

Permanent Electric Dipole and Conformation of Unsolvated Tryptophan

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We have coupled a matrix assisted laser desorption source to an electric beam deflection setup to measure the permanent electric dipole of tryptophan isolated in a molecular beam. The permanent electric dipole is sensitive to the geometry and can be used as a tool to probe the conformation. For tryptophan, the dominant conformation present in the molecular beam has a dipole moment that agrees with the lowest energy conformation found at the B3LYP-DFT/6-31G* and MP2/6-31G* levels of theory. This conformation is stabilized by (COOH- - NH₂) hydrogen bonding and through a favorable interaction between the NH₂ group and the indole ring. Other low-energy conformations found in the calculations have dipole moments that are either too small or too large to account for the experimental results.

In recent years there has been considerable interest in obtaining structural information for unsolvated and partially solvated biomolecules.¹ These studies can provide detailed information about intramolecular interactions and solvent interactions that determine the conformations in all environments. In particular, a variety of sophisticated spectroscopic methods have been employed to examine isolated amino acids such as glycine,^{2,3} alanine,⁴ arginine,⁵ phenylalanine,⁶ tryptophan^{7–9} and tyrosine.^{8,10} The first spectroscopic results for tryptophan isolated in a molecular beam were obtained in 1985 by Levy and collaborators.⁷ They identified six different conformations in the resonantly enhanced two-photon ionization spectrum of jet-cooled tryptophan. More recently, high-resolution vibronic spectra were recorded for tryptophan at 0.38 K in helium droplets.⁸ However, the equilibrium structure has still not been determined. In the work described here we have used electric deflection of a molecular beam to directly probe the permanent dipole of tryptophan. Until very recently,¹¹ this approach has mainly been restricted to the

(1) Desfrancois, C.; Carles, S.; Schermann, J. P. *Chem. Rev.* **2000**, *100*, 3943–3962. Jarrold, M. F. *Annu. Rev. Phys. Chem.* **2000**, *51*, 179–207. Robertson, E. G.; Simons, J. P. *Phys. Chem. Chem. Phys.* **2001**, *3*, 1–18.

(2) Brown, R. D.; Godfrey, P. D.; Storey, J. W. V.; Bassez, M. P. *J. Chem. Soc., Chem. Commun.* **1978**, 547–548.

(3) Stepanian, S. G.; Reva, I. D.; Radchenko, E. D.; Rosado, M. T. S.; Duarte, M. L. T. S.; Fausto, R.; Adamowicz, L. *J. Phys. Chem. A* **1998**, *102*, 1041–1054.

(4) Godfrey, P. D.; Firth, S.; Hatherley, L. D.; Brown, R. D.; Pierlot, A. P. *J. Am. Chem. Soc.* **1993**, *115*, 9687–9691.

(5) Chapo, C. J.; Paul, J. B.; Provencal, R. A.; Roth, K.; Saykally, R. J. *J. Am. Chem. Soc.* **1998**, *120*, 12956–12957.

(6) Snoek, L. C.; Robertson, E. G.; Kroemer, R. T.; Simons, J. P. *Chem. Phys. Lett.* **2000**, *321*, 49–56.

(7) Rizzo, T. R.; Park, Y. D.; Peteanu, L.; Levy, D. H. *J. Chem. Phys.* **1985**, *83*, 4819–4820.

(8) Lindinger, A.; Toennies, J. P.; Vilesov, A. F. *J. Chem. Phys.* **1999**, *110*, 1429–1436.

(9) Piuze, F.; Dimicoli, I.; Mons, M.; Tardivel, B.; Zhao, Q. *Chem. Phys. Lett.* **2000**, *320*, 282–288.

(10) Cohen, R.; Brauer, B.; Nir, E.; Grace, L.; de Vries, M. S. *J. Phys. Chem. A* **2000**, *104*, 6351–6355.

(11) Dugourd, P.; Compagnon, I.; Lepine, F.; Antoine, R.; Rayane, D.; Broyer, M. *Chem. Phys. Lett.* **2001**, *336*, 511–517.

