Detailed Ab Initio Studies of the Conformers and Conformational Distributions of Gaseous Tryptophan

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A systematic and extensive conformational search has been performed to characterize the gas-phase tryptophan structures. A total of 648 unique trial structures were generated by allowing for all combinations of internal single-bond rotamers and were optimized at the DFT/B3LYP/6-311G* level of theory. A total of 45 local minima conformers were found. Further optimization of the 45 conformers with B3LYP and MP2/6-311++G** did not produce meaningful structural change, and accurate geometries, dipole moments, rotational constants, harmonic frequencies, and relative energies were then determined. Combined with statistical mechanics principles, the conformational distributions of gas-phase tryptophan at different temperatures are shown. The results clearly support the conclusion drawn by Compagnon et al. that only one dominant isomer existed in the molecular beam at 85 K and add further evidence that the supersonic jet expansion or embedding helium droplets did not produce an equilibrium distribution.

1. Introduction

The photophysics of tryptophan is of special importance due to its dominant role in the near-ultraviolet absorption and fluorescence of many proteins. The molecular structural information is critical to the understanding of the photophysics of tryptophan,[1] and an accurate account of the molecular conformation of tryptophan is highly desirable.

Levy and collaborators[2] carried out an extensive set of studies of the UV spectroscopy of jet-cooled tryptophan and identified six different conformations in the resonantly enhanced two-photo ionization spectrum. Five of the six conformers were confirmed nicely by the high-resolution vibronic spectra of tryptophan in 0.38 K cold helium droplets.[3] More recently, Compagnon et al.[4] 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 at 85 K. Compagnon et al. found the dominant conformation present in the molecular beam has a dipole moment that agrees with the lowest energy conformation determined at the B3LYP/6-31G* and MP2/6-31G* levels of theory and concluded that the six conformers identified by the Levy group are in contradiction to their dipole measurement at equilibrium.

Notice, however, the conformer search by Compagnon et al. was based upon an initial search by the semiempirical AM1 method, and that is prone to miss important conformers.[5] To be more complete and more convincing, it is helpful to carry out a more systematic and reliable theoretical study of the tryptophan conformations. The goal of this study is to locate all possible gaseous tryptophan conformers, to obtain precise knowledge about the relative stabilities of different conformers on the energy surface, and to provide theoretical results such as rotational constants, vibrational frequencies, and dipole moments of conformers and conformer distributions at various temperatures that may be helpful to future experimentalists. As DFT/B3LYP method has been widely applied to obtain the conformational behavior, theoretical vibrational frequencies, and infrared intensities of amino acids, which are in excellent agreement with the experimental data for glycine,[6] alanine,[7] proline,[8] and valine.[9] B3LYP is used in this work as the main computational method in the systematic search of the tryptophan conformers.

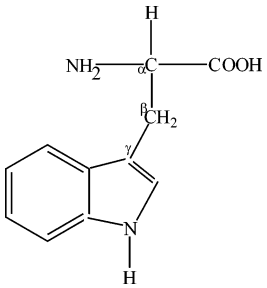

Figure 1. Schematic structure of the tryptophan molecule.

2. Computational Methods

The planar structure of tryptophan is shown in Figure 1. The rigid bonds of the indole ring do not provide any internal rotamer. Different conformers of the tryptophan molecule result from rotating the five internal axes, that is, the $C_\alpha-N$, $C_\alpha-C$, $C-O$, $C_\alpha-C_\beta$, and $C_\beta-C_\gamma$ bonds, and the conformational structure of the gaseous tryptophan molecule may be characterized with five internal torsion parameters. A series of trial structures can be consequently generated for the amino acid of tryptophan by allowing for all combinations of internal single-bond rotamers, as shown in Scheme 1: (a) The carboxyl group possesses syn- or anti-periplanar corresponding to 0° and 180° torsions. (b) One of three groups on the α-carbon will be separated from the other two groups by the plane of the carboxyl. Because of the four different groups or atom on the α-carbon and asymmetry in the carboxyl, each torsion leads to different structures. The torsion around the single bond between the α-carbon and the carboxyl leads to six different possibilities. (c) Because of the same character of the two hydrogen atoms

