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Electric dipole moments and conformations of isolated peptides

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Abstract. The electric dipole moments of the isolated amino acid tryptophan and small glycine-based peptides (WG_n, $n = 1-5$, W = tryptophan, G = glycine) have been measured by deflection of a molecular beam in an inhomogeneous electric field. The measurements are compared to the results of *ab initio* calculations and Monte-Carlo simulations. The conformation and the flexibility of the peptides, at different temperatures, are discussed. The WG_n peptides are much more floppy than an isolated tryptophan, even a single glycine is enough to make the peptide floppy on the timescale of the electric deflection measurements.

PACS. 87.15.-v Biomolecules: structure and physical properties – 33.15.Kr Electric and magnetic moments (and derivatives), polarizability, and magnetic susceptibility

1 Introduction

Electrostatic forces are long-range forces, which play a crucial role in defining the structures and properties of biomolecules. An important contribution to these forces is due to permanent electric dipole moments. Particular arrangements of biomolecules such as the α-helix have large macro-dipoles, which induce strong electric fields [1,2]. More generally, the fluctuations of polar groups in proteins in response to a charge, an electric field or a conformational change, play a key role in defining the structure and binding properties. Experimentally, electric dipole properties of proteins have been studied in solution, but it is difficult to separate the contribution due to the protein from the effects of the solvent [3–5]. By removing a polypeptide into the vapor phase, it is possible to resolve the intramolecular properties from the properties of, or induced by, the solvent, and to determine the intrinsic dipole moments. The measurements described here are the first to examine these intrinsic electrostatic properties. Ultimately they should permit a better understanding of the role of electrostatics in defining the properties of proteins. The dipole of a polypeptide strongly depends on its conformation and so it can be used as a probe of the geometry and the conformational dynamics. This provides a new powerful approach to study the geometries of neutral gas phase biomolecules, that is complementary to spectroscopic techniques [6] (which are usually restricted to small systems) and to ion mobility measurements that are performed on ions [7].

Recently, we coupled a matrix assisted laser desorption (MALD) source to a molecular beam deflection (MBD) experiment and used it to measure the average electric dipole moments of small isolated neutral peptides [8,9]. In this paper, we present the results of electric deflection measurements performed on isolated tryptophan molecules and on glycine-based WG_n peptides ($n = 1-5$, W = tryptophan, G = glycine) at room temperature and at 85 K. Our previously reported results for tryptophan at 85 K (Ref. [8]) and for WG_n peptides at room temperature (Ref. [9]) are summarized and we discuss the influence of the temperature on the average dipole moment and on the flexibility of the peptides.

2 Experiment and experimental results

Figure 1 shows a schematic of our experimental set-up. The apparatus consists of a matrix-assisted laser desorption (MALD) source coupled to an electric beam deflection experiment that incorporates a position sensitive time-of-flight mass spectrometer. High purity cellulose or nicotinic acid are used as matrix materials in the MALD source. Tryptophan, WG, and WG_2 were purchased from commercial sources (Sigma and Bachem), WG_3, WG_4, WG_5 peptides were synthesized using *FastMoc* chemistry an Applied Biosystems Model 433A peptide synthesizer.

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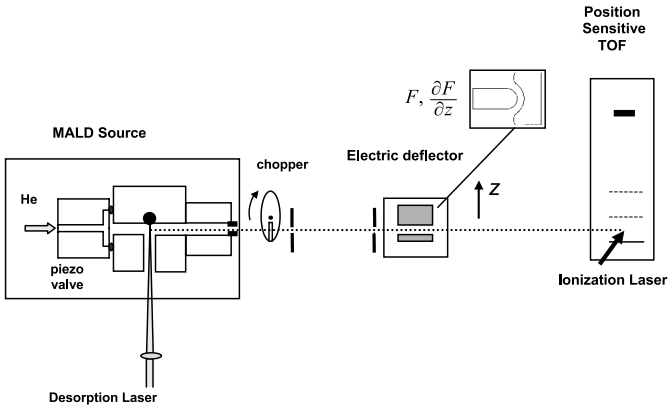

Fig. 1. Schematic diagram of the experimental setup.

