Salt Bridges: Aggregation, Hydration, and Fragmentation of Peptides and Oligonucleotides

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U. Arizona: Linda Breci, Vicki Wysocki

Heidelberg: Bela Paizs

$$ NSF \ (UCSB)$$
Structure of the talk

- Structure of small peptides
  H/D exchange vs. ion mobility
- Aggregation of peptides
- Salt bridges in oligonucleotides?
Structure of small peptides: H/D exchange vs. ion mobility
Motivation

• Building accurate computer models for protein identification from MS/MS data requires knowledge of fragmentation mechanisms.
• Is the structure/conformation related to fragmentation pattern/mechanism?
• Does H/D exchange give information on peptide structure/conformation?
• Can H/D exchange and ion mobility data be structurally correlated?
Approach

- Look at several series of peptides where important groups are systematically varied.
- Do H/D exchange and ion mobility studies on each group.
- Measure hydration energies.
- Do detailed ab initio/DFT calculations on selected systems.
- Here we will discuss RAAAA, AARAA, AAAAR but focus on AARAA.
AARAA

- University of Arizona, Tucson
- University of California, Santa Barbara
- German Cancer Research Center Heidelberg
AARAA
with and without blocked termini
H/D exchange in the Ion Trap (UA)
3 fast exchanges

AARAA

AARAA-O-CH₃

acetyl-AARAA

m/z
D$_2$O: Relay Mechanism for H/D exchange

Campbell, Rodgers, Marzluff, Beauchamp,
JACS, 1995, 12840-12854
<table>
<thead>
<tr>
<th></th>
<th>AARAA</th>
<th>AARAA-OMe</th>
<th>Ac-AARAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exchange</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>

Both termini involved
Salt bridge?
[AARAA]H+
Lowest energy structure (AMBER)

Charge solvation

Zwitterion (salt bridge)
Ion mobility method
(UCSB)
Ion mobility method

Drift cell

1–5 torr He

E
Ion Mobility (UCSB)

[Ac-AARAA]H+

[AARAA]H+

Experiment
○ Calculated (zwitterion)
● Calculated (charge solvation)

Cross section (Å²)
<table>
<thead>
<tr>
<th>Peptide</th>
<th>$\sigma$ (Experiment)</th>
<th>$\sigma$ (AMBER Calculation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neutral Terminus</td>
</tr>
<tr>
<td>[RAAAA]H$^+$</td>
<td>136.43</td>
<td>139.38</td>
</tr>
<tr>
<td>[AARAA]H$^+$</td>
<td>145.11</td>
<td>146.52 ★</td>
</tr>
<tr>
<td>[AAAAR]H$^+$</td>
<td>147.51</td>
<td>148.77 ★</td>
</tr>
<tr>
<td>[AcRAAAA]H$^+$</td>
<td>144.16</td>
<td>144.25</td>
</tr>
<tr>
<td>[AcAARAA]H$^+$</td>
<td>151.38</td>
<td>148.29</td>
</tr>
<tr>
<td>[AcAAAAR]H$^+$</td>
<td>151.84</td>
<td>151.64</td>
</tr>
</tbody>
</table>
H/D-exchange: salt bridge (?)
(AARAA)H⁺ + H₂O

Ion mobility: no salt bridge
(AARAA)H⁺
Calculations
(Heidelberg)
B3LYP/6-31g(d)
<table>
<thead>
<tr>
<th>Structure</th>
<th>Energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(AARAA)H⁺</td>
</tr>
<tr>
<td>Charge solvation</td>
<td>0.0</td>
</tr>
<tr>
<td>Zwitterion (–NH₃⁺)</td>
<td>2.3</td>
</tr>
<tr>
<td>Zwitterion (&gt;C=OH⁺)</td>
<td>12.4</td>
</tr>
</tbody>
</table>
(AARAA)H⁺ + H₂O

