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Sequential Hydration of Small Protonated Peptides

Dengfeng Liu, Thomas Wyttenbach, Perdita E. Barran,† and Michael T. Bowers*

Contribution from the Department of Chemistry and Biochemistry, University of California at Santa Barbara, Santa Barbara, California 93106

Received February 12, 2003; E-mail: bowers@chem.ucsb.edu

Abstract: The sequential addition of water molecules to a series of small protonated peptides was studied by equilibrium experiments using electrospray ionization combined with drift cell techniques. The experimental data were compared to theoretical structures of selected hydrated species obtained by molecular mechanics simulations. The sequential water binding energies were measured to be of the order of 7−15 kcal/mol, with the largest values for the first water molecule adding to either a small nonarginine containing peptide (e.g., protonated dialanine) or to a larger peptide in a high charge state (e.g., triply protonated neurotensin). General trends are (a) that the first water molecules are more strongly bound than the following water molecules, (b) that very small peptides (2−3 residues) bind the first few water molecules more strongly than larger peptides, (c) that the first few water molecules bind more strongly to higher charge states than to lower charge states, and (d) that water binds less strongly to a protonated guanidino group (arginine containing peptides) than to a protonated amino group. Experimental differential entropies of hydration were found to be of the order of −20 cal/mol/K although values vary from system to system. At constant experimental conditions the number of water molecules adding to any peptide ion is strongly dependent on the peptide charge state (with higher charge states adding proportionally more water molecules) and only weakly dependent on the choice of peptide. For small peptides molecular mechanics calculations indicate that the first few water molecules add preferentially to the site of protonation until a complete solvation shell is formed around the charge. Subsequent water molecules add either to water molecules of the first solvation shell or add to charge remote functional groups of the peptide. In larger peptides, charge remote sites generally compete more effectively with charge proximate sites even for the first few water molecules.

Introduction

Although opinions about the origin of life may diverge, there is little doubt that the large abundance of liquid water on our planet had a profound influence on the evolution of the processes of life on earth. A liquid medium provides a high molecular mobility, a condition required for molecules to encounter each other with a frequency suitable for the processes of metabolism and synthesis to occur. The characteristic properties of the water molecule (large dipole moment and ability to act both as an excellent hydrogen bond donor and acceptor) lead to a strong preference of water to interact with and dissolve molecules with functional groups that are polar, acidic, basic, hydrogen bond donors, and/or hydrogen bond acceptors. As a consequence of the strong water−solute interaction the aqueous environment has a significant influence on the shape (folding) of floppy solute molecules and on the solute charge distribution (state of protonation of acidic and basic sites). As shape and biological activity of biomolecules are intimately related, the solvent water is an integral part of a functioning biological system.

Modern nuclear magnetic resonance[1] (NMR) and high-resolution X-ray crystallography[2] techniques are sophisticated enough to address the issue of specific hydration sites of biopolymers in solution and in the crystal, respectively. Also Fourier transform infrared spectroscopy,[3] neutron scattering,[4] and theoretical methods[5] including molecular mechanics in combination with NMR and/or X-ray have been used to obtain information about the location of individual water molecules. However, nothing can be learned from these methods about the influence of an individual water molecule on the structure of the biomolecule or about the contribution of the water molecule to the system energetics. Calorimetric techniques[6] can be used to evaluate bulk hydration enthalpies, but these methods do not provide any information about the effect of an individual water molecule.

Gas-phase spectroscopy methods along with high level ab initio calculations[7] provide the most detailed information on

† Present address: SIRCAMS, School of Chemistry, The University of Edinburgh, Joseph Black Building, The King's Buildings, West Mains Road, Edinburgh EH9 3JJ, United Kingdom.

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molecular structure, but these methods are limited to smaller model systems such as phenyl containing alcohols,[8] amines,[9] and amides,[10] 2-pyridone,[11] guanine,[12] and similar molecules[13] with typically one or two water molecules attached. For glycine the question of zwitterion formation has been addressed theoretically as a function of solvent molecules added.[14] Polar solvents such as water strongly stabilize zwitterions, and for glycine it is found that at least three (probably more) water molecules are required to make the zwitterion more stable than the neutral amino acid form.[14]

Computer aided molecular modeling is a powerful technique to model larger biological systems in solution, and the method can be used to answer certain questions regarding the stepwise addition of water molecules as well. However, certain important processes that are known to occur during hydration such as changes in the charge distribution and particularly proton transfers are not accounted for by these simple molecular modeling methods. On the experimental side, the most promising techniques to study systems with molecular weights in the 100−10 000 amu range appear to be mass spectrometry (MS) based. Fourier transform mass spectrometers have been used to examine the dynamics of de-hydration of extensively hydrated (>100 water molecules) biomolecules formed by electrospray ionization (ESI).[15,16,17] Other MS experiments include the examination of the stepwise hydration of organic amines,[18] amino acids,[19] and a hand full of peptides[20,21] and proteins[22] under high-pressure equilibrium conditions.

