Electron Autodetachment of Oligonucleotide Anions in the Gas Phase

Allison S. Danell and Joel H. Parks

ROWLAND INSTITUTE AT HARVARD, 100 EDWIN LAND BLVD., CAMBRIDGE, MA 02142

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This poster was originally presented in large format, but it was modified for easier reading/downloading from the web.

INTRODUCTION

Trapped oligonucleotide anions have been observed to undergo sequential loss of charge as a function of temperature.

- No changes in the deconvoluted mass spectra occur with the formation of lower charge state ions, indicating that the charge loss is the result of electron autodetachment detachment.
- Electron autodetachment rates vary as a function of temperature, charge state, and sequence.
- The variation of the rates suggest that conformational fluctuations of single-stranded oligonucleotides play a role in the decay process.

EXPERIMENTAL

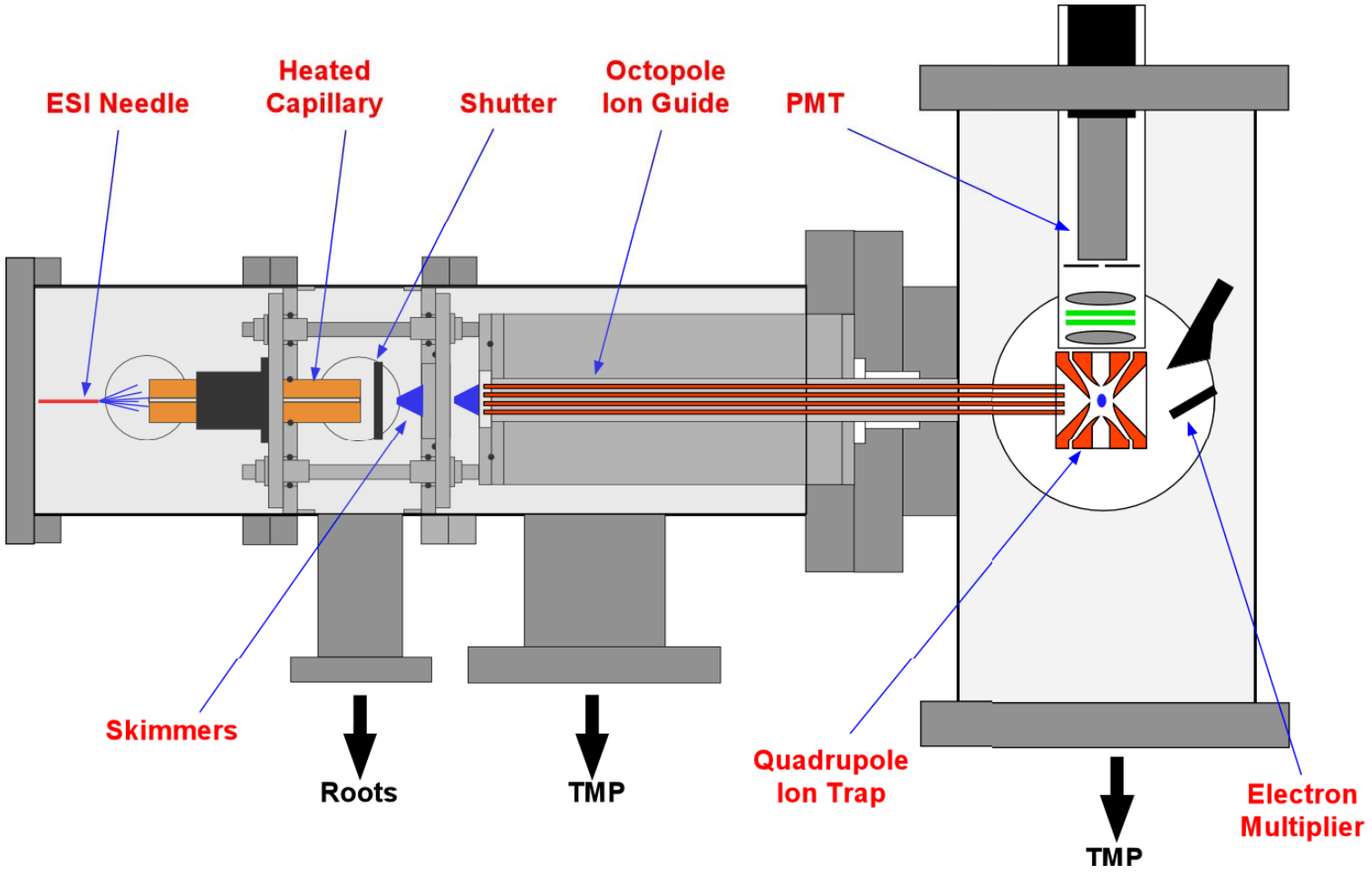
Analytes

- Oligonucleotides purchased from Synthegen (Houston, TX)
- 5 to 10 μM in 70/20/10 MeOH/H_2O/trifluoroethanol

