J|A|C|S
ARTICLES
Published on Web 05/17/2002

Electric Susceptibility of Unsolvated Glycine-Based Peptides

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

Contribution from the Laboratoire de Spectrométrie Ionique et Moléculaire, UMR No. 5579, Université Lyon I et CNRS, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France, and Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois, 60208

Received December 4, 2001

Abstract: The DC electric susceptibilities of unsolvated glycine-based peptides, WG_n (W = tryptophan and G = glycine) with $n = 1-5$, have been measured by deflection of a molecular beam in an electric field. These are the first electric deflection measurements performed on peptides. At 300 K the susceptibilities are in the range of 200−400 Å^3. By far the largest contribution to the susceptibilities is from the permanent dipole moment of the peptides. The results indicate that the peptides do not have rigid conformations with fixed dipoles. Instead the dipole is averaged as the peptides explore their energy landscape. For a given WG_n peptide, all molecules have almost the same average dipole, which suggests that they all explore a similar energy landscape on the microsecond time scale of the measurement. The measured susceptibilities are in good overall agreement with values calculated from the average dipole moment deduced from Monte Carlo simulations.

Introduction

Dielectric relaxation plays a major role in determining the structures and functions of proteins. The charge redistribution that occurs in response to a conformational change may modify the relative stabilities of the low-energy conformations, and so understanding the nature of this charge redistribution is probably one of the keys to understanding protein structure and folding. The protein and solvent may also relax or reorganize in response to an external field, charge separation, or charge transfer, and this reorganization may influence the biological functions of the protein. Thus, one of the challenges in theoretical and experimental studies of proteins is to evaluate correctly these electrostatic interactions. In a protein, relaxation and charge screening are due mainly to the electronic polarizability and to the reorientation of polar or charged groups.[1−7] These effects may be examined for solvated or unsolvated molecules at the microscopic level with molecular dynamics simulations.[8−11] From a macroscopic point of view, particularly for macromolecular modeling, the relaxation may be taken into account by attributing a dielectric function to the protein.[2−11] The protein is generally described as a uniform sphere of low dielectric, surrounded by a solvent of high dielectric. In more refined models, different values may be used for different parts of the protein and one can estimate the dielectric constant of a given site in the protein. For an in-depth discussion of electrostatic properties and dielectric constants of proteins see, for example, the recent review of Schutz and Warshel.[12] Experimentally, dielectric relaxation has been widely studied, but it is difficult to separate protein relaxation from solvent relaxation in solution. By removing a protein to the gas phase, it is possible to separate the intramolecular properties from properties of or induced by the solvent.

A variety of techniques have recently been used to study gas-phase peptides and proteins.[13−16] In addition to mass spectrometry, spectroscopy experiments have been used to determine the structure of amino acids, and mobility measurements have given information on the structure of charged peptides and proteins. Recently, we coupled a matrix-assisted laser desorption source to a molecular beam deflection experiment and used it to measure the electric dipole of unsolvated tryptophan.[17] Here, we present the results of the first electric deflection measurements performed on polypeptides. We have determined the DC electric susceptibilities of unsolvated glycine-based peptides,

* Corresponding author. E-mail: dugourd@lasim.univ-lyon1.fr; mfj@chem.nwu.edu.
[†] Université Lyon I et CNRS.
[‡] Northwestern University.