The present study, based on this latter approach and supported by molecular mechanics studies, is a report on the sequential hydration of a series of small protonated peptides with up to twenty water molecules. The peptides used in this study focus on the small alanine (A) based model peptides di-, tri-, penta-alanine, and some arginine (R) containing penta-peptides such as RAAAA. Somewhat larger bioactive peptides bradykinin (RPPGFSPFR, 9 residues), LHRH (Luteinizing hormone releasing hormone, EHWSYGLRPG, 10 residues), and neurotensin (ELYENKPRRPYIL, 13 residues) will be briefly discussed but details will be reported elsewhere.[23] The choice of this set of peptides allows us to address issues such as the effect of peptide size, the effect of the charge-carrying group (amine vs guanidine), and the effect of peptide charge state on hydration properties. In general (and in the application here), peptide ions formed by electrospray ionization in the positive ion mode are found to be singly or multiply protonated species. Small peptides ($\leq$5 residues) are typically singly protonated, whereas increasingly higher charge states are preferred for increasingly larger peptides: bradykinin and LHRH are singly and doubly protonated, neurotensin is singly, doubly, and triply protonated.

Experimental Section

The experimental method is analogous to that previously used by our group[24] and by others[22,25] for measuring equilibrium properties of a variety of ion-ligand systems and the instrumentation employed here has previously been described in detail.[26] Briefly, 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).

Mass spectra obtained at different drift times (1−2 ms) are identical, confirming that equilibrium is established inside the cell under the conditions used. A potential source of error is water dissociation in the quadrupole after the drift cell. The effect is minimized by tuning the quadrupole bias (controlling the ion kinetic energy) for maximum intensity of hydrated ions. Too much kinetic energy increases dissociation due to collisional activation, and very slow water complexes undergo unimolecular dissociation. Systematic errors in water pressure (1%) and temperature (~1 K) result in a much smaller error of the thermodynamic quantities presented in the results section than statistical errors. Stability of pressure and temperature during an experiment contributes to the statistical error evident in van't Hoff plots. The relatively narrow temperature range available to carry out the experiments of this study is the major source of statistical errors reported in the tables below.

All of the samples were purchased from Sigma (St. Louis, MO) and used without further purification with the exception of the arginine containing pentapeptides, which were synthesized at the University of Arizona at Tucson.[27] Samples were typically sprayed from a ~100 μM solution (water: acetonitrile: trifluoroacetic acid, 50%:50%:0.1%) using a metal coated glass tip in a nano-electrospray arrangement.[26]

In an attempt to theoretically understand experimental trends, the hydration process has also been studied by computer simulations using molecular mechanics methods on small model peptides. For these studies, the AMBER 6 suite of programs has been employed together with the standard AMBER force field.[28] For the water molecule, the

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TIP3P model[29] is employed with modified charges of +0.329 on hydrogen and −0.658 on oxygen to account for the gas-phase water dipole moment of 1.85 D.[30] Model structures are obtained by a simulated annealing protocol identical to that previously used.[31] Model structures in the lowest ~1 kcal/mol energy range are considered reasonable and analyzed for common features.

Selected structures obtained by molecular mechanics are further optimized by density functional theory (DFT) methods on the B3LYP/6-311++G** level using the GAUSSAIN98 software package.[32] All water-binding energies reported here are corrected by the counterpoise correction[33] to account for basis set superposition errors (BSSE). Zero point energies (ZPE) are taken into account for naked and hydrated $CH_3NH_3^+$, where frequency calculations on the B3LYP/6-311++G** level are feasible. No attempt was made to calculate frequencies for the dialanine system. However, in our experience water binding energies decrease by 1−3 kcal/mol after ZPE correction.[34]

Results

Figure 1 shows mass spectra obtained for the peptide neurotensin at 260 K and 1.3 torr H_2O pressure. The electrospray ion source produces charge states +1, +2, and +3 of neurotensin, corresponding to the singly, doubly, and triply protonated peptide. The peak labeled with "0" in Figure 1a corresponds to the bare peptide ion $(M+H)^+$, whereas the most intense peak "2" corresponds to $(M+H)^+\cdot(H_2O)_2$, an ion with a mass of 36 mass units above $(M+H)^+$, and peak "7" corresponds to $(M+H)^+\cdot(H_2O)_7$. Similar labeling occurs for other charge states.

Because the intensity I_n of the $(M+H)^+\cdot(H_2O)_n$ peak is proportional to the ion concentration $[(M+H)^+\cdot(H_2O)_n]$, the ratio I_n/I_{n-1} yields the equilibrium constant K_n

$$(M + zH)^{z+} \cdot (H_2O)_{n-1} + H_2O \rightleftarrows (M + zH)^{z+} \cdot (H_2O)_n \quad (1)$$

$$K_n = \frac{I_n}{I_{n-1}} \frac{1}{P(H_2O)} \quad (2)$$

where $P(H_2O)$ is the known water pressure. Figure 2 shows plots of I_n/I_{n-1} versus the water pressure for neurotensin $(M + 2H)^{2+}$ at 271 K for $n = 1-6$. It can be seen that the data are on a straight line for a given n with the slope being K_n. Measuring K_n as a function of temperature yields the values for the enthalpy ΔH_n° and entropy ΔS_n° of hydration, using eqs 3 and 4

