Time Resolved H/D Exchange of Gas-Phase Protein Ions in a Linear Ion Trap

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OVERVIEW

- Understand the factors that contribute to conformations and structures of biological molecules in the gas phase, and the relation of these to solution structure.
- A pulse of D_2O added at the end of a trapping period in a linear ion trap (LIT) in order to detect the fastest exchanging hydrogens.
- Time-resolved gas-phase H/D exchange of cytochrome *c* (cyt *c*) ions with D_2O in a linear ion trap.
- Folding pathways of gas-phase cyt *c* produced by electrospray ionization have been studied.

INTRODUCTION

- H/D exchange, a method widely used to study protein conformation in solution, has also been used to study the structures of gas phase ions.
- Recently, gas-phase cyt *c* ions produced by electrospray ionization showed evidence of unfolding and refolding over extended timescales (10 ms-10 s) in a Paul trap combined with an ion mobility technique.[1]
- Partially folded or partially unfolded (cyt *c*)$^{n+}$ ions are generated in the orifice-skimmer region by varying the voltage difference (ΔV_{OS}) followed by trapping in the LIT (0.001- 5 s) and time-resolved gas-phase H/D exchange.
- Time-resolved folding and unfolding of the cyt *c* ions are detected.

METHODS

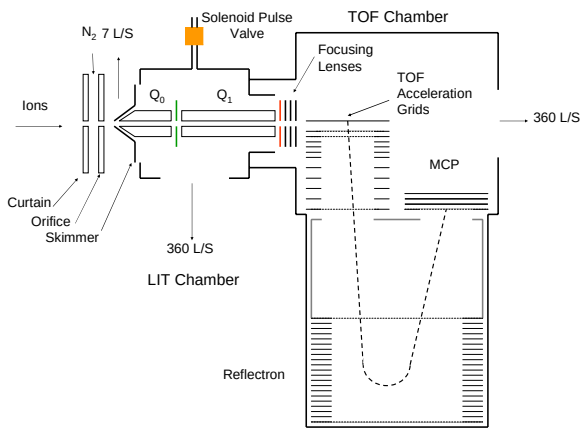

Figure 1. A schematic diagram of the LIT/TOF. Quadrupole, Q_1, is used as a linear ion trap, where the entrance lens is shown in green and the exit lens is red.

METHODS

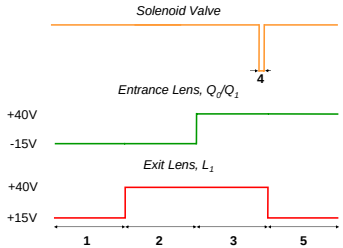

Figure 2. Voltage and Timing Sequence of the LIT and Solenoid Valve.

1. **Drain** (50-2500 ms): eliminates ions accumulated in Q_0 before allowing ions into the trap
2. **Injection** (20 ms): allows ions into the trap.
3. **Trap** (1-2500 ms): stores ions.
4. **Pulse:** Solenoid valve opens during the last 100 ms of trap time for reaction with a **D_2O pulse**.
5. **Detection:** (50 ms) mass analysis and detection of ions in the TOF after the reaction.

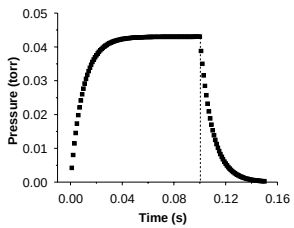

Figure 3. Calculated 100 ms D_2O pulse pressure change in the LIT. (Background pressure in the trap: 2 mtorr, vapor pressure of 50 °C of D_2O: 83.3 torr, pump speed at 360 L/s, time constant = 9.5X10^{-3}, P(steady state) = 0.043 torr.[2] This pulse is equivalent to a D_2O pressure of 39 mtorr applied for 100 ms.

Reagents: 20 μM horse heart cytochrome *c* (Sigma, C-7752) in 49/49/2, water/methanol/acetic acid; D_2O (99.9%D) (Cambridge Isotope Laboratories, Inc.).