Zwitterion setup for relay mechanism:
–14.1 kcal/mol

C-terminus
N-terminus
H₂O
(AARAA)H⁺ + D₂O → (AARAA)D⁺ + HOD
Water binding energy

$(\text{AARAA})\text{H}^+ \cdots \text{H}_2\text{O}$

Theory (Heidelberg): 16 kcal/mol
Experiment (UCSB): 10 kcal/mol

(BSSE correction? Larger basis set?)
Summary

H/D-exchange: salt bridge (?)
(AARAA)H⁺···H₂O

Ion mobility: no salt bridge
(AARAA)H⁺

Theory:
• no salt bridge for (AARAA)H⁺
• salt bridge for (AARAA)H⁺···H₂O
• low TS for H/D-exchange for all 3 N-terminus hydrogens from salt bridge form
• no exchange possible for blocked termini since TS for proton transfer to >C=O groups high in energy
Peptide aggregation

- Important in certain diseases
  - Alzheimers
  - Mad Cow (TSE)
  - Diabetes type II
- Important for chaperone formation
- Observed as (common) occurrence in electrospray of peptides
- Energetics and mechanism not yet known
Self aggregation of peptides in ESI

- Beauchamp et al.: penta-, hexa-peptides
- Clemmer et al.: BK, Insulin chain A, ala$_{12}$
- Jarrold et al.: ac-lys-ala$_{19}$, ac-(gly-ala)$_{7}$-lys
- Bowers et al.: BK, LHRH, neurotensin

- But little detailed understanding of factors that control this aggregation
Bradykinin 9 residue peptide

2 Arg

1 Acidic functional group

Zwitterion formation

LHRH 10 residue peptide

1 Arg

no acidic group

Blocked Termini
Bradykinin

**Ion Arrival Time Distribution**

- **m/z = 354**
- **m/z = 531**
- **m/z = 1061**

**Mass Spectrum**

- **(M+3H)^3+**
- **(M+2H)^2+**
- **(M+H)^+**
Conclude:

\[A = (3BK+3H)^{3+}\]
\[B = (2BK+2H)^{2+}\]
\[C = (BK+H)^{+}\]
Increased Cell Temperature

\[(2M+2H)^{2+} \rightarrow 2(M+H)^+\] dissociation

- Fitting the ATDs yield rate constants
- Binding energies \((E_a)\) are obtained from an Arrhenius type of analysis
$[2\text{BK}+2\text{H}]^{2+} \rightarrow 2[\text{BK}+\text{H}]^+$
BK Fit

--- Fit

---- Data
Arrhenius Plot

\((2M+2H)^{2+} \rightarrow 2(M+H)^{+}\)

- Bradykinin
  - \(E_a = 35.1 \pm 1 \text{ Kcal/mol}\)
  - \(A = 5.1 \times 10^{19} \text{ s}^{-1}\)
  - \(\Delta S^\ddagger = 28.8 \text{ cal mol}^{-1} \text{ K}^{-1}\)
Arrhenius Plot

\[(2M+2H)^{2+} \rightarrow 2(M+H)^{+}\]

**Bradykinin**
- \(E_a = 35.1 \pm 1 \text{ Kcal/mol}\)
- \(\Delta S^\ddagger = 28.8 \text{ cal mol}^{-1} \text{ K}^{-1}\)

**LHRH**
- \(E_a = 51.7 \pm 2 \text{ Kcal/mol}\)
- \(\Delta S^\ddagger = 69.0 \text{ cal mol}^{-1} \text{ K}^{-1}\)
Molecular modeling

- Simulated annealing
- Dynamics simulations (300 K)
Bradykinin

(2M+2H)^{2+} \xrightarrow{(TS)^\dagger} 2(M+H)^{+}

LHRH

Bradykinin

LHRH
Monomers only slightly rearranged in dimer

Electrostatic binding between charged groups dominates
LHRH Monomer vs. Dimer

- Intermolecular interactions in dimer force open the backbone of the monomer
- Causes repulsion of the 2 N-termini and attraction between opposing N and C termini
Hypothesized Potential Energy Surface

\[
\begin{align*}
\text{Rxn Coordinate} & \quad 2[\text{BK+H}]^+ \\
\text{Energy} & \quad E_a = 35.1 \text{ kcal/mol (exp)} \\
\text{Rxn Coordinate} & \quad 2[\text{LHRH+H}]^+ \\
\text{Energy} & \quad E_a = 51.7 \text{ kcal/mol (exp)} \\
\end{align*}
\]