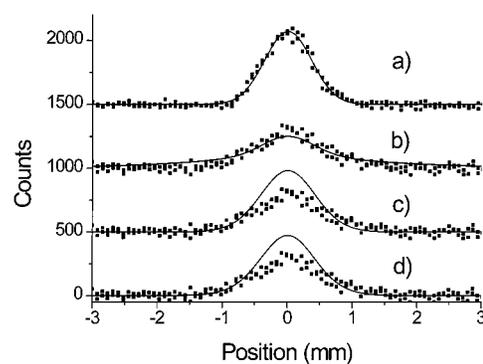


Figure 1. Profiles of tryptophan beams with $F_z = 0 \text{ V m}^{-1}$ (a) and $F_z = 6.7 \times 10^6 \text{ V m}^{-1}$ (b, c, and d). The squares correspond to experimental values and the full line to simulations (b, c, and d have been calculated with the rotational constants and dipole components of isomers I, II, and III, respectively). The profiles for a, b, and c have been offset vertically for clarity.

study of alkali halide dimers.¹² This is the first application of the electric deflection method to a biomolecule.

The experimental 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. Tryptophan and nicotinic acid were mixed in a 1:10 molar ratio and pressed to form a rod. A pulsed molecular beam of tryptophan was produced by desorbing molecules from the rod with the third harmonic of a Nd:YAG laser into a helium carrier gas. At the exit of the source, the tryptophan molecules are thermalized to 85 K in a 50 mm long nozzle cooled by liquid nitrogen. The molecular beam is collimated by two slits, and then goes through an electric deflector with a “two-wire” electric field configuration.¹³ One meter after the deflector, the molecules are ionized with the fourth harmonic of a Nd:YAG laser (266 nm) in the extraction region of a position-sensitive time-of-flight mass spectrometer.¹³ The tryptophan is two-photon ionized. The wavelength used for ionization (266 nm) is close to the resonance band of the indole moiety in tryptophan. There is relatively little fragmentation, roughly 80% of the ion signal is observed at the parent mass channel. Measurements of the molecular beam profile were performed as a function of the electric field in the deflector. The beam velocity was determined with a chopper.

Figure 1 shows examples of the molecular beam profiles obtained without and with an electric field of $6.7 \times 10^6 \text{ V/m}$ in the deflector. The profiles were averaged over 10 000 laser shots. A symmetrical broadening of the profile and a decrease in the intensity on the beam axis are observed when the field is turned on. Similar profiles with a regular increase in the broadening are observed as the electric field is increased from 0 to $1.5 \times 10^7 \text{ V/m}$. The force in the deflector is due to the interaction between the electric field F_z and the dipole μ of the molecule. It can be written as:¹⁴

$$f = \langle \mu_z \rangle \frac{\partial F_z}{\partial z} = \langle \mu_a \cos(az) + \mu_b \cos(bz) + \mu_c \cos(cz) \rangle \frac{\partial F_z}{\partial z} \quad (1)$$

where μ_a , μ_b , and μ_c , are the component 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

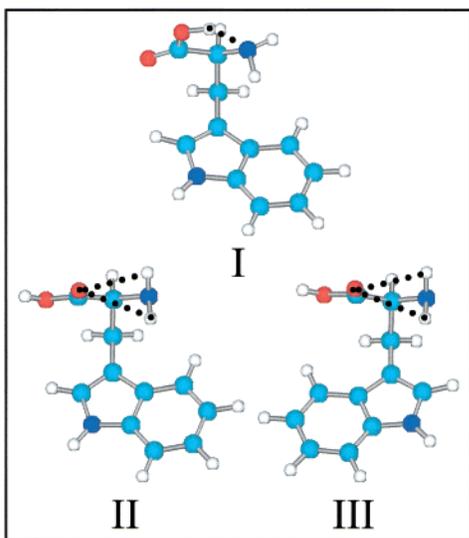
(12) Story, T. L.; Hebert, A. J. *J. Chem. Phys.* **1976**, *64*, 855–858.

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

(14) Townes, C. H.; Shawlow, A. L. *Microwave Spectroscopy*; McGraw-Hill Book Company: New York, 1955.

Table 1. Relative Energies, Dipole Moments, and Rotational Constants for Conformers I to VI

conformer	relative energy in kcal/mol		dipole moment (MP2/6.31G*) in debye (rotational constants in cm ⁻¹)			
	DFT/6-31G*	MP2/6-31G*	A	B	C	total
I	0.0	0.0	-3.37 (0.0409)	-2.12 (0.0134)	-0.29 (0.0118)	3.99
II	2.205	1.258	0.60 (0.0389)	3.00 (0.0141)	-0.48 (0.0122)	3.10
III	2.398	1.330	0.65 (0.0333)	0.96 (0.0165)	-0.21 (0.0130)	1.18
IV	1.150	1.605	0.88 (0.0324)	7.27 (0.0162)	0.96 (0.0126)	7.38
V	1.124	2.139	5.63 (0.0431)	-4.64 (0.0112)	2.47 (0.0096)	7.70
VI	1.756	2.561	6.44 (0.0454)	1.30 (0.0112)	2.69 (0.0095)	7.10

**Figure 2.** Geometry of the three lowest energy conformers found at the MP2/6-31G* level of theory.

principal axes of the molecule and the axis of the electric field. The average force depends on the rotational level of the molecule.¹¹ It is this force that induces the broadening of the beam that is experimentally observed.