(1) Wada, A. *Adv. Biophys.* **1976**, *9*, 1.
(2) Warshel, A.; Russel, S. T. *Q. Rev. Biophys.* **1984**, *17*, 283.
(3) Gilson, M. K.; Honig, B. H. *Biopolymers* **1986**, *25*, 2097.
(4) Simonson, T.; Perahia, D.; Bricogne, G. *J. Mol. Biol.* **1991**, *218*, 859.
(5) Warshel, A.; Aqvist, J. *Annu. Rev. Biophys. Biophys. Chem.* **1991**, *20*, 267.
(6) Laberge, M. *Biochim. Biophys. Acta* **1998**, *1386*, 305.
(7) Simonson, T. *Int. J. Quantum Chem.* **1999**, *73*, 45.
(8) King, G.; Lee, F. S.; Warshel, A. *J. Chem. Phys.* **1991**, *95*, 4366.
(9) Simonson, T.; Perahia, D. *J. Am. Chem. Soc.* **1995**, *117*, 7987.
(10) Löffler, G.; Schreiber, H.; Steinhauser, O. *J. Mol. Biol.* **1997**, *270*, 520.
(11) Boresch, S.; Höchtl, P.; Steinhauser, O. *J. Phys. Chem. B* **2000**, *104*, 8743.
(12) Schutz, C. N.; Warshel, A. *Proteins: Structure, Function, Genetics* **2001**, *44*, 400.
(13) Robertson, E. G.; Simons, J. P. *Phys. Chem. Chem. Phys.* **2001**, *3*, 1.
(14) Jarrold, M. F. *Annu. Rev. Phys. Chem.* **2000**, *51*, 179.
(15) Desfrancois, C.; Carles, S.; Schermann, J. P. *Chem. Rev.* **2000**, *100*, 3943.
(16) Bowers, M. T.; Marshall, A. G.; McLafferty, F. W. *J. Phys. Chem.* **1996**, *100*, 12897.
(17) Compagnon, I.; Hagemeister, F. C.; Antoine, R.; Rayane, D.; Broyer, M.; Dugourd, P.; Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **2001**, *123*, 8440.

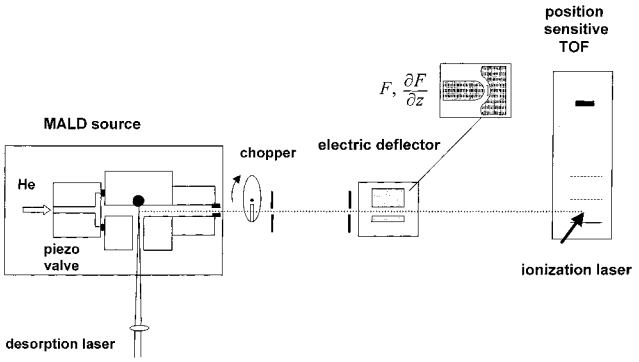

Figure 1. Schematic diagram of the experimental setup.

WG_n (W = tryptophan and G = glycine) with $n = 1-5$, at 300 K. The electric susceptibility characterizes the response of a system to an applied electric field. It is clear that the gas-phase susceptibility cannot be directly compared to the solution-phase susceptibility, but it may be directly compared to calculations performed for isolated molecules and new insights into electrostatic properties of peptides or proteins and the role of the solvent are expected.

Materials and Methods

Peptide Synthesis. Tryptophan was purchased from SIGMA; WG and WG_2 peptides were purchased from BACHEM biochimie SARL. WG_3, WG_4, and WG_5 peptides were synthesized by using FastMoc (a variant of Fmoc) chemistry with an Applied Biosystems Model 433A peptide synthesizer. After synthesis, the peptides were cleaved from the solid substrate with a solution of 95% trifluoroacetic acid, 2.5% water, and 2.5% ethane dithiol, precipitated with cold diethyl ether, washed, and lyophilized. The peptides were used without further purification.

Molecular Beam Deflection Measurements. Figure 1 shows a schematic of our experimental setup. The apparatus consists of a matrix-assisted laser desorption (MALD) source coupled to an electric beam deflection setup that incorporates a position-sensitive time-of-flight mass spectrometer. High-purity cellulose powder is used as the matrix in the MALD source. The peptides and cellulose are mixed in a 1:3 mass ratio and pressed to form a rod. The rod is rotated and translated in a screw motion inside the source, and peptides are desorbed from the rod with the third harmonic of a pulsed Nd:YAG laser (355 nm). They are desorbed into a pulsed helium flow generated with a piezoelectric valve that is synchronized with the desorption laser pulse. A molecular beam of the target peptide leaves the source through a 5 cm long nozzle. The beam is skimmed and tightly collimated by two slits. Then it travels through the electric deflector, which has a "two-wire" electric field configuration.[18] The geometry of the deflector provides both an electric field F and a field gradient $\partial F/\partial z$ which are nearly constant over the width of the collimated molecular beam. One meter after the deflector, the molecular beam is irradiated with the fourth harmonic of a Nd:YAG laser (266 nm) in the extraction region of a position sensitive time-of-flight mass spectrometer.[18] Measurements of the molecular beam profile are performed as a function of the electric field in the deflector. The velocity is selected and measured with a mechanical chopper synchronized with the ionization laser pulse.