24.5 kcal/mol (AMBR)

11.2 kcal/mol (AMBER)
Do our calculated structures agree with experiment? Compare Cross Sections (Å²)

<table>
<thead>
<tr>
<th></th>
<th>Experiment</th>
<th>Theory*</th>
<th>Δσ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[LHRH+H]⁺</td>
<td>257</td>
<td>261</td>
<td>+1.5</td>
</tr>
<tr>
<td>[2LHRH+2H]²⁺</td>
<td>409</td>
<td>417</td>
<td>+1.9</td>
</tr>
<tr>
<td>[BK+H]⁺</td>
<td>240</td>
<td>242</td>
<td>+0.8</td>
</tr>
<tr>
<td>[2BK+2H]²⁺</td>
<td>384</td>
<td>376</td>
<td>−2.1</td>
</tr>
</tbody>
</table>

* minimized structures (lowest 10 kcal/mol)
Conclusions

- Peptides tend to form aggregates of the form \((zM+zH)^{z+}\).
- Energy barriers for dimer dissociation are of the order of 30-50 kcal/mol both for zwitterion (BK) and charge solvation structures (LHRH).
- Entropies (and energies) of activation indicate a major structural change occurs in TS for LHRH (i.e. a loose TS) while the TS for BK is much more dimer like (i.e. tighter).
Are salt bridges involved in the fragmentation of oligonucleotides?
Mass spectrometric analysis of DNA crucial for obtaining rapid information on small samples

Problem: DNA fragments during the sampling processes

Solution: Understand the fragmentation mechanism

Proposed mechanism:
1. protonation of base
2. weaken/break base-sugar bond
3. eliminate base and induce backbone fragmentation
Proton affinities of the 4 DNA bases:

\[ G > A \quad C >> T \]

Hillenkamp, Gross:
Look at fragmentation of oligonucleotides with T and G bases

should see preferential loss of G
dGT, dTGTT, dTTG, dTTGG

positive ions: see loss of G in all cases

but for negative ions …
MALDI PSD Mass Spectra for
Deprotonated Tetranucleotides

J. Gross, F. Hillenkamp, K.X. Wan, M.L. Gross
Possible explanation: dTGGT and dTTGT form salt bridges

J. Gross, F. Hillenkamp, K.X. Wan, M.L. Gross

Look at a simpler model with same possibility of salt bridge formation

Is dTGT$^-$ a salt bridge?
ATDs for dTGT$^{-}$

300 K

80 K

$\Delta \sigma = 17 \text{ Å}^2$
Scatter Plot for dTGT (salt bridge)
Scatter Plot for dTGT $^-$
(singly deprotonated)

2 families of low-energy conformers

"folded"  smaller cross-section
$\Delta\sigma \sim 18 \text{ Å}^2$

"open"  larger cross-section
$\Delta\sigma_{\text{expt}} \sim 17 \text{ Å}^2$
Similar ATDs for dGTT and dTTG

(which should not be salt bridges)

\[ \text{dGTT} \quad \sigma = 194 \, \text{Å}^2 \]
\[ \text{dTGT} \quad \sigma = 196 \, \text{Å}^2 \]
\[ \text{dTTG} \quad \sigma = 195 \, \text{Å}^2 \]
Conclusions:

- dTGT\(^{-}\) not a salt bridge

  \[\therefore\] dTGTT\(^{-}\) and dTTGT\(^{-}\) also not salt bridges

  \[\therefore\] Gross / Hillenkamp mechanism may not be correct

  (or salt bridge is type of transition state)

- Preliminary calculations indicate salt bridge structure is higher in energy

- Hydration Studies being initiated
Small peptides and oligonucleotides are generally not salt bridges.
However, salt bridges can be energetically close to charge solvation structures.
Salt bridges are stabilized (compared to CS) by additional solvation.
Salt bridges are important intermediates (H/D exchange, fragmentation).
Little structural change occurs within a salt bridge unit when units aggregate.

Final conclusions: salt bridges in the gas phase