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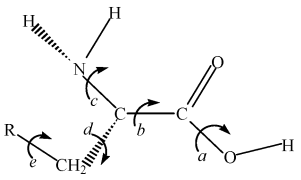
TABLE 1: Relative Energies, Relative Zero-Point Vibrational Energies, Bonding Schemes, Relative Gibbs Free Energies, Rotational Data, and Dipole Moments of Tryptophan Conformers[a]

	relative energies		relative	bonding	relative Gibbs	rotational constants			dipole
conformer	B3LYP	MP2++	ZVPE	scheme[b]	free energy	A	B	C	(D)
1	0.00	0.00	0.00	A	0.00	1.251	0.380	0.335	3.96
2	1.01	1.29	−0.09	A	0.90	1.303	0.328	0.283	7.49
3	1.23	1.32	−0.03	A	1.37	0.983	0.462	0.362	7.33
4	2.04	1.39	−0.45	B	0.55	1.178	0.398	0.345	3.00
5	2.22	1.48	−0.46	B	0.58	0.998	0.462	0.367	1.30
6	1.61	1.85	−0.08	A	1.50	1.366	0.327	0.279	6.85
7	2.85	2.05	−0.35	D	1.18	0.984	0.475	0.372	3.17
8	2.52	2.25	−0.34	E	1.35	1.185	0.393	0.342	2.14
9	1.37	2.33	−0.24	A	1.58	1.264	0.325	0.286	7.42
10	3.45	2.53	−0.54	C	1.48	1.068	0.438	0.359	3.20
11	2.92	2.97	−0.54	C	1.83	1.282	0.331	0.282	3.42
12	1.74	2.99	−0.30	A	2.08	1.376	0.318	0.277	6.52
13	4.68	3.11	−0.68	C	2.06	1.013	0.468	0.374	3.12
14	3.00	3.30	−0.51	F	1.83	1.170	0.362	0.321	2.02
15	4.58	3.38	−0.60	C	2.27	1.072	0.437	0.359	4.04
16	5.00	3.41	−0.54	E	2.57	0.991	0.485	0.379	4.35
17	4.75	3.50	−0.70	C	2.08	1.214	0.394	0.344	3.35
18	3.52	3.57	−0.58	C	2.41	1.374	0.325	0.278	4.31
19	4.35	3.76	−0.64	C	2.02	1.164	0.372	0.333	2.33
20	4.06	3.89	−0.62	E	2.46	1.294	0.329	0.280	4.71
21	3.75	4.09	−0.50	C	3.01	1.425	0.328	0.305	1.70
22	5.10	4.11	−0.50	E	3.18	1.180	0.401	0.349	2.42
23	2.63	4.26	−0.56	C	2.76	1.403	0.303	0.256	1.51
24	4.50	4.31	−0.59	E	2.98	1.371	0.326	0.279	3.25
25	4.031	4.42	−0.31	A	3.36	1.299	0.352	0.303	3.52
26	5.89	4.79	−0.76	E	3.41	1.074	0.423	0.358	3.24
27	5.54	4.80	−0.74	E	3.23	1.177	0.370	0.330	3.68
28	5.74	5.04	−0.39	A	4.24	1.095	0.425	0.348	6.25
29	4.26	5.09	−0.54	C	3.78	1.337	0.330	0.293	3.27
30	3.58	5.17	−0.55	C	3.96	1.370	0.325	0.271	3.27
31	3.69	5.54	−0.10	A	5.27	1.463	0.316	0.273	4.00
32	5.88	5.55	−0.70	G	4.20	1.204	0.367	0.320	4.93
33	3.85	5.91	−0.56	E	4.09	1.475	0.296	0.251	3.45
34	8.12	6.80	−0.59	G	5.81	1.206	0.395	0.344	3.27
35	7.45	6.81	−0.76	G	5.00	1.178	0.380	0.333	5.77
36	7.79	6.89	−0.79	H	5.24	1.219	0.369	0.325	5.89
37	5.02	7.03	−0.59	D	5.16	1.354	0.324	0.268	0.94
38	8.79	7.57	−0.70	G	6.34	0.982	0.468	0.365	4.26
39	9.07	8.01	−0.83	H	6.05	1.164	0.388	0.339	6.60
40	9.09	8.63	−0.76	H	7.22	1.290	0.329	0.281	5.99
41	10.49	8.89	−0.84	H	7.28	1.301	0.377	0.333	3.98
42	9.42	9.18	−0.61	H	8.10	1.435	0.328	0.304	2.03
43	9.85	9.30	−0.79	H	7.96	1.365	0.327	0.279	6.93
44	8.37	9.42	−0.72	H	7.96	1.382	0.306	0.258	4.46
45	11.30	9.58	−0.80	H	8.49	0.985	0.477	0.370	5.71