$$\Delta G_n^\circ = -RT\ln K_n \quad (3)$$

$$\Delta G_n^\circ = \Delta H_n^\circ - T\Delta S_n^\circ \quad (4)$$

The expected linear relationship between ΔG_n°, and temperature T is demonstrated in Figure 3 for the example of neurotensin $(M + 2H)^{2+}\cdot(H_2O)_n$, $n = 1$ and 2, with the intercept yielding ΔH_n° and the slope ΔS_n°. Values for ΔH_n° and ΔS_n° thus obtained are summarized in Tables 1−3 for the molecules included in this study with values of n in the range of 1−10. It can be seen that ΔH_n° values are in the range from −7 to −15 kcal/mol and ΔS_n° from −13 to −37 cal/mol/K with on average more

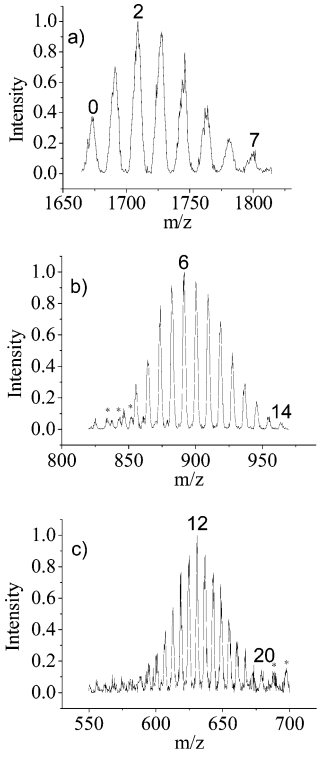

Figure 1. ESI mass spectra of singly (a), doubly (b), and triply (c) protonated neurotensin recorded after exposure to 1.3 Torr of water vapor at 260 K. Numbers above the peaks indicate the number of water molecules added, an asterisk (*) labels peaks due to impurities.

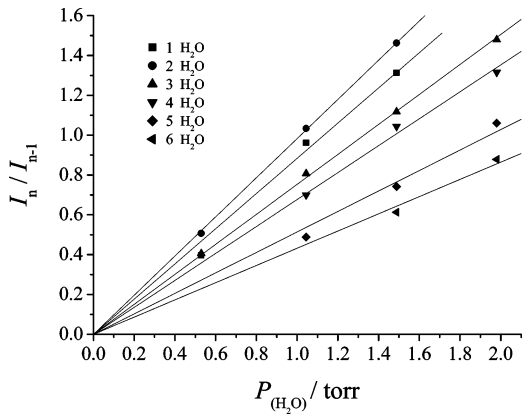

Figure 2. Ratio of peak intensities of the two features corresponding to neurotensin (charge state +2) with $n-1$ and n water molecules attached, respectively, as a function of water vapor pressure in the drift cell which is held at 271 K. The different symbols used for the different values of n are indicated in the legend inserted in the figure.

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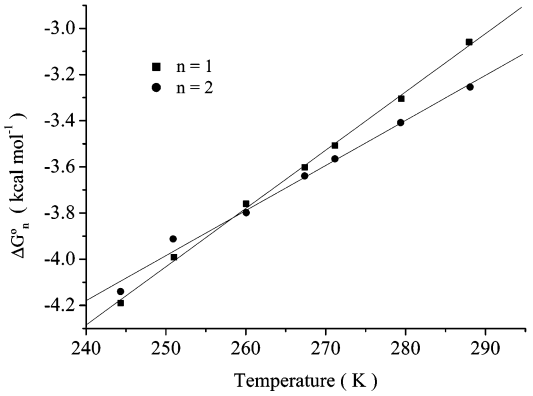

Figure 3. ΔG°_n of hydration vs temperature for neurotensin in charge state +2. Squares: $n = 1$, circles: $n = 2$.

Table 1. Experimental ΔH°_n, ΔS°_n, and ΔG°_n Values for the Sequential ($n-1 \rightarrow n$) Hydration of Neurotensin for Various Charge States

charge state	n	$-\Delta H^\circ_n$[a] (kcal/mol)	$-\Delta S^\circ_n$[b] (cal/mol/K)	$-\Delta G^\circ_n$ (298 K)[c] (kcal/mol)
+1	1	9.2	21	2.9
	2	9.8	24	2.6
	3	(9)	(20)	2.6
	4	(9)	(23)	2.3
+2	1	10.3	25	2.8
	2	8.9	20	3.1
	3	9.6	23	2.8
	4	9.4	22	2.8
	5	8.5	20	2.7
	6	(8)	(19)	2.6
	7	(9)	(21)	2.5
+3	1	(14.8)	(37)	3.7
	2	(11.9)	(28)	3.7
	3	9.5	20	3.5
	4	9.3	21	3.2
	5	9.4	21	3.2
	6	9.8	24	2.8
	7	8.8	20	2.9
	8	(10)	(24)	2.7
	9	(9)	(22)	2.6
	10	(9)	(21)	2.7

[a] Estimated error of ±0.3 kcal/mol. For numbers in brackets the estimated error is ±1 kcal/mol. [b] Estimated error of ±1 cal/mol/K. For numbers in brackets the estimated error is ±4 cal/mol/K. [c] Estimated error of ±0.1 kcal/mol.

negative values for small values of n, although this trend does not hold rigorously for all molecules.

