Abstract

The hydration of the naturally occurring decapeptide LHRH has been explored in a gas phase study. The primary objective of this work was to determine values of ?H° and ?S° for the process:

$$(M + zH)^{zH}(H_2O)_n + H_2O \hat{U} (M + zH)^{zH} + (H_2O)_{n+1}$$

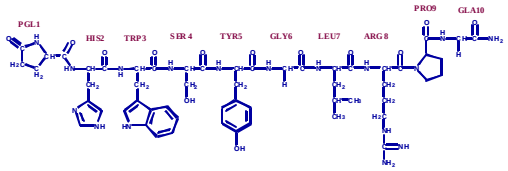
where, M = LHRH, z = 1 and 2, and n = 1, 2, 3 . . .

A continuous beam of peptide ions was introduced into a temperature regulated copper reaction cell containing water vapor at a known pressure ranging from 0.5 to 2.0 torr. A quadrupole mass spectrometer situated after the cell was used to measure signal intensities of the bare and hydrated peptide ions. Within the reaction cell, equilibrium was rapidly established and the ratio of reactant to product peak intensities was used to yield ?G°, the free energy change at the cell temperature. Mass spectra were obtained at a variety of temperatures ranging from 265-300 K and a plot of ?G° versus T was used to determine ?H° and ?S°. These values have been obtained for the addition of up to 7 water molecules to $(M+2H)^{2+}$ and 4 to $(M+H)^{+}$.

Preliminary results show that the first water is both more strongly bound to the peptide and has a significantly more negative entropy of association than do subsequent additions. A series of molecular dynamics calculations have been performed to determine conformational changes accompanying water association. Initial findings are presented here, and discussed along with the implications for connecting these results to solution phase studies.

Experimental Technique

Luteinizing Hormone Releasing Hormone (LHRH)



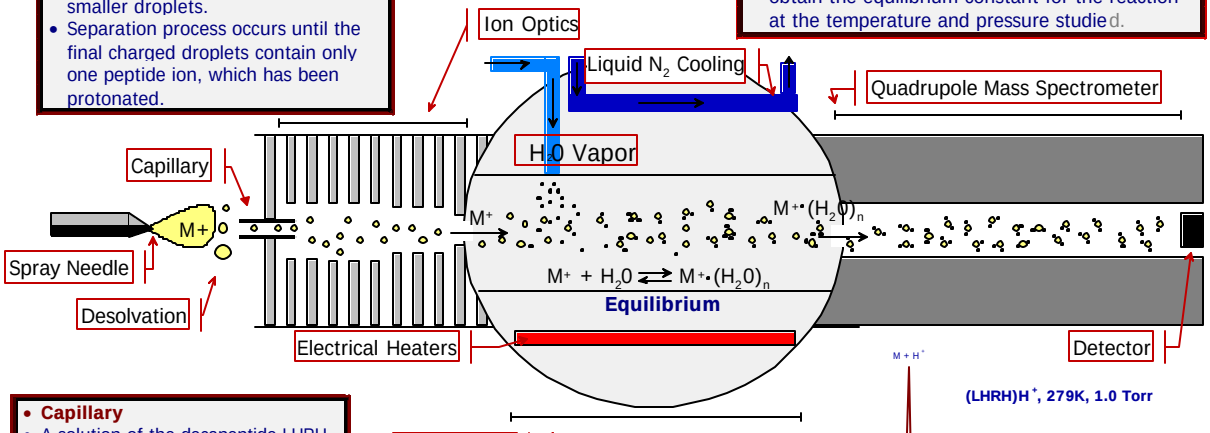
- Mass: 1182 amu; 10 residues
- Sprayed from 100µM solution
- Detected as $(LHRH)H^+$ and $(LHRH)2H^+$

These experiments used an Electrospray Ionization Mass Spectrometer. This newly developed instrument allows a range of solvated species to be examined as ions in the gas phase. Different concentrations of hydrated peptide ions produced from the electrospray are measured using a mass spectrometer. Both the physical processes and the machinery used are outlined in the following diagram:

- **Desolvation**
- The imposed electric field causes excess charge to accumulate in the solution droplets released from the spray needle.
- Because of Coulombic repulsion, the sprayed drops break into smaller and smaller droplets.
- Separation process occurs until the final charged droplets contain only one peptide ion, which has been protonated.

