Electron Autodetachment of Oligonucleotide Anions in the Gas Phase
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This poster was originally presented in large format, but it was modified for easier reading/downloading from the web.
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.
**Analytes**
- Oligonucleotides purchased from Synthegen (Houston, TX)
- 5 to 10 µM in 70/20/10 MeOH/H₂O/trifluoroethanol

**Instrumentation**
- Custom-built quadrupole ion trap
  - Ω = 600 kHz, z₀ = 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
METHODS

Eject and detect

q_z (4- ion) = 0.50

Typical scan function

Static P of He
~ 0.4 mTorr

Hold ions in trap
(Varied 0 – 900 s)

Pulsed P of He
~1 mTorr

Inject

Isolate

Time

tTo f amplitude

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.

Rates of $4- \rightarrow 3-$ and $3- \rightarrow 2-$ for $T_7\text{-dye}$ as $f(T)$

4- $\rightarrow$ 3- $\rightarrow$ 2- for $T_7\text{-dye}$ as f(t) at 123°C
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- $\rightarrow$ 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)
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 \( M^{n-} \rightarrow M^{(n-1)-} \) 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

![Structures illustrating flexibility](image)
Understanding parameters affecting electron autodetachment rates

- **Fluctuations in conformation**
  - increased fluctuations $\rightarrow$ decreased $<\text{charge separation}>$ $\rightarrow$ decreased detachment energy barrier

- **Charge State**
  - increased charge state $\rightarrow$ increased Coulomb repulsion

- **Temperature**
  - increased population of higher vibrational modes $\rightarrow$ 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

   For more information on FRET experiments, see TPK # 197.