A more qualitative analysis of Figure 1 indicates that charge state +1 of neurotensin is hydrated to a lesser extend than charge state +2, which is in turn less hydrated than charge state +3. The maximum number of water molecules adding to neurotensin $(M + H)^+$, $(M + 2H)^{2+}$, and $(M + 3H)^{3+}$ at 260 K and 1.3 torr of water vapor is 7, 14, and 20, respectively, if peaks with at least 2% intensity are considered. Similar results are obtained for the other peptides (Table 4): 5−7 water molecules add to singly protonated peptides, 13−14 to doubly protonated peptides.

Discussion

One of the most striking results of this study is that the maximum number n_{max} of water molecules adding to a peptide ion is ~6 for $(M + H)^+$, ~13 for $(M + 2H)^{2+}$, and ~20 for $(M + 3H)^{3+}$, respectively (260 K, 1.3 Torr H_2O, see Table 4). Hence, n_{max} is roughly proportional to the number z of charges

Table 2. Experimental ΔH°_n, ΔS°_n, and ΔG°_n Values for the Sequential ($n-1 \rightarrow n$) Hydration of the Singly Protonated Peptides Indicated[a]

peptide	n	$-\Delta H^\circ_n$ (kcal/mol)	$-\Delta S^\circ_n$ (cal/mol/K)	$-\Delta G^\circ_n$ (298 K) (kcal/mol)
AAA	1	12.3	23.3	5.4
	2	11.3	22.6	4.6
	3	8.7	16.8	3.7
	4	7.1	13.0	3.2
	5	7.6	16.2	2.8
AAAAA	1	10.5	21.3	4.2
	2	8.5	17.3	3.3
	3	9.0	20.4	2.9
	4	8.6	20.6	2.5
RAAAA	1	9.3	20.2	3.3
	2	7.8	17.6	2.6
	3	7.1	14.8	2.7
AARAA	1	10.2	23.1	3.3
	2	8.4	18.3	2.9
Ac-AARAA	1	9.5	20.7	3.3
	2	8.1	17.6	2.9
AARAA-OCH_3	1	9.4	21.2	3.1
	2	8.4	17.5	3.2
	3	7.6	17.0	2.5
AAAAR-OCH_3	1	9.2	20.6	3.1
	2	7.8	15.2	3.3

[a] Error levels are ±0.3 kcal/mol for ΔH°_n, ±1 cal/mol/K for ΔS°_n, and ±0.1 kcal/mol for ΔG°_n.

Table 3. Theoretical Binding Energies E_n and Experimental ΔH°_n, ΔS°_n, and ΔG°_n Values[a] for the Sequential ($n-1 \rightarrow n$) Hydration of the Protonated Species Indicated

	n	$-E_n$ (MM)[b] (kcal/mol)	$-E_n$ (DFT) (kcal/mol)	$-\Delta H^\circ_n$ (kcal/mol)	$-\Delta S^\circ_n$ (cal/mol/K)	$-\Delta G^\circ_n$ (298 K) (kcal/mol)
n-decylamine	1	13.9	16.9[c]	14.8	23.1	7.9
	2	12.6	13.9[c]	12.1	22.2	5.5
	3	11.4	11.9[c]	9.6	18.8	4.0
	4	7.8	9.3[c]	7.5	14.2	3.3
	5	7.7		6.7	13.0	2.8
Dialanine	1	16.5	19.2[d]	14.8	24.7	7.4
	2	12.8	13.7[d]	10.5	17.9	5.2
	3	11.7		8.9	16.8	3.9
	4	9.5		7.8	15.5	3.2
	5	8.9		6.8	13.2	2.9

[a] Error levels are ±0.3 kcal/mol for ΔH°_n, ±1 cal/mol/K for ΔS°_n, and ±0.1 kcal/mol for ΔG°_n. [b] Obtained by molecular mechanics (AMBER). [c] Obtained by DFT on $CH_3NH_3^+$ (B3LYP/6-311++G** including corrections for BSSE and ZPE). [d] Obtained by DFT (B3LYP/6-311++G** including BSSE correction). On the basis of the $CH_3NH_3^+$ results, the ZPE correction is expected to decrease the values in the table by 1-2 kcal/mol.

on the peptide, clearly indicating that the charge is a dominant factor in the hydration process of these peptide ions. This is also evident in Figure 4a, where ΔG°_n (273 K) is plotted as a function of the number n of water molecules. For a given value of n the triply charged ions have generally the largest $|\Delta G^\circ_n|$ values, whereas singly charged ions have the smallest values. If ΔG°_n is plotted as a function of water molecules added per charged site (Figure 4b) the data of the different charge states are now nearly superimposed. Both Figure 4 and Tables 1−3 show that water binding energies generally decrease with increasing number of water molecules already added, an observation previously reported for virtually any ionic system.[35]

The result that n_{max} is roughly proportional to z suggests that the water molecules may form little water clusters around each

(35) Keesee, R. G.; Castleman, A. W., Jr. *J. Phys. Chem. Ref. Data* **1986**, *15*, 1011.

