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Direct Probing of Zwitterion Formation in Unsolvated Peptides

Rodolphe Antoine,[†] Michel Broyer,[†] Philippe Dugourd,*[,†] Gary Breaux,[‡] Frederick C. Hagemeister,[‡] David Pippen,[‡] Robert R. Hudgins,[‡] and Martin F. Jarrold*[,‡]

Laboratoire de Spectrométrie Ionique et Moléculaire, UMR No. 5579, CNRS et Université Lyon 1, 43 bd du 11 novembre 1918, 69622 Villeurbanne Cedex, France, and Chemistry Department, Indiana University, 800 East Kirkwood Avenue, Bloomington, Indiana 47405-7102

Received May 2, 2003; E-mail: dugourd@lasim.univ-lyon1.fr; mfj@indiana.edu

Amino acids exist as zwitterions in solution and in their canonical forms in the gas phase.[1−5] The zwitterionic form can be stabilized in the gas phase by salt bridge interactions when a net charge is present,[6,7] or by shielding of the charges, either by the addition of water molecules[8,9] or by complex formation.[10] Calculations suggest that two water molecules are sufficient to stabilize a zwitterion in glycine,[11] the simplest amino acid. In peptides, the charge shielding function can be accomplished through self-solvation. At present, there is very little information available about the minimum peptide size required to stabilize a zwitterion,[12−14] and what information is available has been obtained on charged peptides using indirect methods. Here, we report studies of zwitterion formation in small neutral alanine-based peptides using, for the first time, a direct probe of the charge distribution. We have used molecular beam electric deflection measurements to determine the electric dipole susceptibility of unsolvated WA_n and $Ac-WA_n-NH_2$ (W = tryptophan and A = alanine) peptides with up to five alanines (the capped $Ac-WA_n-NH_2$ peptides cannot form zwitterions). We find that the WA_n peptides remain in the canonical form at room temperature.

The measurements were performed on an apparatus consisting of a matrix-assisted laser desorption (MALD) source coupled to an electric beam deflection setup with a position-sensitive time-of-flight mass spectrometer.[15] The peptides were synthesized using *FastMoc* chemistry with an Applied Biosystems model 433A peptide synthesizer. MALD targets were prepared by pressing a 1:3 ratio of the peptide and high purity cellulose powder. Peptides were desorbed from the target with the third harmonic of a pulsed Nd:YAG laser (355 nm) into a helium flow generated with a piezoelectric valve that is synchronized with the desorption laser. A molecular beam leaves the source through a 5 cm long nozzle. Both ions and neutrals are produced in the desorption step. The ions are ejected from the beam with a transverse electric field, and the resulting neutral beam is tightly collimated by two slits before it travels through the 15 cm long electric deflector. The deflector provides both an electric field F ($F = 0$ to 2×10^7 V m^{-1}) and a field gradient $\partial F/\partial z$. The direction of the field, z, and its gradient are perpendicular to the beam axis. One meter after the deflector, the peptides are photoionized with the fourth harmonic of a Nd:YAG laser (266 nm) in the extraction region of a position-sensitive time-of-flight mass spectrometer. The velocity of the peptides in the neutral beam is selected and measured with a mechanical chopper that is synchronized with the ionization laser.

The beam profile of rigid molecules is symmetrically broadened in the deflector, while for nonrigid molecules (i.e., when one cannot separate the vibrational and rotational Hamiltonians such as for those molecules studied here), the beam is globally deflected toward the high field.[16] The deflection:

$$d = \frac{K}{mv^2}\langle \mu_z \rangle \frac{\partial F}{\partial z} \tag{1}$$

where $\langle \mu_z \rangle$ is the average z component of the induced plus permanent dipole moment. m and v are the mass and the velocity of the peptide, respectively, and K is a geometrical factor obtained by calibration with the sodium atom whose polarizability is known with high accuracy from atomic interferometry.[17] The beam profile was monitored as a function of the electric field, and the deflection d shows the expected linear dependence on the square of the applied field. Electric susceptibilities $\chi = \langle \mu_z \rangle / F$ are obtained from plots of the deflection against F^2.

To discriminate between zwitterionic and canonical structures, one needs to estimate susceptibilities for both structures. The susceptibility of a molecule is related to its permanent dipole moment and its electronic polarizability by the Langevin−Debye formula:[16,18]

$$\chi = \frac{\langle \mu^2 \rangle_T}{3kT} + \alpha_e \tag{2}$$

where $\langle \mu^2 \rangle_T$ is the average value of the dipole at temperature T (without the electric field), and α_e is the static electronic polarizability. The temperature T is equal to the nozzle temperature which was fixed to 300 K (there is no significant cooling in the mild expansion as the molecular beam exits the source). The electronic polarizability α_e is obtained by using an empirical method based on molecular additivity.[19] The canonical average value of the dipole squared $\langle \mu^2 \rangle_T$ is obtained using simulated tempering (ST), a Monte Carlo-based method, following the scheme proposed by Mitsutake and Okamoto.[20] In ST, the temperature is a dynamic variable. The temperature is discretized in M different values T_m ($m = 1, ..., M$), and the simulation is realized in two steps: Monte Carlo at fixed temperature T_m and a temperature update to neighboring values $T_m \pm 1$. The conformation and the temperature are updated with a weight:

$$W(E,T_m) = e^{-E/kT_m + g_m} \tag{3}$$

The g_m values are chosen to perform a free random walk in temperature space, which induces a random walk in potential energy space and allows the simulation to escape local minima and to explore effectively the conformational landscape. In this work, we used $M = 15$ with upper and lower temperatures of 1250 and 100 K, respectively. The energy E of each structure was obtained from the Amber force field with AMBER96 parameters.[21] The permanent dipole of each structure was obtained using the partial charges defined in AMBER96. Comparison with dipoles obtained by more sophisticated methods showed this to be a good approximation. In

[†] Université Lyon 1.
[‡] Indiana University.

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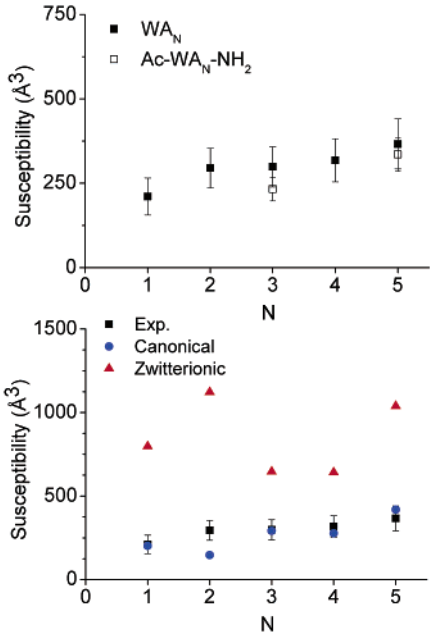

Figure 1. (Top) Measured electric susceptibility of unsolvated WA_n and $Ac-WA_n-NH_2$ peptides. (Bottom) Comparison of measured susceptibilities for WA_n peptides with calculated values for canonical and zwitterionic structures.

each Monte Carlo step, we updated every dihedral angle in the backbone and in the tryptophan side chain. Every 30 steps, two temperature updates were attempted. A total of 10^7 steps was performed for each peptide. The energy and μ^2 values were monitored at every MC step after a 10 000 step initialization run. Canonical averages of μ^2 at $T = 300$ K were obtained through a weighted histogram analysis method.[22] g_m parameters were determined using the replica exchange method prior to the ST run.

The measured susceptibilities for WA_n ($n = 1-5$) and $Ac-WA_n-NH_2$ ($n = 3, 5$) are plotted in the upper half of Figure 1. The experimental susceptibilities are in the range from 250 to 400 Å^3 and show a small overall increase with the number of alanine residues. The susceptibilities for the capped and uncapped peptides are very similar. In the capped peptides, an acetyl group at the N-terminus and amide group at the C-terminus prohibit zwitterion formation. The lower part of Figure 1 compares measured susceptibilities for WA_n peptides to the results of the simulations for canonical and zwitterionic structures. As expected, the calculated susceptibilities for the zwitterionic forms are much larger than those for the canonical ones, and the results for the canonical forms are in good agreement with the experimental results. This provides conclusive evidence that unsolvated WA_n ($n = 1-5$) peptides do not form zwitterions.

Examples of low free energy structures obtained during the ST runs for WA_n ($n = 1-5$) are shown in Figure 2. The peptides are floppy, and many different conformations are explored during the course of the simulations. At room temperature, the backbones tend to be extended for all peptide sizes. For $n = 1-3$, the lowest potential energy structures obtained during the simulations also have extended backbones. Yet, for $n = 4$ and 5, the lowest potential energy structures are hairpin-like, while the peptides are unfolded at room temperature. The lowest free energy structure found for zwitterionic WA_5 is also shown in Figure 2. The peptide is folded to bring the two charged ends together. There are also favorable interactions between the NH_3^+ group and backbone carbonyl groups and between the COO^- group and backbone amide NH groups. These interactions strongly constrain the backbone and make the

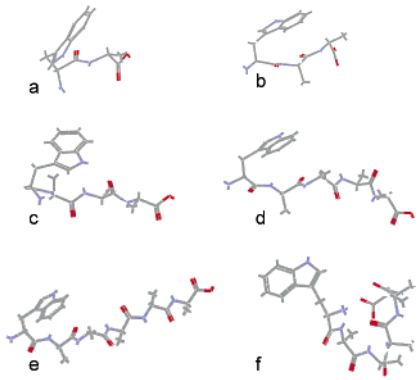

Figure 2. Examples of low free energy structures (at $T = 300$ K) found during the course of the simulated tempering calculation for canonical WA_n, $n = 1-5$ (a−e), and zwitterionic WA_5 (f).

zwitterionic form entropically unfavored at room temperature. Thus, even if the zwitterion had a lower potential energy than the canonical form, it still may not have the lowest free energy at room temperature.

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