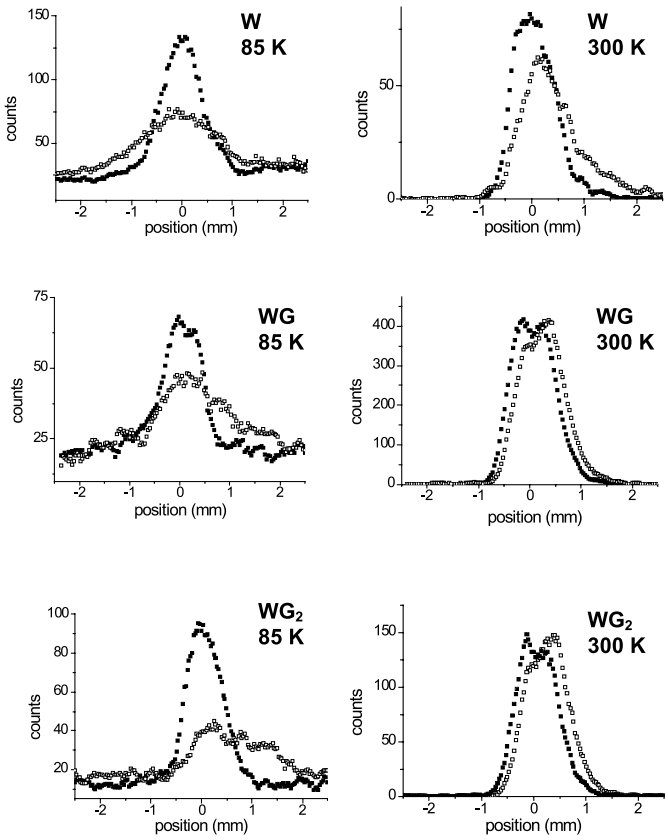

Fig. 2. Beam profiles of W, WG, and WG_2 peptides measured with $F = 0$ Vm^{-1} (■) and with $F = 1.2 \times 10^7$ Vm^{-1} (□) in the deflector, at $T = 85$ K and $T = 300$ K.

The peptides and matrix are mixed in a 1:5 to 1:3 mass ratio and pressed to form a rod. The rod is rotated and translated in a screw motion inside the source. The peptides are desorbed from the rod with the third harmonic of a Nd:YAG laser (355 nm). They are entrained by 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 50 mm long nozzle. The nozzle diameter is 2 mm and the source pressure is a few torr. The temperature of the nozzle can be adjusted from 300 K to 85 K. The molecular beam is skimmed and tightly collimated by two slits. Then, it travels through the electric deflector which has a "two-wire" electric field configuration [10]. This configuration 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 (the z-direction is perpendicular to the beam axis and collinear with the axis of the time-of-flight mass spectrometer as shown in Fig. 1). The value of the electric field is 1.5×10^7 V/m for a voltage of 25 kV across the two cylindrical poles of the deflector. 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 [10]. For tryptophan and the glycine-based WG_n peptides, the parent mass is always the dominant peak but the amount of fragmentation increases as the size of the peptide increases [9]. 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.

In the deflector, a molecule with an electric dipole moment $\boldsymbol{\mu}$ is submitted to an instantaneous force along the z-axis of $f = \boldsymbol{\mu}\partial\mathbf{F}/\partial z$. The deviation d of a molecule of mass m and velocity ν is then given by:

$$d = \frac{K}{m\nu^2}\langle f\rangle = \frac{K}{m\nu^2}\langle\mu_z\rangle\frac{\partial F}{\partial z} \qquad (1)$$

where K is a geometrical factor. The deviation of a molecule is proportional to the average value of the projection of its dipole on the z-axis in the deflector. The electric field leads to either a broadening and/or a global deviation of the molecular beam depending on the conformational flexibility of the molecule.

Figure 2 shows beam profiles measured for W, WG, and WG_2 with an electric field $F = 1.2 \times 10^7$ V/m (20 kV across the deflector) and with $F = 0$ V/m in the deflector. These measurements were performed at two different nozzle temperatures: 85 K and 300 K. The beam profiles measured without the electric field are nearly symmetric and can be fit with a Gaussian. The beam profiles measured with the electric field are strongly temperature dependent. At 85 K, the profiles measured with the electric field are all broader than those measured without the field. For tryptophan, the profile is almost symmetric. For WG and WG_2 peptides, the profiles are asymmetric with a tail to the right. At 300 K, the profiles measured with the electric field are shifted to the z-positive direction (*i.e.* towards the high field region in the deflector). In addition, there is a slight broadening of the profile for tryptophan with a tail to the right, but no significant broadening for WG and WG_2. The shape of the profile is directly related to the rigidity of the molecule. In the following section, we discuss simulations of the profiles for rigid and floppy molecules.