Table 4. Maximum Number n_{Max} of Water Molecules Adding to the Corresponding Peptide in Charge State $+z$ at 260 K and 1.3 Torr of Water Vapor Pressure[a]

peptide	+z	n_{max}	n_{max}/z
neurotensin	+1	7	7
	+2	14	7
	+3	20	6.7
LHRH [b]	+1	6	6
	+2	13	6.5
bradykinin [b]	+1	7	7
	+2	13	6.5
AA	+1	7	7
AAA	+1	7	7
AAAAA	+1	7	7
RAAAA	+1	6	6
Ac-AARAA	+1	5	5
AARAA-OCH_3	+1	5	5
AAAAR-OCH_3	+1	5	5

[a] Only species with >2% intensity are considered. [b] Ref 23.

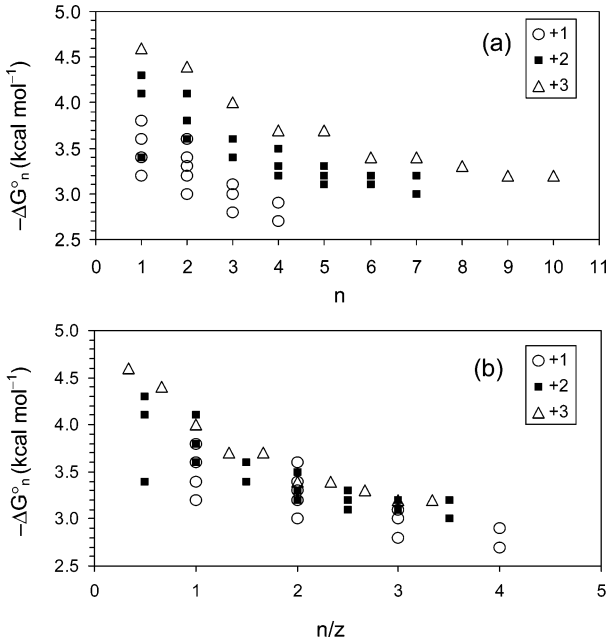

Figure 4. $\Delta G°_n$(273 K) of hydration for all the arginine containing peptides included in this study. (a) $\Delta G°_n$ vs the number n of water molecules added to peptides in charge states +1 (open circles), +2 (solid squares), and +3 (open triangles). (b) Same as (a) but x-axis normalized by charge state z.

charge and therefore an $(M + 3H)^{3+}$ ion adds as many water molecules as three $(M + H)^+$ ions under identical conditions. For protonated organic amines such as *n*-decylamine this is obviously a very likely scenario. Water molecules will most likely bind to the protonated amino group and not at all to the hydrophobic hydrocarbon tail. In FT-ICR studies of extensively hydrated species of such molecules, "magic" numbers for the number of water molecules have been observed, indicating that the particularly stable water clathrate structures (water microdroplets) bound to the protonated amine are preferred structures.[16] The water binding energies measured for doubly protonated diamines (e.g., $NH_3-(CH_2)_{12}-NH_3^{2+}$) show an interesting pairwise pattern: $\Delta H°_n \cong \Delta H°_{n+1}$, $n = 1, 3, 5...$, indicating that the n^{th} water molecule adds to one amine and the $(n+1)^{th}$ to the other.[18]

Our molecular mechanics and DFT calculations on *n*-decylamine with up to seven water molecules indicate that the first three water molecules prefer to make a strong hydrogen bond to the three hydrogen atoms available at the $-NH_3^+$ group. The fourth water molecule binds to one of the first three water molecules, thus starting a second solvation shell (3−1 arrangement).[36] The fifth H_2O molecule adds to any one of the first four water molecules yielding the nearly iso-energetic arrangements 3−2 (with two H_2O molecules in the second solvation shell) and 3−1−1 (with the fourth H_2O bound to one of the first three H_2Os, and the fifth H_2O bound to the fourth H_2O). For the $(M + H)^+\cdot(H_2O)_7$ cluster the most typical arrangement is 3−3−1 (Figure 5). Table 3 indicates that theoretical water binding energies obtained by molecular mechanics and by DFT calculations agree reasonably well with each other and with the experimental values of $\Delta H°_n$ (within 2−3 kcal/mol). Experimental $\Delta H°_n$ and $\Delta S°_n$ values are in excellent agreement with literature values reported for *n*-propylamine and *n*-hexylamine, although there are some differences in $\Delta S°_n$ for $n \geq 3$.[18,35,37]

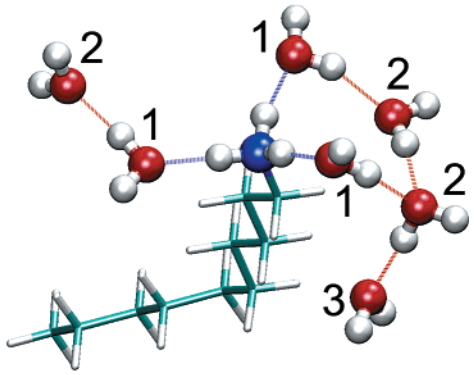

Figure 5. Low energy model structure of protonated *n*-decylamine hydrated by seven water molecules obtained by molecular modeling. Water molecules in the first solvation shell are labeled with "1", those in the second and third with "2" and "3", respectively.

