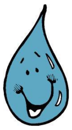
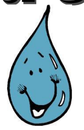
Hydration of Small Peptides

Thomas Wyttenbach,
Dengfeng Liu, and
Michael T. Bowers





Department of Chemistry and Biochemistry
University of California Santa Barbara
Santa Barbara, California 93106

Introduction

Why study hydration?

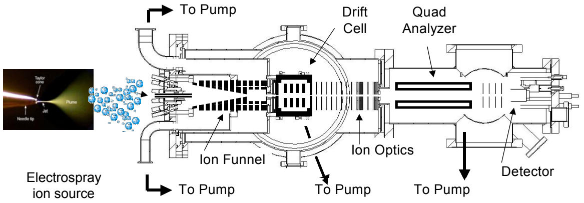
Bridge gas phase and solution phase

Study solvation on a molecular level (not much is known)
i.e. the effect of individual water molecules on solute molecules
in terms of

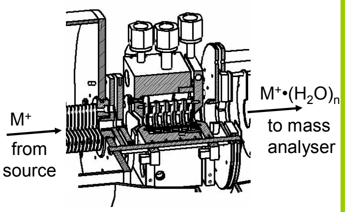
- energetics (water binding energy)
- structure
  - ❑ conformations, folding
  - ❑ zwitterion formation
  - ❑ hydration sites

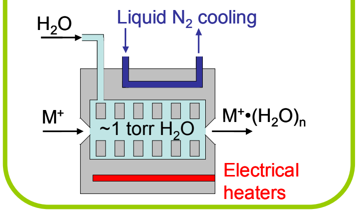
Methods

1 Experimental



Drift cell





Ions are formed by ESI, transported into a high vacuum chamber via an ion funnel, and injected into a drift cell filled with ~0.1 to 2.3 torr of water vapor (no additional buffer gases added). The water pressure (measured by a baratron) is limited by the pumping capacity and for cold temperatures by the water vapor pressure over ice. Ions travel through the cell under the influence of a weak electric field (5-10 Vcm^{-1} at 1 torr of H_2O) and quickly equilibrate with water vapor. The cell temperature is increased and lowered by electrical heaters and a flow of liquid nitrogen, respectively, and is measured by a Pt-resistor and three thermocouples in various places in and around the copper cell. The amount of water uptake is analyzed in the quadrupole mass filter following the drift cell. Maximum water pickup is achieved at ~260 K, a compromise between low temperature and still reasonable water vapor pressure (~1.3 torr).

2 Equilibrium concepts

•Hydration (water uptake) is detected as an increase of mass.
•The ratio
$[M^+\bullet(H_2O)_n] / [M^+\bullet(H_2O)_{n-1}]$
is determined from relative peak intensities in the mass spectrum.

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

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

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

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

3 Calculations

•Model structures are generally generated by AMBER molecular mechanics (MM).
•For selected small model systems density functional theory (DFT) calculations are carried out including the NBO calculations on $CH_3NH_3^+$ mentioned in the results section.

3 cont'd

Experimental water binding energies –ΔH°:
Charge remote hydration vs. charge solvation
Ac-AAAAK vs. Ac-AAKAA

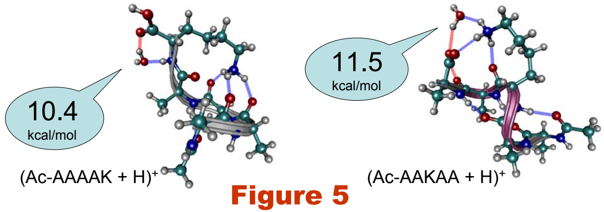


Figure 5

4 Deprotonated carboxylic acids

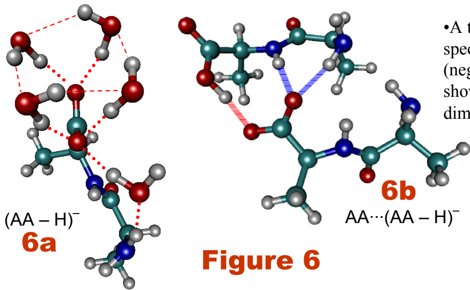


Figure 6

•A typical ESI mass spectum of dialanine (negative ion mode) shows exclusively dimer AA···$(AA - H)^-$
•CID yields monomeric $(AA - H)^-$.