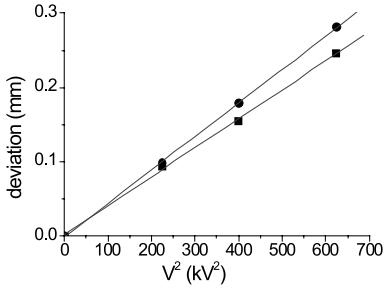

Fig. 3. Deviation of the beam as a function of the square of the voltage across the deflector for WG (■) and WG_2 (●) at $T = 300$ K. The solid line corresponds to a linear fit of the data.

3 Discussion

3.1 Rigid molecules: Tryptophan at 85 K

For a rigid molecule the dipole moment is locked to a particular direction within the molecular framework and the molecule is assumed to be a rigid rotor. The Hamiltonian for such a rigid molecule in the electric field is:

$$H = H_{\rm rot} - \boldsymbol{\mu} \cdot \mathbf{F} \tag{2}$$

where $H_{\rm rot}$ is the Hamiltonian for rotation of the molecule. The resulting force in the deflector is due to the interaction between the electric field F and the permanent dipole μ of the molecule. It can be written for an asymmetric top as [11]:

$$f = \langle \mu_z \rangle \frac{\partial F}{\partial z} = \langle \mu_a \cos(az) + \mu_b \cos(bz) + \mu_c \cos(cz) \rangle \frac{\partial F}{\partial z} \tag{3}$$

where μ_a, μ_b, μ_c, are the components of the dipole moment along the three principal axes of the molecule and $\cos(az)$, $\cos(bz)$ and $\cos(cz)$ represent the cosines of the angles between the principal axes of the molecule and the axis of the electric field. The force can be calculated by diagonalization of equation (2) or by perturbation methods. The average force depends on the rotational level of the molecule and this induces a broadening of the beam (different molecules experience a different force).

The shape of the experimental profile measured for the tryptophan molecule at 85 K is in good agreement with the results of simulations for a rigid molecule [8]. The lowest energy geometry of the tryptophan molecule obtained at the MP2/6-31G* level and the result of simulations of the beam profile using the dipole components ($\mu_a = 3.37$ D, $\mu_b = 2.12$ D, $\mu_c = 0.29$ D) obtained for this geometry, are shown in Figure 4. The calculated profile is in reasonably good agreement with experimental data. The intensity at the maximum of the normalized beam profile provides a convenient measure of the amount of broadening. In the insert in Figure 4, we have plotted this quantity against the voltage across the deflector. A near-exponential decrease in the maximum intensity is observed with increasing the deflector voltage. The solid line represents the results of a simulation of this quantity using the

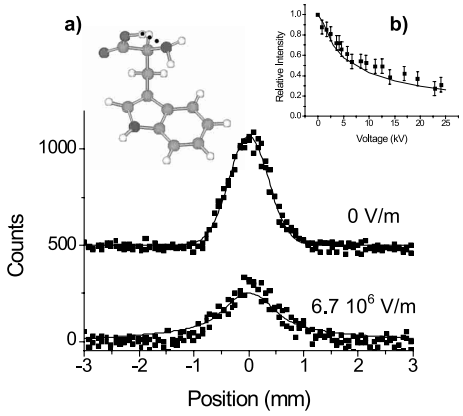

Fig. 4. Beam profiles of tryptophan with $F = 0$ Vm^{-1} and $F = 6.7 \times 10^6$ Vm^{-1}. The squares correspond to experimental values and the full line to simulations with $\mu_a = 3.37$ D, $\mu_b = 2.12$ D, $\mu_c = 0.29$ D. The second profile has been offset vertically for clarity. Insert (a): geometry of the lowest energy conformer found at the MP2/6-31G* level of theory and which is used in the simulation. Insert (b): plot of the relative intensity at the maximum of the normalized peak as a function of the voltage across the deflector (the intensity at the maximum of the normalized peak is related to the amount of broadening). (■) Experimental results, (—) results of simulations for the lowest energy isomer ($\mu_a = 3.37$ D, $\mu_b = 2.12$ D, $\mu_c = 0.29$ D).

Table 1. Calculated values of the dipole moment for the structure of lowest energy of tryptophan.