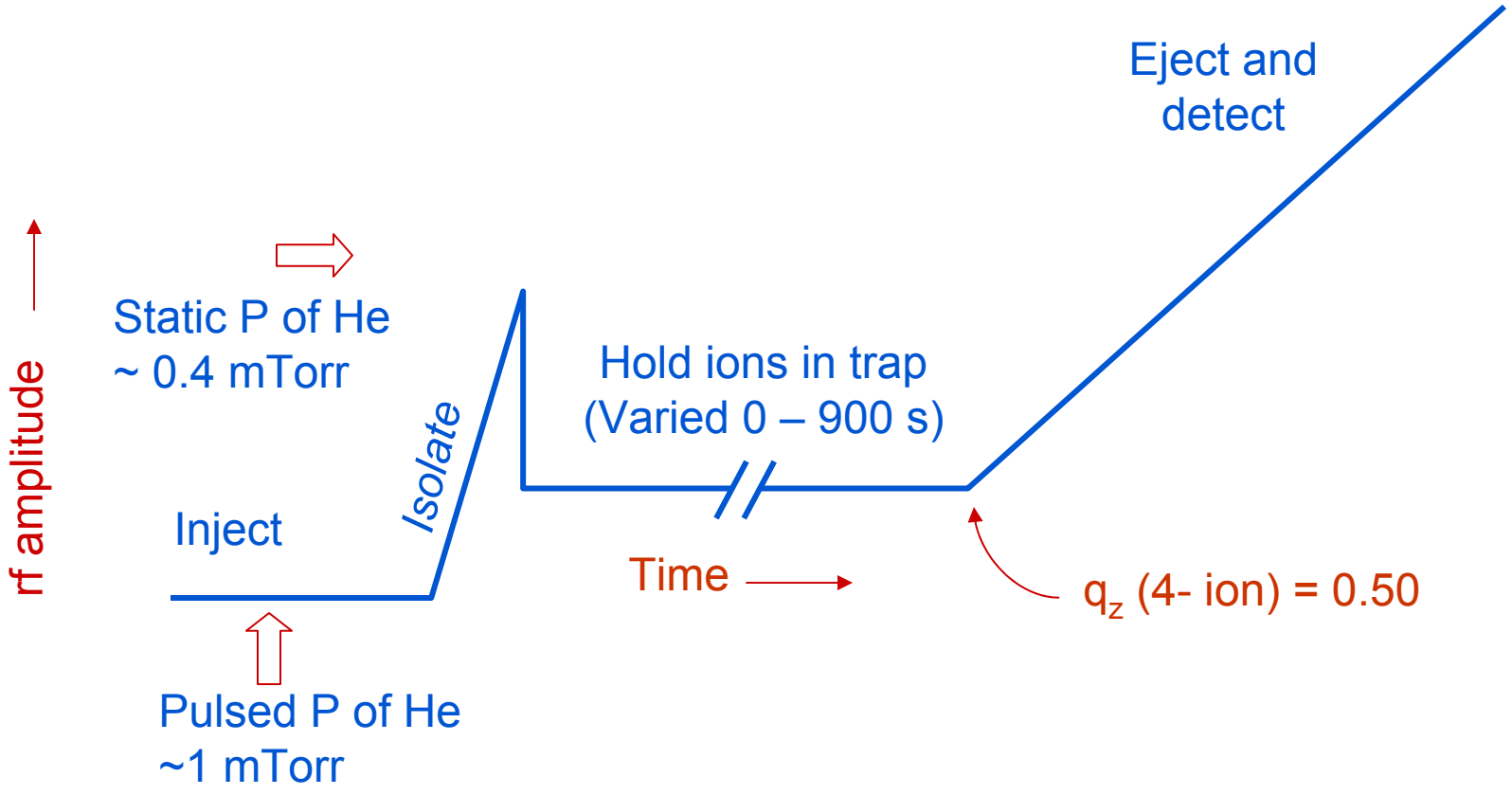
Instrumentation

- Custom-built quadrupole ion trap
  - Ω = 600 kHz, z_0 = 3 mm
  - nanoESI source
- Temperature-controlled trap assembly and He background gas
  - Electrodes and He inlet seated in copper housing
    - Copper housing resistively heated with Watlow model 965 temperature controller
  - Aluminum oxide spacers
  - Heating to ~ 170°C
- Leybold Inficon Quadrex 200 Residual Gas Analyzer (RGA) mounted on vacuum system