[a] Geometries were optimized at the B3LYP/6-311G* level, and relative energies in kcal/mol were optimized at the B3LYP/6-311G* (B3LYP) and MP2/6-311++G** (MP2++) levels. Relative zero-point vibrational energies (ZPVE) in kcal/mol and rotational constants in GHZ were obtained at the B3LYP/6-311G* level, and dipole moment (D) was obtained at the MP2 level. [b] See Figure 2 for the bonding schemes. Gibbs free energies in kcal/mol are the electronic energies obtained at the MP2/6-311++G** level plus the thermal free energies at room temperature obtained at the B3LYP/6-311G* level.

SCHEME 1[a]

R=indole ring



[a] (a) 2-fold: 0, 180. (b) 6-fold: 30, 90, 150, 210, 270, 330. (c) 3-fold: −120, 0, 120. (d) 6-fold: −120, −60, 0, 60, 120, 180. (e) 3-fold: 60, 120, 180.

on the nitrogen atom, the orientation of the amino group allows for three rotamers. (d) Because the electronic density distribution of the indole ring is not symmetrical, we take it into account that there are six possible rotamers about the $C_\alpha-C_\beta$ bond so that all possible local minima conformers may be located. (e) Finally, because the relative position of the two hydrogen atoms and the other group on the β-carbon is stationary, placement of the unique group above or below the indole ring plan leads to the same conformers. Therefore, the interaction of the β-carbon with the indole ring represents three possible rotamers. This leads to a total of 648 possible structures for the tryptophan.

In the process of the calculation, an extensive set of trial structures was necessary. If any rotamers were excluded, some local minima conformers may be lost.[5] Consequently, all of the 648 trial structures were used as the guess structures and fully optimized at the B3LYP/6-311G* level using the Gaussian 98 quantum chemistry package,[10] and a set of unique conformers was found in the calculations. The frequencies of these unique conformers were subsequently calculated. All of the conformers were then reoptimized at the MP2/6-311++G** level with no

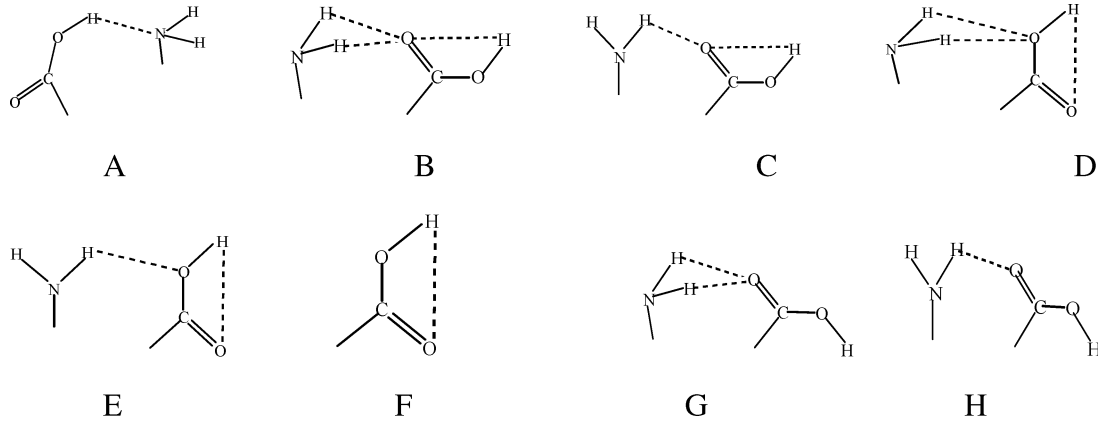


Figure 2. H-bonding types between the amino group and the carboxyl functional group of the tryptophan.

noticeable structural changes, although substantial changes in the conformer energies were observed.