An extended conformational search was performed for tryptophan with the semiempirical AM1 method. The 20 lowest energy conformers found with AM1 were submitted to full geometry optimization with density functional theory (DFT) using the Becke3-Lee-Yang-Parr functional and the 6-31G* basis set (B3LYP-DFT/6-31G*).^{15,16} The six lowest energy conformers were then re-optimized at the Hartree-Fock level with second-order Møller-Plesset corrections with the same basis set (MP2/6-31G*).¹⁷ Relative energies and the components of the permanent dipole are listed in Table 1. The conformations of the lowest energy isomers (I to III) are shown in Figure 2. The lowest energy conformation, conformation I, is stabilized by (COOH...NH₂) hydrogen bonding and through a favorable interaction between the NH₂ group and the indole ring. Conformations II and III both have (NH₂...OCOH) hydrogen bonding, and differ by a flip of the indole ring.

Beam profiles were calculated for every isomer in Table 1 and compared to experimental results. Briefly, the beam profiles were determined by an exact calculation of the rotational levels of the asymmetric rotors. The force due to the electric field was obtained using first-order perturbation theory.¹⁴ The final beam profile is

obtained by taking into account every rotational level with a Boltzmann weight factor. A similar calculation for symmetrical top molecules using a classical approach is described in ref 11.

The broadening of the beam profile depends mainly on the value of the dipole moment along the principal axis of the molecule (μ_a). Calculated profiles for isomers I to III are compared to experimental results in Figure 1. The profile calculated for structure I is in very good agreement with experimental data. Similar agreement is observed for every value of the electric field in the range that we have investigated. On the other hand, conformers II, III, and IV (not shown in the figure) have a smaller dipole (μ_a component) than the experimental value. The profiles calculated for isomers V and VI (not shown) are significantly broader than the experimental beam profiles.

These results clearly demonstrate that conformer I is the dominant isomer in the molecular beam. This is in agreement with the relative energies obtained from the DFT and MP2 calculations. With the large energy gap between isomers I and II in the calculations, the vast majority of molecules are expected to be in the isomer I at 85 K if equilibrium is attained. It is important to note that with our experimental setup molecules are not rapidly quenched, but slowly cooled to 85 K.¹⁸ This temperature is significantly higher than that expected in the experiments of Levy and collaborators where six different tryptophan conformations were identified. It is possible that there are minor amounts of other conformations present under our conditions. But our results cannot easily be explained by a mixture of conformations, with dipole moments much larger and much smaller than found here; the resulting beam profile would be quite different from that shown in Figure 1. Profiles calculated for isomer I are in agreement with the measured profiles over a wide range of deflection voltages (up to 1.5×10^7 V m⁻¹) and at low voltages the beam profile is very sensitive to the value of the dipole.

We have demonstrated that measurement of the permanent electric dipole can be used as a tool to probe the conformation of unsolvated biomolecules. For tryptophan, the dominant conformation present in the molecular beam has a dipole moment that agrees with that for the lowest energy conformation found in calculations. The approach described here can be applied to much larger peptides. Indeed we have already performed electric deflection measurements on peptides with more than 20 amino acids.¹⁹ We are currently using this approach in an effort to measure the macrodipole of α -helices and to obtain evidence for zwitterionic forms of amino acids and peptides.

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(18) Significant further cooling is not expected to occur when the molecules exit the source: the source pressure is a few Torr and the nozzle aperture is 2 mm.

(19) Compagnon, I.; Antoine, R.; Rayane, D.; Broyer, M.; Dugourd, Ph.; Breaux, G.; Hagemester, F. C.; Hudgins, R. R.; Jarrold, M. F. To be submitted for publication.

(15) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098–3100.

(16) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789.

(17) Pople, J. A.; Binkley, J. S.; Seeger, R. *Int. J. Quantum Chem. Quantum Chem. Symp.* **1976**, *10*, 1.