SCHEMATIC OF THE QUADRUPOLE ION TRAP ASSEMBLY



METHODS

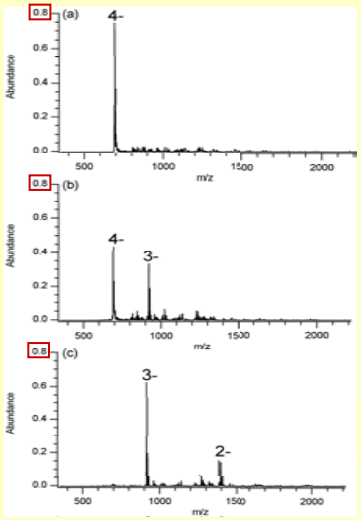


Typical scan function

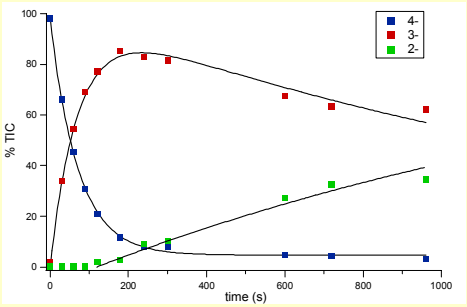
RESULTS

Initial observation of charge state conversion

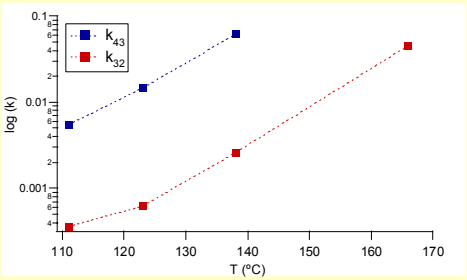
- Initially observed conversion of $M^{n-} \rightarrow M^{(n-1)-}$ under thermal and temporal conditions used for FRET experiments [1]
  - Ion population conserved (see absolute intensity scales for mass spectra below)
  - Laser excitation not required
- Rate equations solved to describe first order exponential decay/growth curves observed
- Rates increased with charge state and temperature



Mass spectra of $(T_7\text{-dye})^{4-}$ at varied heating times at 123°C. (a) 0 s (isolation of 4- charge state); (b) 30 s; (c) 960 s.



