approach could increase the population of low-abundance product ions for more effective multistage analyses.

Figure 9 shows the demonstration of this approach for the dissociation of melittin molecular ions of different charge states. An initial mass spectrum for ions in the accumulation trap is shown in Figure 9a. $(M + 3H)^{3+}$ ions were then selected and axialized by use of a single-frequency QE (4 V_{p-p} amplitude for the period of 10 s). After their transfer to the analyzer trap, these ions were subjected to SORI dissociation (20 V_{p-p} at the frequency of 1500 Hz above the reduced cyclotron frequency) in the presence of dry nitrogen injected into the analyzer at the pressure of $\sim 10^{-7}$ Torr. Simultaneously with the SORI excitation in the analyzer

(21) *Tandem Mass Spectrometry*; McLafferty, F. W., Ed.; Wiley: New York, 1983.

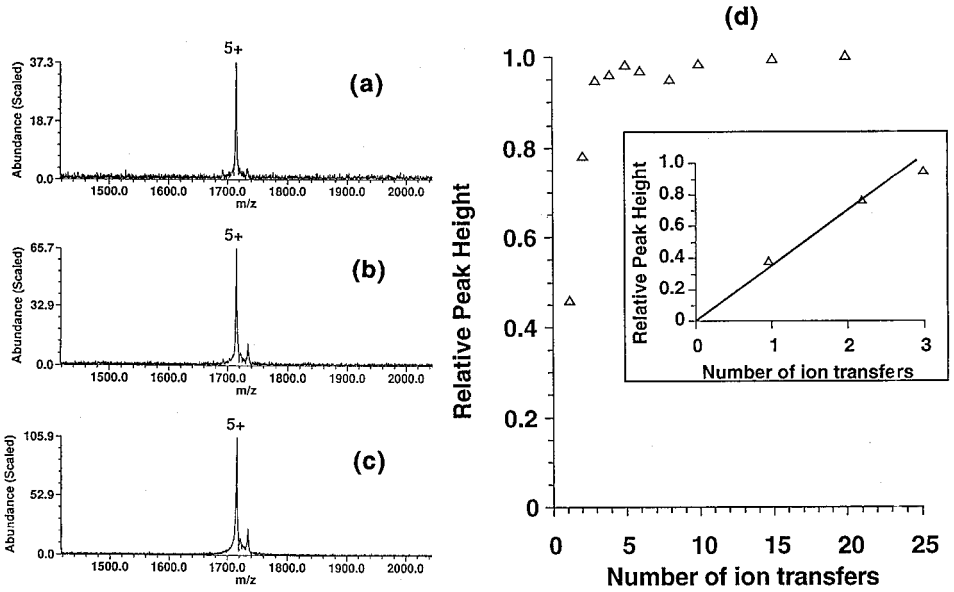

Figure 7. ESI-FTICR mass spectrum of $(M + 5H)^{5+}$ ubiquitin ions subjected to stepwise selective ion accumulation in the analyzer trap. The ion population is built up proportionally with the number of transfers until the effective space charge trap limit: (a) one-transfer accumulation; (b) three-transfer accumulation; (c) five-transfer accumulation; (d) experimental dependence of the FTICR signal amplitude from ion population in the analyzer on the number of transfers of the ions from the same *m*/*z* range.

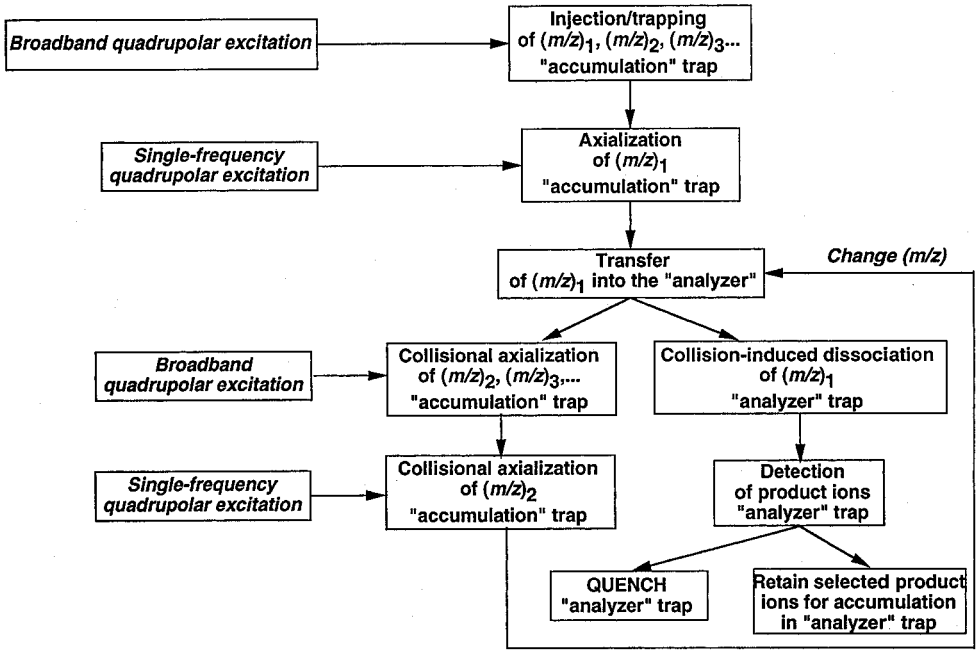

Figure 8. Experimental steps used for sequential ion transfers between the accumulation and the analyzer traps followed by collision-induced dissociation of transferred ions in the analyzer. Potentially, product ions at *m*/*z* much different from the parent ions collisionally dissociated may be retained in the analyzer trap and accumulated over additional cycles.

trap, tailored noise broad-band QE (20 V_{p-p} amplitude, 1000−2000 *m*/*z* range) was applied to the ions still stored in the accumulation trap to prevent their loss. After the SORI event, a mass spectrum of $(M + 3H)^{3+}$ melittin dissociation products was obtained in the analyzer trap (Figure 9b), and single-frequency QE was used for collisional axialization of $(M + 2H)^{2+}$ ions in the accumulation trap. These ions were then transferred to the analyzer trap for the next SORI dissociation step. Figure 9c shows the high-resolution product ion mass spectrum obtained in the analyzer trap after dissociation of the $(M + 2H)^{2+}$ melittin ions. Note, that no additional ion trapping from the ESI source was involved in this experimental sequence.