RESULTS

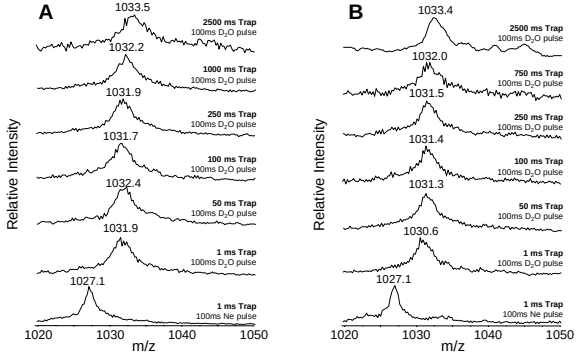

Figure 4. Mass spectra of (cyt *c*)$^{12+}$ (100-2500 ms drain, 20 ms inject, 1-2500 ms trap,100 ms pulse of Ne or D_2O, 50 ms detect) with (**A**) ΔV_{OS}=100 V and (**B**) ΔV_{OS}=150 V. A pulse of Ne was used as a control at each trapping time (data not shown) to calibrate the TOF by accounting for the changes in ion temperature and internal energies. Ne was used since it has a mass similar to D_2O.

RESULTS

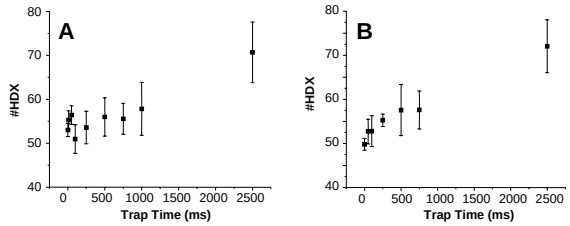

Figure 5. (**A**) Number of hydrogens exchanged (#HDX) in 100 ms vs. trap time for (cyt *c*)$^{10+}$ (100-2500 ms drain, 20 ms inject, 1-2500 ms trap, 50 ms detect) with ΔV_{OS}=100 V. (**B**) Number of hydrogens exchanged (#HDX) in 100 ms vs. trap time for (cyt *c*)$^{10+}$ (100-2500 ms drain, 20 ms inject, 1-2500 ms trap, 50 ms detect) with ΔV_{OS}=150 V.

Cytochrome *c* folding and unfolding in the gas-phase

- **ΔV_{OS}=100 V (A):**
  - Increasing #HDX is observed up to 50 ms of trap time.
  - This apparent cyt *c* unfolding step, is followed by 50 ms of decreasing number of hydrogen exchanges, indicating fewer surface hydrogens available for exchange.
  - With trapping times greater than 100 ms, a gradual increase in #HDX is observed with a plateau between 2.5 and 5 s (data not shown).
- **ΔV_{OS}=150 V (B):**
  - A gradual increase in #HDX is observed with respect to trapping time. Under these conditions, the #HDX plateaus between 2.5 and 5 s of trapping time (data not shown).
- Similar trends were observed for all positively charged cyt *c* ions [(cyt c)$^{7+\text{-}17+}$].

CONCLUSIONS

- A short pulse at the end of the trapping cycle in the LIT allows for the exchange of the fastest exchanging hydrogens in cyt *c* in the gas phase.
- Using D_2O vapor at a pressure of 83.3 torr backing the valve, a 100 ms pulse allowed for the exchange of ~50 hydrogens.
- With a skimmer-to-orifice voltage ratio of 100 V, cyt *c* ions showed a refolding step in the millisecond time range followed by a slower unfolding step in the trap in seconds.
- Increasing the ions' internal energies in the orifice-skimmer region eliminated this refolding process and only the slower unfolding step was observed in the trap.
- Both shorter-time scale (ms) and longer term (up to minutes) gas phase processes can be observed using time-resolved H/D exchange with the current setup.

ACKNOWLEDGEMENTS

Supported by the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1. E.R. Badman, S. Myung and D.E. Clemmer, *J. Am. Soc. Mass Spec.*, 2005, **16**, 1493.
2. D. J. Douglas and J. B. French, *J. Anal. At. Spectrom.*, 1988, **3**, 743.