4- → 3- → 2- for T_7-dye as f(t) at 123°C

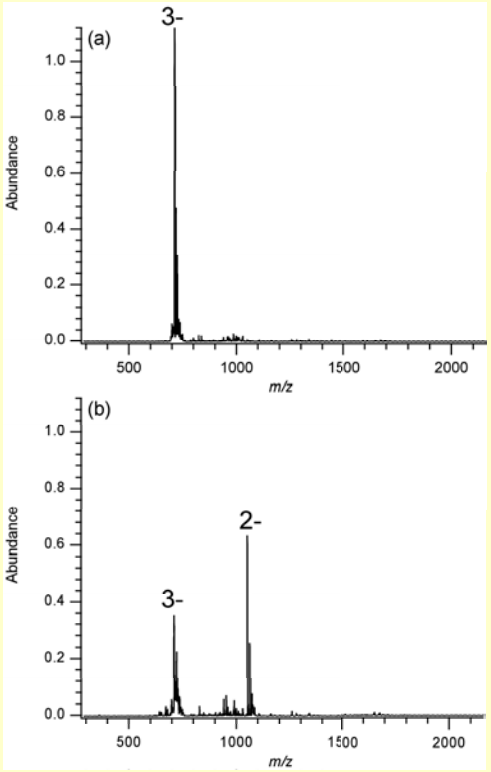


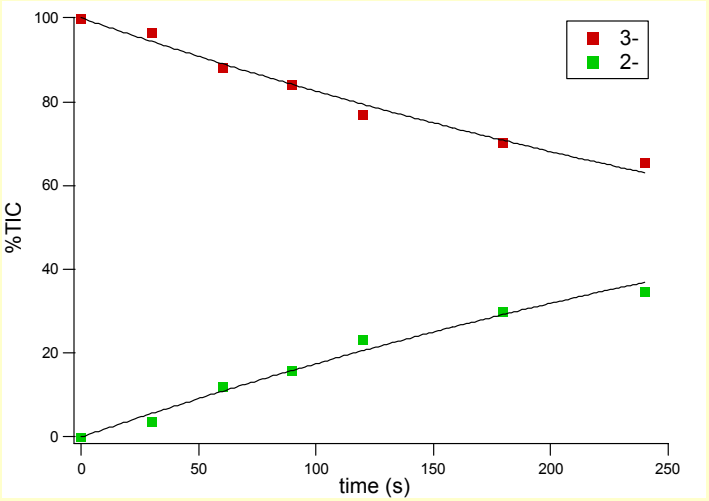
Rates of 4- → 3- and 3- → 2- for T_7-dye as f(T)

RESULTS

Charge state conversion of underivatized oligonucleotides

- Process of $M^{n-} \rightarrow M^{(n-1)-}$ also observed from underivatized oligonucleotides (see $A_7^{3-} \rightarrow A_7^{2-}$ below)



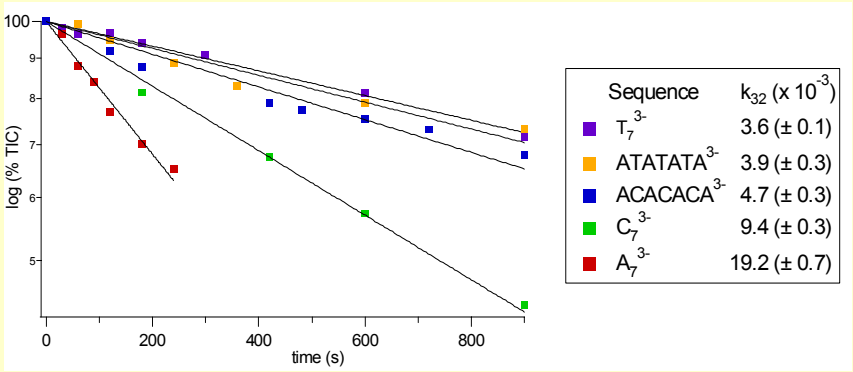


Mass spectra of $(A_7)^{3-}$ at varied heating times at 102°C. (a) 0 s (isolation of 3- charge state); (b) 240 s.

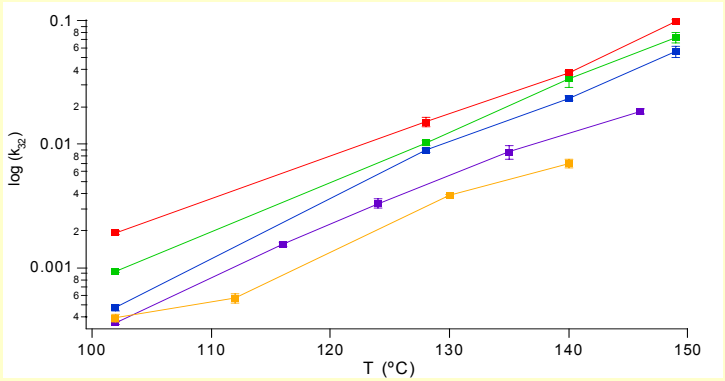
3- → 2- for A_7 as f(t) at 102°C

RESULTS

- All 7-mer oligonucleotides studied underwent charge state conversion
- For most sequences (except ATATATA), charge state conversion was only process observed
- Rates of charge state conversion varied with sequence
- Sequence dependence persisted over T range



3- → 2- for various oligonucleotide 7-mers as f(t) at 102°C



3- → 2- rates for 7-mers as f(T) (see legend above left)

RESULTS

Electron autodetachment

- $M^{n-} \rightarrow M^{(n-1)-}$ phenomenon proposed to occur via electron autodetachment
- Electron autodetachment occurs due to Coulomb repulsion in multiply charged anions
  - In small rigid molecules, has been shown to vary for different structural isomers having different electron charge separations [2, 3]
  - Has been shown to occur following vibrational heating via multiphoton infrared absorption [4]
  - Theoretical calculations describe transfer of vibration to electronic energy [5]
- Electron autodetachment from oligonucleotides dominated by conformation-induced changes in charge separation
  - Single-stranded oligonucleotide conformations very flexible and vary as a function of
    - charge state
    - temperature
    - base composition