Method	μ_a (D)	μ_b (D)	μ_c (D)	μ total (D)
MP2/6-31G* reference [8]	3.37	2.12	0.29	3.99
MP2/6-311G(d,p) this work[a]	3.28	2.09	0.06	3.89
MP2/6-311+G(d,p) reference [12][b]				3.63

[a] Single point calculation performed on a B3LYP/6-311G(d,p) optimized structure. [b] Single point calculation performed on a B3LYP/6-31+G(d) optimized structure.

MP2/6-31G* dipole values given above. The agreement is reasonably good. The decrease observed in the insert of Figure 4 depends mainly on the μ_a value, and it is possible to deduce a better value for μ_a by adjusting it to fit the data. This approach leads to a value of $\mu_a = 2.6 \pm 0.6$ D. This is significantly smaller than the value obtained at the MP2/6-31G* level of theory (3.37 D) for the lowest energy geometry. The dipole components from calculations for this conformation with a more-extended basis set are given in Table 1. In the table we have included the dipole moment obtained by Snoek *et al.* from *ab initio* calculation related to their spectroscopic study of tryptophan [12]. They also found the structure shown in Figure 4 to be the global minimum. The addition of diffuse functions to the basis set tends to decrease the calculated dipole slightly, which improves the agreement with the μ_a

value deduced above. While the agreement is still somewhat lacking, as described in references [8,12], the experimental data is completely inconsistent with the dipole moments calculated at the MP2/6-31G* level of theory for the next five lowest energy conformations found in our search. So there is little doubt that the structure shown in Figure 4 is responsible for the measured peak broadening. The experimental results are consistent with the presence of a single dominant conformation (the lowest energy conformation found in the *ab initio* calculations) rather than a mixture, though minor amounts of other conformations cannot be ruled out. Levy and collaborators found six different conformations in their 1985 spectroscopic studies of jet cooled tryptophan [13]. Simons and collaborators using infrared and ultraviolet ion dip spectroscopy and high level *ab initio* calculations, have recently assigned the most strongly populated isomer to the lowest energy conformation found in their calculations (which is the same as the one shown in Fig. 4) [12]. The absence of a significant population of other conformers in our experiments is presumably related to the slower cooling rate in our source, which allows more equilibration between the conformations during cooling. There is a fairly large energy gap between the lowest energy geometry and the next lowest energy isomer in the calculations, so the overwhelming majority of the molecules are expected to be in the lowest energy conformation at 85 K, if equilibrium is attained.

3.2 Floppy molecules: WG_n peptides at 300 K

When the molecule is floppy, the situation is different. The molecule may fluctuate or/and interconvert easily between different conformations with different dipole moments pointing in different directions. The motion of the molecule is no longer described by equation (2). In particular, coupling with vibrational terms cannot be neglected. In general, the calculation of the average value of the projection of the dipole on the axis of the electric field is not possible. However, if the fluctuations of the molecules are such that during the microsecond time scale of the measurement, all the molecules explore a similar energy landscape, with a probability of sampling a particular conformation given by a canonical distribution, it is possible to have a very simple formulation for the average dipole. Assuming a linear response, the average dipole of the molecule is [14]:

$$\langle \mu_z \rangle = \chi(0) F \tag{4}$$

with the DC susceptibility $\chi(0)$ given by:

$$\chi(0) = \alpha_{\mathrm{e}} + \frac{1}{kT} \left\langle \mu_z^2 \right\rangle_0 \tag{5}$$

where T is the temperature of the molecules, α_{e} is their electronic polarizability 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, calculated with the unperturbed distribution (*i.e.* without the electric field). The deflection can be written:

$$d = \frac{K}{m\nu^2} \chi(0) F \frac{\partial F}{\partial z} = \frac{K'}{m\nu^2} \chi(0) V^2 \tag{6}$$

where V is the voltage in the deflector. All the molecules are deflected by the same amount. The beam is shifted and not broadened. In Figure 2, this is the case for WG and WG_2 at 300 K. Plots of deviation against V^2 for WG and WG_2 at 300 K are shown in Figure 3. A linear relationship is observed in agreement with the predictions of equation (6). The DC electric susceptibility $\chi(0)$ is deduced from a linear fit to the plot of the measured deviations against V^2 (Fig. 3). The measured susceptibilities for WG_n ($n = 1$–5) polypeptides are plotted in Figure 5. The susceptibilities are in the range of 200 to 400 Å^3 and they increase with the number of glycine residues in the peptide.

