The Structure of Small Protonated Peptides Containing Arginine and the Effect of Hydration

Thomas Wyttenbach, Denfeng Liu, Perdita Barran, Michael T. Bowers
University of California, Santa Barbara

Vicki Wysocki, Linda Breci
University of Arizona, Tucson, AZ

Bela Paizs
German Cancer Research Center, Heidelberg, Germany
Background

• 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.
• Obtain
  ◦ H/D exchange
  ◦ ion mobility
  ◦ hydration energy
  ◦ detailed ab initio/DFT data.
• Example series: RAAAA, AARAA, AAAAR
• This talk: AARAA.
AARAA

zwitterion / salt bridge
AARAA

blocked termini
H/D exchange in the Ion Trap
3 fast exchanges

AARAA

AARAA-O-CH₃

acetyl-AARAA
AARAA: H/D exchange
AARAA-OMe: No H/D exchange
Ac-AARAA: No H/D exchange

Both termini involved in (AARAA)H⁺ H/D exchange

Is (AARAA)H⁺ a salt bridge???
Relay Mechanism for H/D exchange with D₂O

Campbell, Rodgers, Marzluff, Beauchamp,
JACS, 1995, 12840-12854
Relay Mechanism for H/D exchange with D$_2$O

Campbell, Rodgers, Marzluff, Beauchamp, JACS, 1995, 12840-12854
Relay mechanism involving salt bridge
Ion mobility method

ESI Ion Source → Ion Funnel → Drift Cell → MS → Detector

1–5 torr He Drift cell

In → E → out
Experimental cross sections

Cross Section (Å²)

AARAA

AARAA-OMe

Ac-AARAA
Experiment: drift time $\rightarrow$ ion mobility $\rightarrow$ experimental cross section

Calculation: model structures $\rightarrow$ calculated cross section

Molecular modeling: AMBER
(AARAA)H$^+$

Lowest energy structure (AMBER)

Charge solvation open

Zwitterion (salt bridge) compact
Cross Sections

Cross Section (Å²)

- Experiment
- Calculation: Charge Solvation
- Calculation: Salt Bridge
H/D-exchange (✓):
salt bridge (?)
\((\text{AARAA})\text{H}^+ + \text{H}_2\text{O}\)

Ion mobility (✓):
no salt bridge
\((\text{AARAA})\text{H}^+\)
Calculations

MM scan / DFT optimization
B3LYP/6-31+G(d,p)

<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>4.8</td>
</tr>
<tr>
<td>Zwitterion (&gt;C=OH⁺)</td>
<td>10.9</td>
</tr>
</tbody>
</table>
Charge solvation
\[ \sigma = 151 \, \text{Å}^2 \]

Salt bridge
\[ \sigma = 137 \, \text{Å}^2 \]

Experiment:
\[ \sigma = 145 \, \text{Å}^2 \]
(AARAA)H⁺•H₂O

global minimum
(charge solvation)
Zwitterion (+5 kcal/mol)

set up for
H/D exchange
relay mechanism

(AARAA)H⁺•H₂O

C-terminus
N-terminus
Charge solvation

(AARAA)H⁺•H₂O

C-terminus

N-terminus
Transition state

\((\text{AARAA})H^+ \cdot H_2O\)
Zwitterion

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

C-terminus

N-terminus
3 fast exchanges

AARAA

AARAA-O-CH₃

acetyl-AARAA

Relative Intensity

m/z

MH⁺
(AARAA)H⁺ + D₂O  \rightarrow  (AARAA)D⁺ + HOD

2 kcal/mol  \rightarrow  11 kcal/mol

(AARAA)H⁺ \cdots \text{D}_2\text{O}

B3LYP/6-31+G(d,p)
(AARAA)H⁺ + D₂O → (AARAA)D⁺ + HOD

Relay mechanism

ZW → CS

5 kcal/mol

2 kcal/mol

B3LYP/6-31+G(d,p)
Ion mobility instrumentation:
Hydration under equilibrium conditions
Mass spectrum

\[ \text{MH}^+ + n \text{H}_2\text{O} \quad \leftrightarrow \quad \text{MH}^+ \cdot (\text{H}_2\text{O})_n \]

1.3 torr H\textsubscript{2}O
260 K

\[ n=0 \]

AARAA
Mass spectra

a) AARAA

b) AARAA-OMe

\[
\text{MH}^+ + n \text{H}_2\text{O} \rightleftharpoons \text{MH}^+ \cdot (\text{H}_2\text{O})_n
\]

1.3 torr H2O
260 K

\[n=0\]
## Experimental $\Delta H^\circ$ and $\Delta S^\circ$ values

\[
MH^+ \cdot (H_2O)_{n-1} + H_2O \quad \rightleftharpoons \quad MH^+ \cdot (H_2O)_n
\]

<table>
<thead>
<tr>
<th>M</th>
<th>n</th>
<th>$-\Delta H^\circ$ (kcal/mol)</th>
<th>$-\Delta S^\circ$ (cal/mol/K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AARAA</td>
<td>1</td>
<td>10.2 ± 0.3</td>
<td>23 ± 1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.4</td>
<td>18</td>
</tr>
<tr>
<td>Ac-AARAA</td>
<td>1</td>
<td>9.5</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.1</td>
<td>18</td>
</tr>
<tr>
<td>AARAA-OMe</td>
<td>1</td>
<td>9.4</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.4</td>
<td>18</td>
</tr>
</tbody>
</table>
Water binding energy

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

Theory (dkfz): 8.9 kcal/mol (after BSSE and ZPE correction)

Experiment (UCSB): 10.2 kcal/mol
H/D-exchange: (AARAA)H⁺ salt bridge?

Ion mobility: (AARAA)H⁺ no salt bridge

Theory: 
- no salt bridge for (AARAA)H⁺
- 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
- H₂O stabilizes (AARAA)H⁺ salt bridge more than charge solvation