Results

Mass Spectra of WG_n Peptides. Figure 2 shows typical mass spectra recorded for neutral tryptophan and WG_n ($n = 1-5$) peptides ionized with 266 nm light. Two photons are needed to ionize tryptophan and the peptides. The photoionization efficiency is enhanced by the fact that the photon energy is close to the resonance band of the indole moiety in tryptophan. With the source and ionization conditions that were used to record the spectra, the parent mass is the dominant peak for all peptides. For tryptophan, very little fragmentation is observed; roughly 90% of the total ion signal is observed at the parent mass. Fragments are observed at masses 117, 130, and 131 Da. These fragments may be assigned to indole derivatives: C_8H_7N (the indole molecule), C_9H_8N (indole + CH), and C_9H_9N (indole + CH_2). Figure 3 shows an assignment of the peaks observed in the mass spectrum of WG. The fragments observed for tryptophan are also observed for WG. In addition we observe peaks at 216 and 246 Da which are assigned respectively to the loss of COOH and NH_2 from the parent. All the observed fragments contain the indole moiety. The deflections (see below)

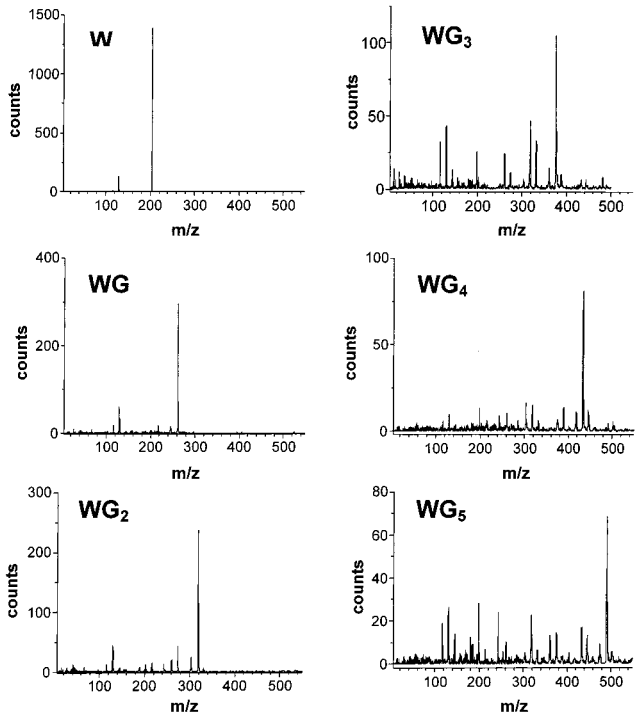

Figure 2. Mass spectra of WG_n peptides ionized with a photon energy of 4.66 eV (266 nm).

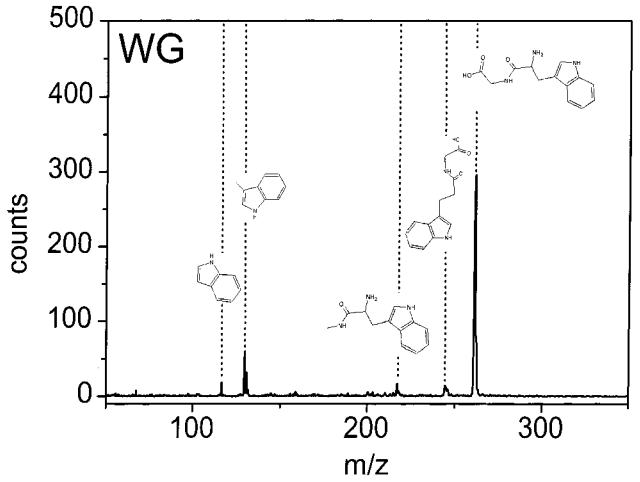

Figure 3. Mass spectrum of neutral WG peptides ionized with a photon energy of 4.66 eV (266 nm). A tentative assignment of the fragment ions is shown.