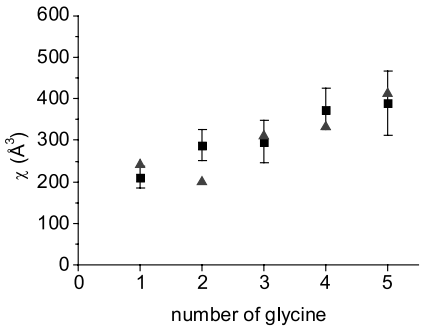

Fig. 5. Measured (■) and calculated (▲) DC electric susceptibility, $\chi(0)$, of WG_n peptides as a function of the number of glycine residues.

For a floppy molecule, the electric susceptibility given by equation (5), is the sum of the electronic polarizability (α_{e}), and of the contribution due to the permanent dipole moment ($\langle \mu_Z^2 \rangle_0 / kT$). A crude but fairly reliable estimate of the electronic polarizability for the WG_n peptides can be obtained using an empirical method based on molecular additivity [15]. The second contribution arises from the permanent dipole moment of the molecules. The value of $\langle \mu_Z^2 \rangle_0$ was estimated for the WG_n peptides using Monte Carlo (MC) simulations. The MC simulations were performed using the CHARMM force field [16]. The overall dipole moment of the peptide was obtained from the vector addition of the dipoles of the peptide bonds, and the dipoles of the polar groups of the isolated amino-acids [9]. The canonical average value of the dipole moments obtained from these simulations are used in equation (5) to give the contribution of the average dipole to the DC electric susceptibility. The calculated values are compared to the measured electric susceptibilities in Figure 5.

The overall agreement between the measured and calculated electric susceptibilities for the WG_n peptides is good. The main contribution to the susceptibility ($\langle \mu_Z^2 \rangle_0 / kT$) was calculated assuming that the peptides are flexible and sample a large conformational landscape. At room temperature, most of the conformations explored in the MC simulations correspond to random-looking structures. Typical structures found for WG_5 during the course

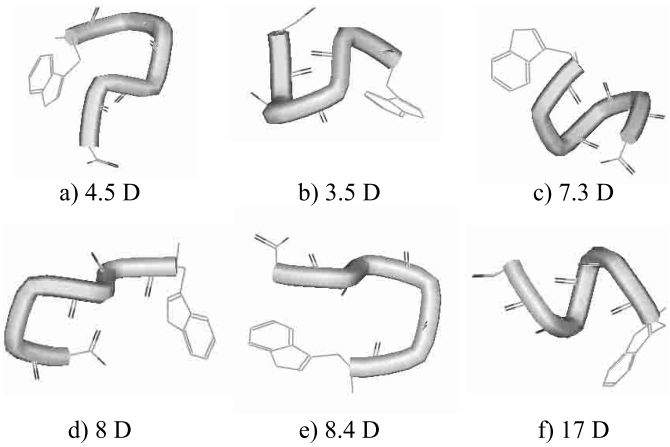

Fig. 6. (a–e) Examples of low energy structures found during the course of the MC simulations for WG_5. (f) α-helical structure of WG_5. The dipole moment of each structure is given.

of the Monte-Carlo are shown in Figures 6a–6e. For comparison, the α-helical structure for WG_5 and its dipole value are shown in Figure 6f. The susceptibility expected for the α-helix (2300 Å^3) is much larger than the average dipole value deduced from the experiments.

3.3 The intermediate case

Finally, it is possible that the molecule converts between different conformations during the microsecond time scale of the measurement, but that all the molecules in the beam do not explore the same energy landscape, due for example, to a high energy barrier which prevents interconversion. Thus all the molecules in the beam do not have the same average dipole. The beam profiles measured with the electric field will be a complex mixture of deviation and broadening. This intermediate case, where peptides are not rigid but not totally floppy is observed for W at 300 K and for WG_n peptides at 85 K.

4 Conclusion

Electric deflection measurements can be used as a tool to probe the conformation and the flexibility of isolated peptides. When the molecule is rigid, as for tryptophan at 85 K, the measured dipole moment corresponds to the dipole of the lowest energy equilibrium geometry. Whereas, when the molecule is very floppy, the measurements provide the average value of the square of the projection of the dipole moment on the axis of the electric field. Comparison of the results for the isolated tryptophan molecule and for the WG_n peptides shows that incorporation of a single glycine is enough to make the peptide floppy on the time scale of the electric deflection measurements. The increased conformational freedom in the WG_n peptides presumably results because incorporation of the glycine provides new degrees of freedom and new hydrogen bonding partners.

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