For peptides, however, the situation is not as clear-cut. Peptides contain many hydrophilic groups besides the site(s) of protonation, and it is not obvious whether hydration will follow the pattern of organic amines discussed above. Magic numbers have been observed for hydrated gramicidin S ions in FT-ICR studies indicating that water clathrate structures might be forming in this case as well.[15,16] Molecular mechanics studies on hydrated doubly protonated gramicidin S indicate that water molecules tend to cluster around the two protonated amino groups, but they also add to a lesser degree (2 H_2O out of 14) to charge remote sites.[15] Molecular mechanics calculations on protonated dialanine with up to seven water molecules carried out in our lab indicate that hydration occurs preferentially at the protonated amino group, but once the first solvation shell is filled the remaining water molecules either start a second solvation shell or they bind charge remotely to the carboxyl group or the backbone amide. Water molecules often insert between a hydrogen bond donor and an acceptor, such as

(36) In the AMBER calculations, the fourth water molecule does one of two things, which are energetically equally favorable. It either binds to one of the first three water molecules (3−1 arrangement), or it squeezes between two of the first three water molecules trying to find a place in the first solvation shell (4−0 arrangement). In the second scenario, the interaction between the fourth H_2O and the $-NH_3^+$ group appears to be mostly electrostatic, although in some cases, two water molecules appear to be sharing one of the amino hydrogen atoms for hydrogen bonding. However, in DFT calculations on $CH_3NH_3^+$ (B3LYP/6-311++G**) the 4−0 arrangement is not a minimum and it converts to a 3−1 arrangement upon geometry optimization. Hence, the 4−0 arrangement is most likely an artifact of the AMBER parametrization used here.

(37) Meot-Ner, M. *J. Am. Chem. Soc.* **1984**, *106*, 1265.

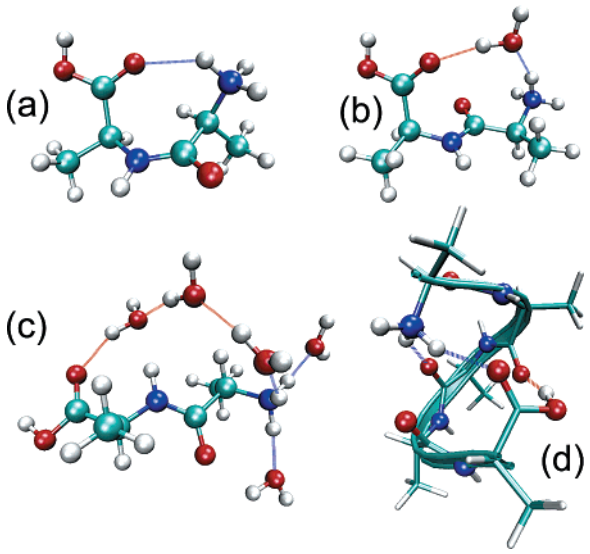

Figure 6. Model structures obtained by molecular mechanics calculations for (a) $(AA + H)^+$, (b) $(AA + H)^+ \cdot H_2O$, (c) $(AA + H)^+ \cdot (H_2O)_5$, and (d) $(AAAAA + H)^+$.

between $-COOH$ and $-NH_3^+$. In the lowest energy $(AA + H)^+$ structure there is a weak hydrogen bond (2.1 Å) between the C- and N-termini (Figure 6a). The first water molecule always inserts into this hydrogen bond to form a $>C{=}O\cdots H_2O\cdots H_3N^{+-}$ bridge (Figure 6b). By the time five water molecules are added the structural feature $>C{=}O\cdots H_2O\cdots H_3N^{+-}$ is still observed in most structures, but a chain of three water molecules $>C{=}O\cdots H_2O\cdots H_2O\cdots H_2O\cdots H_3N^{+-}$ becomes energetically just as favorable (Figure 6c).