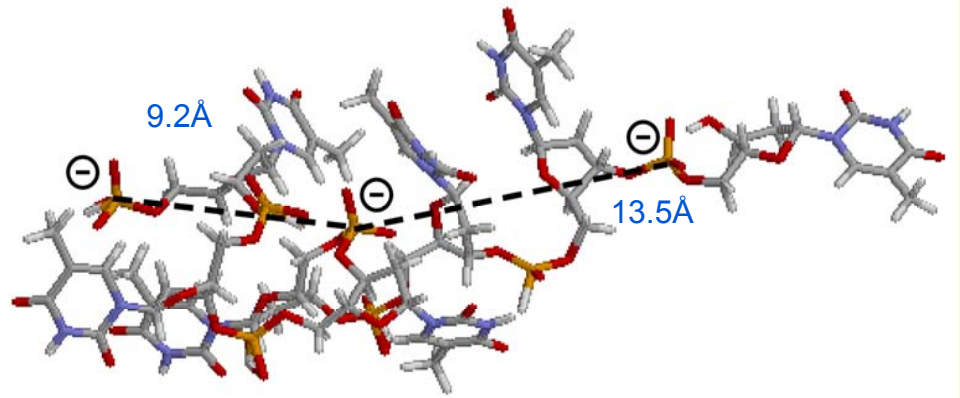
RESULTS

- Other dissociation or ion/molecule reactions are not likely causes of observation of $\mathbf{M}^{n-} \rightarrow \mathbf{M}^{(n-1)-}$ because
  - Total ion population conserved
  - Likely product ions from dissociation of oligonucleotides would appear above cutoff m/z of ~ 300 Da
  - No trend observed in variation of rates of charge loss as a function of He pressure
  - RGA measurements indicate no contaminants present and no change in background gases except ~ 3x increase in water vapor from room to elevated temperatures

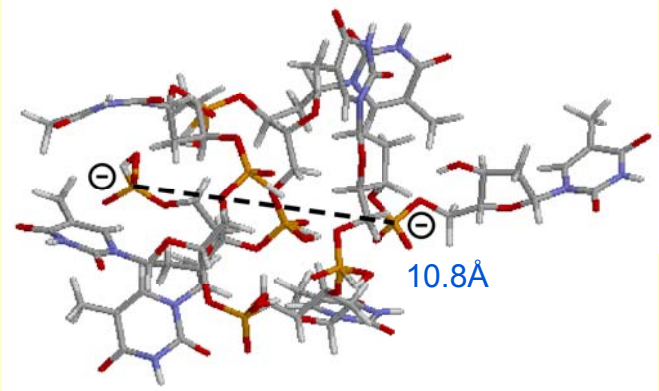
RESULTS

Molecular dynamics simulations

- Initial calculations of $(T_7)^{3-}$ and $(T_7)^{2-}$ performed [6]
  - Insight II software, 298 K, extensible systematic forcefield optimized for vacuum
  - Negative charges localized on phosphate groups
  - Structures illustrate flexibility of single-stranded oligonucleotides



T_7^{3-}



T_7^{2-}

SUMMARY

Understanding parameters affecting electron autodetachment rates

- Fluctuations in conformation
  - increased fluctuations → decreased <charge separation> → decreased detachment energy barrier
- Charge State
  - increased charge state → increased Coulomb repulsion
- Temperature
  - increased population of higher vibrational modes → increased flexibility and increased probability of electronic curve-crossing
  - increased internal energy (RRKM) may contribute energy to excitation of vibrational modes
- Sequence
  - Flexibility of oligonucleotides correlated with base stacking interactions [7]
  - Dielectric screening of Coulomb fields vary with polarizability of nucleotides
- Molecular dynamics simulations required for further understanding

REFERENCES

[1] Danell, A. S.; Parks, J. H. *Int. J. Mass Spectrom.* **2003**, *in press*.
For more information on FRET experiments, see TPK # 197.

[2] Wang, L.-S.; Wang, X.-B. *J. Phys. Chem. A*, **2000**, *104*, 1978-1990.

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[7] Goddard, N. L.; Bonnet, G.; Krichevsky, O.; Libchaber, A. *Phys. Rev. Lett.* **2000**, *85*, 2400-2403.