(18) Bonin, K. D.; Kresin, V. V. *Electric-Dipole Polarizabilities of Atoms, Molecules and Clusters*; World Scientific Publishing Co.: Singapore, 1997.

observed for the two lighter fragments are much smaller than those determined for the parent. This shows that the dissociation processes that lead to these fragments occur during desorption, and not during photoionization. The signal is too small to reach a firm conclusion about the origin of the other peaks in the WG mass spectrum. One should note that for fragments generated in the source during desorption, only the indole-containing ones are expected to be photoionized with a high efficiency. There may be other fragments present that are not detected because they do not contain the indole moiety.

For larger peptides, the number of peaks increases; some of them may be due to traces of smaller peptides or to impurities in the sample. However, fragments that contain the indole residue are dominant in the mass spectra. The peaks at 117, 130, and 131 Da are observed for all WG_n peptides. In addition, a peak at 199 Da is found in the spectra for all WG_n with $n > 1$. This is probably an indole derivative resulting from cleavage of the peptide backbone at the second residue, followed by loss of NH_2 ($C_{12}H_{11}N_2O$). Finally, a peak at 16 Da to the left of the parent peak is observed for every size; these peaks are assigned to the loss of NH_2.

Electric Deflection of the WG_n Peptide Beams. The left-hand side of Figure 4 shows beam profiles measured for WG_n ($n = 1-5$) peptides with an electric field $F = 0$ V/m in the deflector and with $F = 1.5 \times 10^7$ V/m (25 kV across the deflector). The profiles are nearly symmetric and can be fit with a Gaussian. The small irregularities in the beam profiles are not meaningful; they are thought to arise from inhomogeneities in the ionizing laser beam. The peptide beam profiles measured with the electric field are shifted toward the high-field region in the deflector. The deviations were measured for different voltages across the deflector, and the plots on the right-hand side of Figure 4 show the deviation as a function of the square of the applied voltage. A linear behavior is obtained: the measured deviation is proportional to the square of the applied voltage.

In the deflector, the force is proportional to the average (induced plus permanent) dipole $\langle \mu_z \rangle$ of the peptide and to the gradient of the electric field. The deviation is equal to:

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

where m and v are respectively the mass and the velocity of the peptide, and K is a geometrical factor. The values of the geometrical factors, K and K' (see below), were determined by calibration with the polarizability of the sodium atom, which is known to high accuracy from measurements with an atom interferometer.[19] One can define an electric susceptibility $\chi(0)$ for the peptide as:

$$\chi(0) = \frac{\langle \mu_z \rangle}{F} \tag{2}$$

and

$$d = \frac{K}{mv^2} \chi(0)\, F \frac{\partial F}{\partial z} = \frac{K'}{mv^2} \chi(0)\, V^2 \tag{3}$$

(19) Ekstrom, C. R.; Schmiedmayer, J.; Chapman, M. S.; Hammond, T. D.; Pritchard, D. E. *Phys. Rev. A* **1995**, *51*, 3883.