The molecular mechanics results not only provide information about the sites of hydration but also about the peptide conformation as a function of water addition. For completely dehydrated $(AA + H)^+$ the dihedral angle about the peptide bond $C_\alpha - C_{CO} - N - C_\alpha$ as shown in Figure 6a is ~0° (cis), but the structure with a trans configuration which is much more commonly found in condensed phase peptide structures is within 0.1 kcal/mol. DFT calculations confirm that the cis conformer is energetically a reasonable structure, and at the B3LYP/6-311++G** level it is ~1 kcal/mol more stable than the most stable trans conformer. In both the cis and the trans configurations, there is a weak hydrogen bond between the C- and N-termini. The unusual stability of the cis configuration has also been found for protonated diglycine,[38] but it is not found at all for larger protonated oligoglycines[38] or oligoalanines (this work, see below). As the first water molecule is added to $(AA + H)^+$ the dipeptide always ends up in a trans configuration with the water molecule inserted into the hydrogen bond between the N- and C-termini (Figure 6b). These two structural features (inserted water molecule, trans peptide bond) persist as more water molecules are added. Structural changes within the $(AA + H)^+$ moiety of hydrated species do occur and are due to changes in the backbone dihedral angles ϕ and ψ, whereas the dihedral angle about the peptide linkage stays ~180° (see e.g., Figure 6c).

Theoretical water binding energies obtained from molecular mechanics simulations and DFT calculations on the $(AA + H)^+ \cdot (H_2O)_n$ system are compiled in Table 3 along with experimental ΔH_n° and ΔS_n° values. The DFT binding energies are not corrected for zero point energies (ZPE) and are therefore expected to be too large by 1−2 kcal/mol.[39] A correction of that magnitude brings the DFT and the AMBER result in near perfect agreement with each other, although both theoretical methods yield slightly larger values than experimentally observed. However, a maximum deviation of 2 kcal/mol between AMBER and experiment is certainly satisfactory and within the combined error bars of the two methods. Incidentally, experimental ΔH_n° and ΔS_n° values for dialanine lead to ΔG_n° (293 K) values that are identical (within ~1 kcal/mol) to experimental ΔG_n°(293 K) values, $n = 1$ and 2, reported in the literature for diglycine.[20]

For larger peptides the situation is more complicated because in these cases the water molecules have to compete with peptide-internal solvation (self-solvation) of the charged site(s). This is clearly evident in our molecular mechanics calculations for singly and doubly protonated bradykinin and LHRH. In these two cases, the first one or two water molecules have a preference for adding to a charged site, but for the following water molecules charge remote water binding sites compete effectively. Larger peptides are obviously more extensively folded than smaller peptides, and therefore, large peptides are more likely to offer ideal (charge remote) hydration sites in surface crevices where multiple water−peptide hydrogen bonds are possible. A detailed report of the very interesting and insightful findings by experiment and theory on the hydration of bradykinin and LHRH ions is beyond the scope of this paper and will be given elsewhere.[23] We simply conclude here that the formation of water micro-droplets around the charged sites of larger peptides is an even less likely scenario than in dialanine discussed above. This is an interesting observation given the fact that the extent of hydration scales essentially quantitatively with charge (Table 4).

Said another way, the effect of charge state on peptide hydration is not simply due to hydrating the charged sites. The fact that n_{max} is proportional to z in $(M + zH)^{z+} \cdot (H_2O)_{n_{max}}$ appears to have multiple origins. Because charge-dipole interactions are relatively long range interactions, the amount of charge on the peptide probably has a significant influence on the water−peptide binding energy even if the water molecule is not directly bound to any charged site. Furthermore, the degree of unfolding of peptides correlates strongly with the number of charges on the peptide.[40] In the gas-phase, hydrophobic parts of the peptide tend to be exposed to the surface and therefore unfolding the peptide increases hydrophilic hydration sites. In addition, our molecular mechanics simulations indicate that addition of water to the more compact structures of low charge states induces conformational changes yielding more open structures with hydrophilic parts exposed. The energy cost in this opening up process due to loss of self-solvation is offset by the energy gain due to water solvation.

The contention that the water molecules have to compete with self-solvation of the charged site is supported by the water binding energies measured here as a function of peptide size.

(38) Wyttenbach T.; Bushnell J. E.; Bowers, M. T. *J. Am. Chem. Soc.* **1998**, *120*, 5098.

(39) The magnitude of correction is estimated on the basis of the $CH_3NH_3^+$ calculations, where frequency calculations were carried out on the same level of theory.

(40) Any multiply charged peptide is expected to unfold to some degree due to Coulomb repulsion. See for example, the recent reviews: (a) Jarrold, M. F. *Annu. Rev. Phys. Chem.* **2000**, *51*, 179−207. (b) Hoaglund-Hyzer, C. S.; Counterman, A. E.; Clemmer, D. E. *Chem. Rev.* **1999**, *99*, 3037−3079.

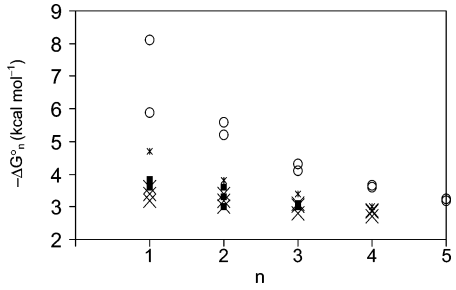

Figure 7. ΔG_n° vs n for the singly protonated species of the small oligoalanines AA and AAA (open circles), of AAAAA (stars), of arginine containing pentapeptides (solid squares), and of the larger peptides bradykinin, LHRH, and neurotensin (×).