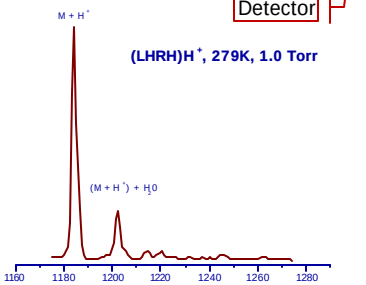
- **Ion Optics**
- Lenses guide and focus the ion beam into the reaction cell.
- The ion signal is optimized by tuning the voltages applied to these elements.

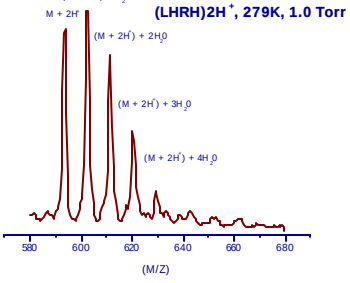
- **Quadrupole**
- The Quadrupole is scanned over a mass range that covers the mass of $(LHRH)zH^{z+}$ and any subsequent water additions.
- Detected data is sent to a computer program interface. Mass spectrums are produced and then analyzed for the different concentrations of hydrated ions.
- The relative intensities are measured in order to obtain the equilibrium constant for the reaction at the temperature and pressure studied.



- **Capillary**
- A solution of the decapeptide LHRH is released from a micro needle source directly in front of the capillary.
- The potential difference between the needle and the capillary is ~1000kV, the distance is variable (1-5 mm).
- Positive ions are drawn to the negative electric field of the capillary.
- As the ions enter the capillary they are also propelled into the vacuum chamber by differential pumping.

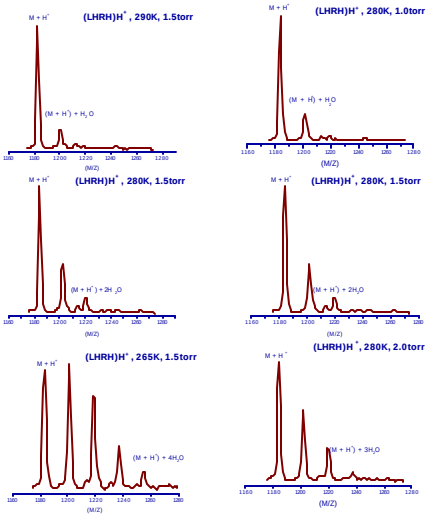
- Reaction Cell
- The reaction cell is a controlled environment of known temperature and pressure.
- Water vapor is introduced. Pressure of water in the cell is measured accurately, and held constant for any one set of measurements.
- The temperature of the cell can be varied through the use of liquid nitrogen cooling and electric heaters. This was crucial to our experiment, because the equilibrium of the hydration was measured at different temperatures and pressures in order to calculate the thermodynamic values of the reaction.