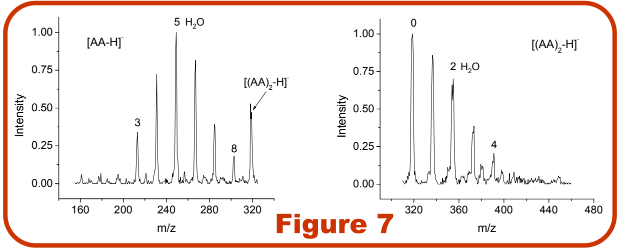


Figure 7

Table 3

Experimental binding energies (–ΔH°) and entropies (–ΔS°) of the *n*th water adding to deprotonated dialanine, dialanine dimer, diglycine, and diglycine dimer.

	n	–ΔH° (kcal/mol)	–ΔS° (cal/mol/K)
$[AA\text{-}H]^-$	1	11.7±0.2	17.1±0.4
	2	9.6±0.2	16.0±0.6
	3	8.6±0.2	15.6±0.4
$[(AA)_2\text{-}H]^-$	1	8.4±0.4	19.1±1.3
$[GG\text{-}H]^-$	1	11.0±0.3	14.5±0.7
	2	9.5±0.3	15.2±0.7
	3	8.2±0.3	14.4±0.7
	4	7.4±0.1	13.7±0.3
$[(GG)_2\text{-}H]^-$	1	7.5±0.3	14.7±1.1

•Hydration experiments indicate that water binds much more readily to the monomer than to the dimer (**Fig. 7**).
•In the dimer the deprotonated carboxylic group is well solvated by the neutral dialanine molecule (**Fig. 6b**).
•In the monomer the deprotonated carboxylic group is not self-solvated at all.
•The first water molecule adding to AA···$(AA - H)^-$ is about equally strongly bound as the third water in $(AA - H)^-$ (**Table 3**).

Conclusions

- The charge carrying group ($-NH_3^+$ or $-COO^-$) is the preferred site of hydration.
- Adding water delocalizes part of the positive charge on $-NH_3^+$ over the water molecules added. As a result the electrostatic interaction between the partly hydrated system and the next water molecule decreases.
- Hence, the water binding energy steadily deceases with increasing amount of hydration.
- Filling the first solvation shell around $-NH_3^+$ is not reflected in the binding energies.
- The intramolecular H-bond in lysine eliminates one site of hydration on the $-NH_3^+$ group, reflected in a smaller binding energy of the first water.
- In peptides with fully solvated (by self-solvation and/or hydration) $-NH_3^+$ groups charge remote water binding sites compete efficiently.

Results

1 Free protonated amines without intramolecular hydrogen bonds: Solvation shells

- Molecular mechanics calculations indicate that 3 water molecules fill the first solvation shell around a free –NH_3^+ group (**Fig. 1** “MM” ■).
- Experimental water binding energies monotonically decrease with increasing number of water molecules (**Fig. 1** “Experiment” ● 💬). No indication for filling the first solvation shell.
- Why is there a decrease of the water binding energy from the first to the third water?
- Why is there no step between the third and fourth water?
- Adding water delocalizes part of the positive charge on –NH_3^+ over the water molecules added (**Table 1** , NBO charges). As a result the electrostatic interaction decreases (**Fig. 2** ●).

Figure 1

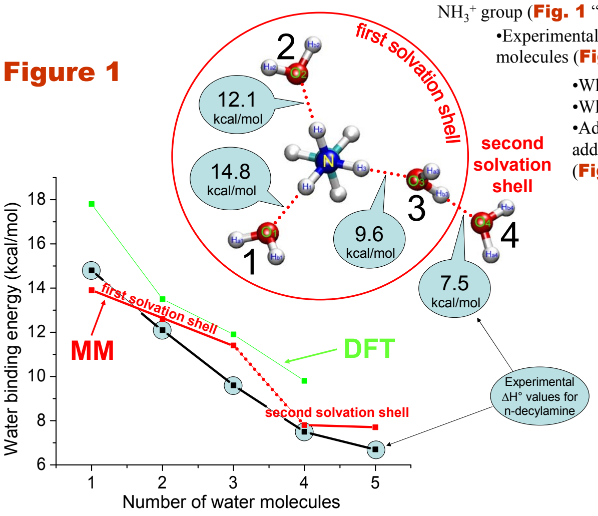


Table 1

NBO charge distribution of naked and hydrated $CH_3NH_3^+$ calculated at the B3LYP/6-311++G** level of theory