Water is most strongly bound to the smaller peptides (Tables 1−3) because the water molecule does not have to displace or compete with any peptide-internal functional groups bound to the charged site, a process requiring energy. For instance, ΔH_1° for protonated dialanine is −14.8 kcal/mol, whereas ΔH_1° = −12.3 and −10.5 kcal/mol for protonated tri- and penta-alanine, respectively. The trend in ΔH_1° is easily rationalized with the increasing interaction between the protonated N-terminus and the rest of the molecule (C-terminus, backbone carbonyl groups) with increasing chain length (Figure 6, parts a and d). Delocalization of part of the charge over the entire peptide might contribute to the size effect as well. The size effect is also reflected in the ΔG_n° values plotted as function of n in Figure 7 for the singly charged ions. The smallest values for $|\Delta G_n^\circ|$ are found for the largest peptides neurotensin, LHRH, and bradykinin and the largest values for the smallest peptide dialanine. The effect of self-solvation has previously been demonstrated for simple molecules such as diamines $NH_2(CH_2)_kNH_3^+$.[20] In the series k = 2−12 the $\Delta G_{n=1}^\circ$ (293 K) values of hydration range from −7.8 kcal/mol for k = 2 to −4.9 kcal/mol for k = 12.[20]

Another interesting observation is that arginine-containing peptides have somewhat smaller water binding energies than peptides without arginine. This is most clearly evident when comparing ΔH_n° of penta-alanine with any of the arginine containing penta-peptides with blocked termini (Table 2). For instance, the ΔH_1° value for the first water molecule adding to (Ac-AARAA + H)$^+$ is −9.5 ± 0.3 kcal/mol whereas the corresponding value for (AAAAA + H)$^+$ is −10.5 kcal/mol. Both molecules feature only one basic site, an amine in AAAAA and the very basic guanidino group on the arginine side chain in Ac-AARAA. The fact that water binds less strongly to the guanidinium group $-NH-C(NH_2)_2^+$ than to a protonated amine $-NH_3^+$ can be rationalized by the more extensive delocalization of the positive charge in the larger guanidinium group compared to the much smaller amine. This interpretation is supported by data on the protonated amino acids arginine[41] and valine and proline[19] reported earlier.

The very similar hydration energies for RAAAA, Ac-AARAA, AARAA-OCH_3, and AAAAR-OCH_3 suggest they are structurally similar. The last three cannot be salt bridges due to blocked termini suggesting RAAAA is probably not a salt bridge. The case is not as clear for AARAA, where the first water is more strongly bound than for the other four peptides (Table 2). For this, and other reasons, this peptide has been studied in greater detail experimentally and theoretically with the results reported elsewhere.[42] In that paper, one focus is the induction of salt bridge formation by hydration. It is clear that in solution AARAA is a salt bridge; the question is how many water molecules does it take to effect the transition?

Conclusions

The conclusions of the present study on the sequential hydration of small protonated peptides are the following:

Water binding energies ($-\Delta H_n^\circ$) are of the order of 7 to 15 kcal/mol. The larger values correspond to addition of the first water to small peptides that do not contain arginine. An exception is the +3 charge state of neurotensin, a large peptide. Values near the lower limit are found for adding a water molecule to a peptide that is already hydrated by several water molecules. Generally, the binding energy decreases with increasing level of hydration and appears to level off at a value of 7−9 kcal/mol.

Entropies of hydration (ΔS_n°) are typically ∼−20 cal/mol/K but values ranging from −13 to −37 cal/mol/K are observed. In general, ΔS becomes less negative as more waters are added reflecting the greater freedom of motion of the H_2O adduct as n increases (Table 3).

The level of hydration at a given temperature (240−340 K) and water vapor pressure is proportional to the level of protonation of the peptide. At 260 K and 1.3 torr of water pressure it is found for any peptide M included in this study that the ratio n_{max}/z is 5−7, where n_{max} is the maximum number of water molecules adding to $(M + zH)^{z+}$.

For larger peptides self-solvation competes with water solvation of the charge site(s). Hence, charge remote solvation sites compete with charge solvation sites for water addition. In addition, water binding energies generally decrease with peptide size reflecting the competition with self-solvation (e.g. AA, AAA, and AAAAA, Tables 2 and 3).

Finally, arginine-containing peptides have relatively small water binding energies. For example, the ΔH_1° values for the pentapeptides (Ac-AARAA + H)$^+$ and (AAAAA + H)$^+$ are −9.5 ± 0.3 and −10.5 ± 0.3 kcal/mol, respectively. When self-solvation is minimized the effect becomes more dramatic as in (Arg + H)$^+$ and (AA + H)$^+$, where the bonding energies are 9.3 and 14.8 kcal/mol, respectively.

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(41) Wyttenbach, T.; Bowers, M. T. *Top. Curr. Chem.*, in press.
(42) Wyttenbach, T.; Paizs, B.; Barran, P. E.; Breci, L.; Liu, D.; Suhai, S.; Wysocki, V. H.; Bowers, M. T., in preparation.