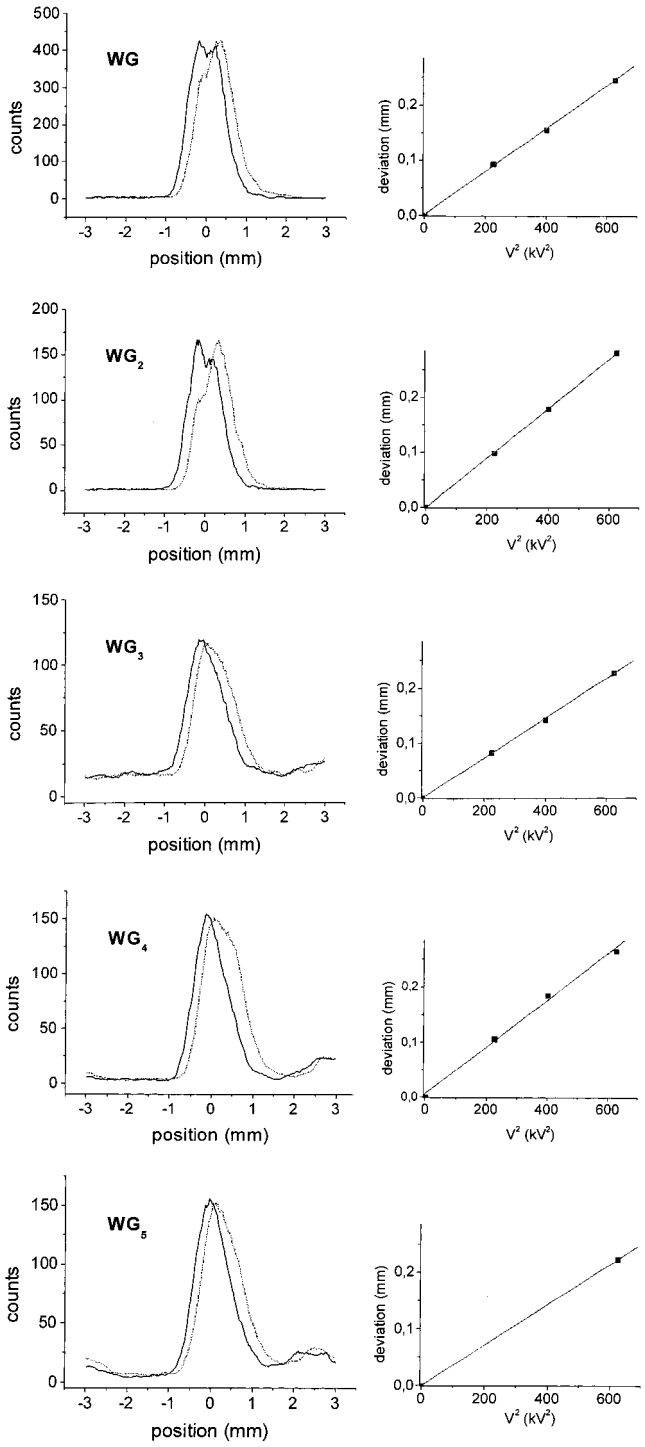

Figure 4. The left-hand side shows beam profiles of WG_n peptides measured without (solid line) and with (dashed line) a voltage (25 kV) across the deflector. The right-hand side shows plots of the deviation of the beam as a function of the square of the voltage across the deflector. The solid line corresponds to a linear fit of the data.

As noted above, the measured deviation is proportional to the square of the applied voltage, and the DC electric susceptibilities are deduced from linear fits to the plots of the deviation against V^2 (Figure 4). The measured susceptibilities for WG_n ($n = 1-5$) polypeptides are plotted in Figure 5 and listed in Table 1. The susceptibilities are in the range of 200−400 Å^3 and they monotonically increase with the number of glycine residues in the peptide.

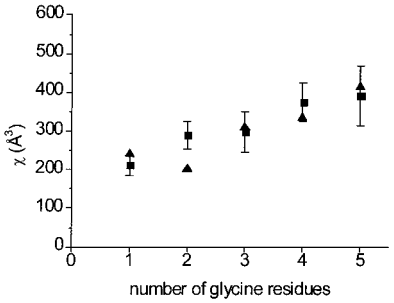

Figure 5. Comparison of the measured DC electric susceptibility, $\chi(0)$, of WG_n peptides as a function of the number of glycine residues (■) with the results of calculations ($\alpha_e + \alpha_{pd}$) (▲) (see text).

Table 1. Measured and Calculated Values for the DC Electric Susceptibilities of WG_n peptides[a]

	WG	WG_2	WG_3	WG_4	WG_5
$\chi(0)$	214 ± 27	289 ± 37	289 ± 37	375 ± 50	391 ± 77
α_e	28.4	33.5	38.6	43.7	48.8
α_{pd}	212	167	271	289	365
$\alpha_{pd} + \alpha_e$	240.4	200.5	309.6	332.7	413.8

[a] $\chi(0)$ are the experimental values, α_e are the contributions from the electronic polarizability calculated from a model based on molecular additivity, and α_{pd} are the contributions to the electric susceptibility from the average dipole calculated from the Monte Carlo simulations. All values are in Å^3.

Discussion

To analyze these results, one needs to know the relevant contributions that give rise to the DC electric susceptibility of a polypeptide. The electric susceptibility characterizes the response of a molecule to an externally applied electric field. Two effects contribute to this response: (i) the electronic polarizability (α_e) and (ii) the reorientation of the whole molecule and/or the reorientation of polar groups in the electric field (α_{pd}).