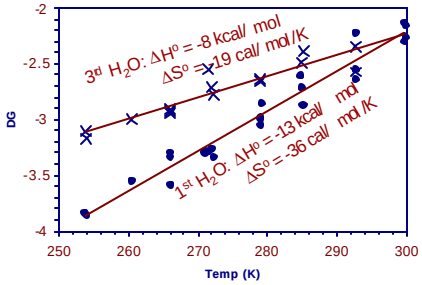


Results

Mass Spectra for Varying Temperature and Pressure



Thermodynamic Values Calculation



At Equilibrium

$$M^+\bullet(H_2O)_{n-1} \overset{H_2O}{\rightleftharpoons} M^+\bullet(H_2O)_n$$

$$K_{eq} = \frac{[M^+\bullet(H_2O)_n]}{p(H_2O)\ [M^+\bullet(H_2O)_{n-1}]}$$

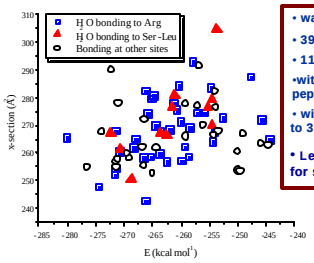
$$\Delta G^o = -RT \ln K_{eq}$$

$$\Delta G^o = \Delta H^o - T\Delta S^o$$

n	ΔH^o [kcal/mol]	ΔS^o [cal/mol/K]
1	-13	-36
2	-10	-26
3	-8	-19
4	-7	-16

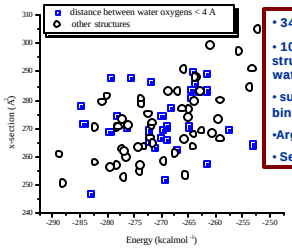
- Calculations
- Using Molecular Dynamics a simulated annealing procedure gives 100 possible structures for the peptide ion with one or two water molecules attached.
- These structures are sorted according to their energy and cross-section.
- Lower energy structures are examined for similarities.
- Binding sites for water are identified.
- Lowest energy structure has site based on Arg Try Gly-NH_2.
- The number of inter -peptide bonds not involving water are counted.

$(M+H^+) + H_2O$: 100 Structures



- water binds to peptide surface
- 39/100 H_2O bound toArg
- 11/100 H_2O bound atLeu-Ser
- without water average intra--peptide H-bonds 42
- with water average decreases to 37 (not including water)
- Less possible conformations for singly hydrated peptide

$(M+H^+) + 2H_2O$: 100 structures

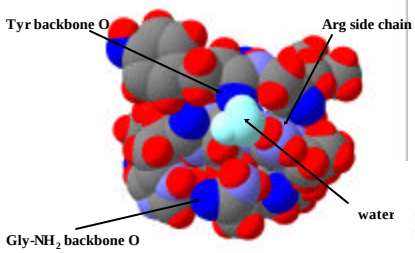


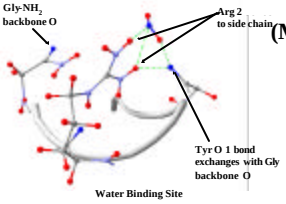
- 34/100 H_2O – H_2O
- 10 lowest energy structures at least one water binds to Arg
- suggests several likely binding sites
- Arg preferred for 1 H_2O
- Second water moves

?E Calculation:

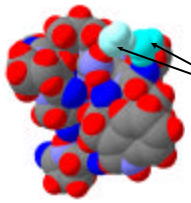
N	E_{avg} (kcal/mol)	?E (kcal/mol)
0	-256.10 ± 2.11	
1	-274.10 ± 2.5	18
2	-283.90 ± 2.7	9

$(M+H^+) + H_2O$ Arg Binding Site

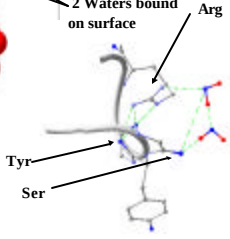




$(M+H^+) + 2H_2O$



- Lowest energy structure with H_2O - H_2O (3rd lowest)



- Conclusions
- Formation of hydrated LHRH ions with up to 15 H_2O molecules under equilibrium conditions is possible using the experimental procedure outlined
- Binding energies and entropies have been determined
- Calculations indicate preferred binding on the surface of peptide
- 1/3 of structures found for $(M+H)^+ + H_2O$ have water binding to Arg
- With subsequent water addition at least one water binds to the Arg site, with a variety of sites available for 2nd water addition, for 1/3 of cases it binds to 1st water