CONCLUSIONS

A dual FTICR trap design based on the use of two separate and rf shielded from each other traps has been developed and experimentally evaluated. Several capabilities that should serve to extend the biological applications of FTICR have been demonstrated including the following:

(1) Multiple Ion Transfers between the Traps. As many as five successful transfers of the same ions between the traps have been obtained. The efficiency of ion transfer between the traps was estimated to be 70%.

(2) Sequential Ion Transfer between the Traps. The sequential transfers of the ions of different *m*/*z*'s from the *same*

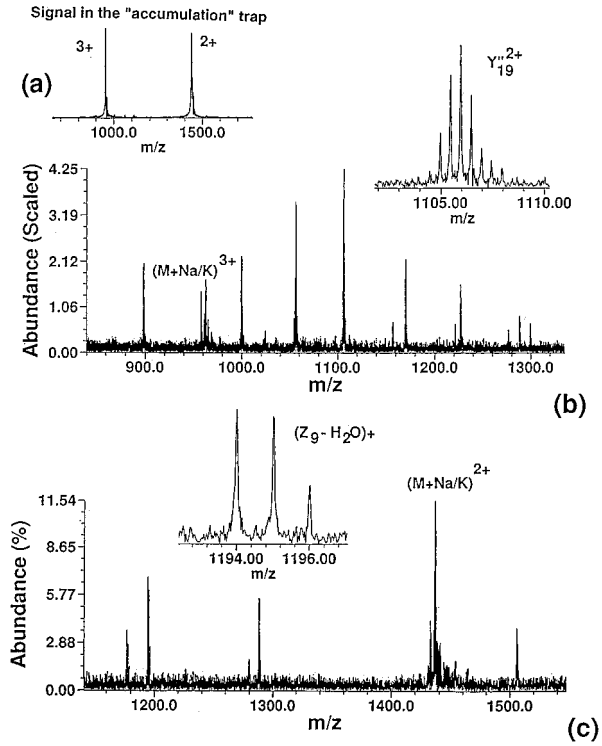

Figure 9. Demonstration of sequential ion transfers and the subsequent use of SORI collisional dissociation for polypeptide sequence determination. (a) $(M + 2H)^{2+}$ and $(M + 3H)^{3+}$ melittin ions detected after initial accumulation in the first trap; (b) product ion mass spectrum after transfer of $(M + 3H)^{3+}$ to the analyzer trap and subjected to SORI dissociation; (c) product ion mass spectrum after transfer of $(M + 2H)^{2+}$ ions to the analyzer followed by their SORI dissociation. After each transfer, the rest of the ions in the accumulation trap were subjected to tailored noise broad-band QE of 20 V_{p-p} amplitude for storage until the next transfer step.

ion population initially trapped in the accumulation trap have been demonstrated. It was shown that by using tailored noise broad-band quadrupolar excitation for collisional axialization, we can maintain effective ion storage in either trap for periods of up to several minutes. This extended trapping period facilitates CID experiments or trapping of newly injected ions in the accumulation trap, and much longer periods should be feasible if necessary.

(3) Mass-Selective Accumulation of the Ions from Several Sequential Transfers. Low-abundance ions (due to low sample concentrations, etc.) can be accumulated in one of the traps using several sequential ion transfers from another trap. The accumulated ion population is proportional to the number of transfer events until being limited by space charge effects.

(4) Ion Accumulation in One Trap Simultaneous with Detection of the Other Ions in Another Trap. It was shown that, by proper rf shielding of the corresponding leads and trap electrodes, the traps can be almost completely insulated from each other. This shielding allows simultaneous broad-band azimuthal quadrupolar excitation at amplitudes of up to 20 V_{p-p} in one trap and detection of the FTICR signal in another trap over the same frequency range. This capability provided by a two-trap assembly is crucial for most of the experiments described in this work.

(5) Collision-Induced Dissociation in Combination with "Sequential" Ion Transfer between the Trap. It was demonstrated that the ions can be successfully stored in one trap for the period of several minutes. During this period, selected ions from the *same* population in one trap can be transferred sequentially (i.e., one after another) to the other trap for subsequent CID experiments and additional measurements.

The multistage MS^n capabilities in combination with the sequential and multiple ion transfers between the traps described have the potential to be useful in a broad range of biological applications of FTICR, increasing both the sensitivity and effectiveness for characterization of large biomolecules and their interactions.[22] We are currently developing an improved multiple-trap design that incorporates better rf shielding, a larger trap size, segmented trapping plates for improved "linearization" of electric fields during excitation, and the introduction of a significantly higher higher magnetic field (11−12 T).

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(22) Wu, Q.; Gao, J.; Sigal, G. B.; Bruce, J. E.; Whitesides, G. M.; Smith, R. D. *J. Am. Chem. Soc.* **1997**, *119*, 1157−1158.

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