The electronic polarizability reflects the relative displacement of electrons with respect to nuclei in a molecule placed in an electric field. It can be enhanced when the molecules have delocalized electrons such as in π-conjugated systems. Thus, for the WG_n peptides, a large contribution to α_e arises from the indole moiety in tryptophan. A crude but fairly reliable estimate of the electronic polarizability for WG_n peptides can be obtained by using an empirical method based on molecular additivity.[20] The polarizabilities obtained from this model are listed in Table 1. The main contribution comes from the tryptophan residue ($\alpha_e = 23.3$ Å^3). The total electronic polarizabilities are in the range of 28−49 Å^3. These values are almost 1 order of magnitude smaller than the experimental values.

The second contribution α_{pd} arises from the interaction of the permanent dipole moment of the molecule with the electric field. If the molecules studied here were rigid with the dipole moment locked to a particular direction within the molecular framework then the interaction of the permanent dipole moment with the electric field would lead to a broadening of the beam profile and not a deflection of the beam.[17,21] The broadening results from the distribution of initial conditions for the rotational motion of the molecule. For the WG_n peptides studied here the electric field causes a deflection and a slight broadening of the peak. Thus all the molecules have approximately the same deviation, which means that the average component of the dipole along the electric field axis is almost the same for every molecule. This indicates that the molecule is floppy and may interconvert between different conformations with different dipole moments pointing in different directions. Fluctuations of the molecule and coupling between rotational motion and vibrational motion lead to a random orientation of the dipole in space. The fact that for each WG_n all molecules have almost the same deviation suggests that they all explore a similar energy landscape. The slight broadening of the beam profiles noted above may be due to the fact that for a given WG_n peptide all molecules do not explore exactly the same landscape. Assuming that there is a linear response, the contribution of the average dipole in a floppy molecule to the susceptibility is given by:[22]

$$\alpha_{pd} = \frac{1}{kT}\langle \mu_Z^2 \rangle_0 \qquad (4)$$

where T is the temperature of the molecule and $\langle \mu_Z^2 \rangle_0$ is the average of the square of the projection of the permanent dipole moment on the axis of the electric field (Z-axis). The distribution of molecules in the molecular beam is assumed to be canonical with a temperature equal to the source temperature. In eq 4, the average value is calculated for an unperturbed distribution (i.e. without the electric field).

$\langle \mu_Z^2 \rangle_0$ was estimated for the WG_n peptides by using Monte Carlo (MC) simulations. The MC simulations were performed with Hyperchem 6.0 using CHARMM force field with the CHARMM22 parameter set.[23,24] Each MC run consisted of 3×10^7 steps at $T = 300$ K. To ensure that the conformational landscape was fully explored the torsion angles of the peptide backbone were completely randomized every 50 000 steps. The resulting structure was locally optimized and then 10 000 MC steps were performed. The exchange was then either accepted or rejected based on a metropolis criterion before resuming the 3×10^7 step MC run. During the course of the MC simulation, the dipole was calculated every 2000 steps by using a simple additive model where the overall dipole moment of the peptide is obtained from the vector addition of the dipoles of the peptide bonds and the dipoles of the polar groups of isolated amino acids. The dipole values that we used were 2.1 D for the indole residue,[25] 3.46 D for the amide group,[26] and 1.3, 0.7, 2.3, and 1.5 D for the −NH, −C═O, −C−O, and −OH bonds in the NH_2 and COOH groups.[27] The canonical average dipole moments obtained from these simulations are used in eq 4 to give the contribution of the average dipole to the electric susceptibility. These values are given in Table 1. The electric susceptibility is the sum of the contributions due to the electronic polarizability (α_e) and the contribution from the average dipole