	Atom	Molecule		
		$CH_3NH_3^+$	H_2O	$CH_3NH_3^+ \bullet H_2O$
	N	-0.672		-0.698
	H_1	0.442		0.472
	H_2	0.442		0.428
	H_3	0.442		0.428
	O_1		-0.916	-0.96
	H_{a1}		0.458	0.504
	H_{b1}		0.458	0.504

	Atom	Molecule		
		$CH_3NH_3^+ \bullet (H_2O)_2$	$CH_3NH_3^+ \bullet (H_2O)_3$	$CH_3NH_3^+ \bullet (H_2O)_4$
	N	-0.722	-0.746	-0.75
	H_1	0.464	0.454	0.45
	H_2	0.464	0.454	0.45
	H_3	0.414	0.454	0.46
1	O_1	-0.958	-0.956	-0.956
	H_{a1}	0.498	0.494	0.492
	H_{b1}	0.498	0.494	0.492
2	O_2	-0.958	-0.956	-0.956
	H_{a2}	0.498	0.494	0.492
	H_{b2}	0.498	0.494	0.492
3	O_3		-0.956	-0.986
	H_{a3}		0.494	0.486
	H_{b3}		0.494	0.518
4	O_4			-0.952
	H_{a4}			0.49
	H_{b4}			0.49

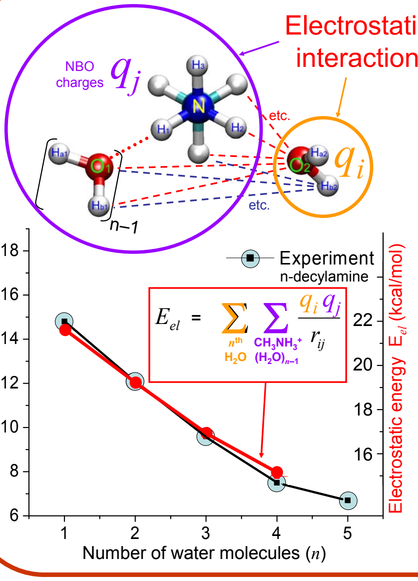
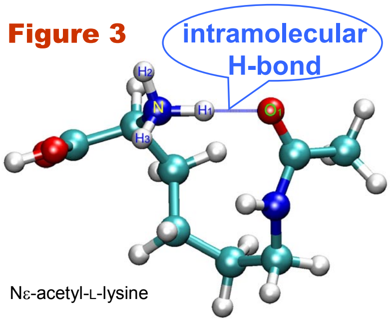


Figure 2

2 Lysine: protonated amine with intramolecular hydrogen bond

- Intramolecular H-bonds weaken amine···water interaction.
- The one intramolecular H-bond in Nε-acetyl-L-lysine is the first site of solvation (“self-solvation”).
- In Nε-acetyl-L-lysine the first water is bound as strongly as the second (or third) water in a free amine (**Table 2**).

Figure 3



Nε-acetyl-L-lysine

Table 2

Experimental binding energies (–ΔH° in kcal/mol) for n^{th} water molecule

n	n-decyl-amine	Nε-acetyl-L-lysine
1	14.8	10.1
2	12.1	8.9
3	9.6	7.7
4	7.5	

3 Fully solvated (by self-solvation or hydration) protonated amines: charge remote hydration sites in peptides

- If the site of protonation is fully hydrated (dialanine, **Fig. 4a**) or self-solvated (Ac-AAAAK, **Fig. 4b**) charge remote hydration sites become competitive.

Figure 4