(20) Miller, K. J. *J. Am. Chem. Soc.* **1990,** *112*, 8533.
(21) Dugourd, P.; Compagnon, I.; Lepine, F.; Antoine, R.; Rayane, D.; Broyer, M. *Chem. Phys. Lett.* **2001**, *336*, 511.
(22) McQuarrie, D. A. *Statistical Mechanics*; Harper & Row Publishers: New York, 1973.
(23) *HyperChem*, v. 6.0; Hypercube, Inc.: 1115 NW 4th Street, Gainesville, FL 32601.
(24) MacKerell, A. D.; Bashford, D.; Bellott, M.; Dunbrack, R. L.; Evanseck, J. D.; Field, M. J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.; Kuczera, K.; Lau, F. T. K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D. T.; Prodhom, B.; Reiher, W. E.; Roux, B.; Schlenkrich, M.; Smith, J. C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M. *J. Phys. Chem. B* **1998**, *102*, 3586.
(25) Carles, S.; Desfrancois, C.; Schermann, J. P.; Smith, D. M. A.; Adamowicz, L. *J. Chem. Phys.* **2000**, *112*, 3726.
(26) Hol, W. G. J.; Duijnen, P. T. V.; Berendsen, H. J. C. *Nature (London)* **1978**, *273*, 443.
(27) Barrow, G. *Physical Chemistry*; McGraw-Hill: New York, 1973.

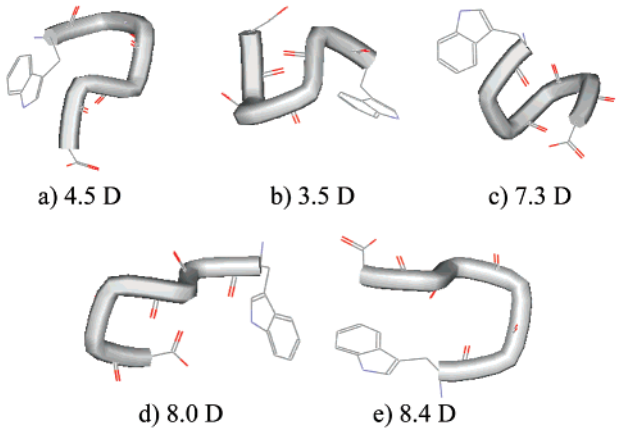

Figure 6. Example of low-energy structures found during the course of the MC simulation for WG_5. The dipole moment determined for each structure from the additive model (see text) is given.

(α_{pd}). These sums are compared to the measured electric susceptibilities in Table 1 and in Figure 5.

The overall agreement between the measured and calculated electric susceptibilities for the WG_n peptides is good. The largest discrepancy is for WG_2, although the origin of the discrepancy for this peptide is unclear at this time. The main contribution to the susceptibility (α_{pd}) was calculated assuming that the peptides have nonrigid conformations that explore their full conformational landscape. The good overall agreement between the measured and calculated values is a strong indication that this assumption is true. For comparison, the values calculated with the additive model for the WG_n peptide in α-helical conformations (with the dipoles of the amide groups aligned) are much larger ($\mu = 2.6$, 5.6, 9.0, 12.7, and 16.9 D and $\alpha_{pd} =$ 53, 250, 649, 1299, and 2300 Å^3 for WG_n, $n = 1-5$). At room temperature, most of the conformations explored in the MC simulations correspond to random-looking coil structures with lower dipole moments. Some typical structures found for WG_5 during the course of the Monte Carlo run are shown in Figure 6. The results of mobility experiments performed on protonated polyglycine ions have also been interpreted in terms of random-looking structures.[28,29] In those studies, however, the conformation appears to be dominated by hydrogen bonding to the protonated group and they are more compact (globule-like) than found for the neutrals here.

Conclusion

We have coupled a matrix-assisted laser desorption source to a molecular beam deflection experiment to determine the average DC electric susceptibility of WG_n ($n = 1-5$) peptides in the gas phase. The main contribution to the electric susceptibility is due to the permanent dipole moment. The results indicate that the WG_n peptides studied here do not have rigid conformations with fixed dipoles at 300 K. Instead the dipole is averaged, on the microsecond time scale of the measurements, as the peptide explores its energy landscape. The measured electric susceptibilities are in good agreement with values calculated for nonrigid peptides exploring their full conformational landscape. The measurements described here can be applied to larger peptides or proteins; we are currently using this approach to examine helix formation in peptides with 10−20 residues.

Acknowledgment. We gratefully acknowledge the support of the National Institutes of Health and the Ministère de la recherche (ACI jeunes chercheurs).

JA012656D

(28) Hudgins, R. R.; Mao, Y.; Ratner, M. A.; Jarrold, M. F. *Biophys. J.* **1999**, *76*, 1591.
(29) Wyttenbach, T.; Bushnell, J. E.; Bowers, M. T. *J. Am. Chem. Soc.* **1998**, *120*, 5098.