3. Results and Discussion

Conformers and Energies. A total of 45 local minima unique conformers of the gaseous tryptophan have been located in our calculations. Table 1 shows the relative energies, relative zero-point vibrational energies (the scale factor of frequency used[11] is 0.96), theoretical rotational data, and dipole moments obtained for all tryptophan conformers.

The relative energies of the tryptophan conformers are determined by the interplay of the different types of H-bonds, the interaction between the amino group and the indole ring plane, the interaction between the carboxyl and the indole ring plane, the steric strain, and the repulsion of lone pairs on the nitrogen and oxygen atoms. To analyze the large amount of data, all conformers were characterized by the interaction of hydrogen bonding. For the purposes of this study, we selected a distance of 2.80 Å as a cutoff for near-atom interaction between the NH_2 group and the carboxyl. This distance is consistent with the typical intramolecular interaction distances found in this system. Because of the steric constrains of the conformer, the length of the intramolecular H-bond in this system is larger than the ordinary intermolecular H-bond as the indole ring is a very big and complex substitution system and possesses a great electronic density. Analyzing all of the stable conformers obtained, we in all find eight types of hydrogen bonds between the amino group and the carboxyl, that is, the bonding schemes A−H as illustrated in Figure 2. See Table 1 for the hydrogen-bonding types of all of the conformers.

Conformer 1 is found to be the global minimum for the tryptophan at both the DFT and the MP2 level. Its total electronic energy is −684.649251 au at the MP2/6-311++G**, level and its conformer is stabilized by hydrogen bonding ($COOH\cdots NH_2$) and through a favorable interaction between the NH_2 group and the indole ring. Four of the six most stable conformers, 1, 2, 3, and 6, display an intramolecular H-bond ($COOH\cdots NH_2$) and an additional H-bonding interaction between the amino group and π-electron system of the aromatic ring. The additional H-bond leads to an increased electron density around the naked nitrogen atom, which makes the O−H···N H-bond stronger. Also, the O−H···N H-bond leads to the partial change at the amino hydrogen involved in the π-electron interaction, promoting a cooperative interaction with the indole ring. It can be noted from Table 1 that ZVPE values of the above four conformers are maximal, which shows that the four conformers have a rigid and compact structure. Conformers 4 and 5 both have the bifurcated ($NH\cdots OCOH$) H-bond and differ by a flip of the indole ring.

TABLE 2: Equilibrium Distributions (%) of Tryptophan Conformers at Various Temperatures

conformer	85 K	298 K	398 K	498 K
1	99.84	48.61	26.86	15.85
2	0.06	9.21	9.21	7.76
3	0.04	4.60	4.48	3.71
4	0.03	9.05	10.31	9.56
5	0.02	8.53	10.16	9.69
6		3.37	4.27	4.15
7		3.69	5.53	6.03
8		2.81	4.53	5.17
9		2.26	3.63	4.07
10		1.63	3.10	3.91

Notice that the conformers I, II, III, IV, V, and VI found at the MP2/6-31G* level in ref 4 correspond to conformers 1, 4, 5, 3, 2, and 6, respectively. Adding polarization function and affiliating diffuse function to the basis set, the relative energies of conformers (relative to conformer 1) have a considerable change.

Conformational Distribution. In the Born−Oppenheimer approximation and neglecting vibro-rotational coupling, the molecular partition function can be factorized into its translational, rotational, vibrational, electronic, and nuclear parts, that is, $q = q_{trans}q_{rot}q_{vib}q_{elec}q_{nucl}$. The translational and nuclear partition functions are identical for all of the species, and therefore they are irrelevant for the equilibrium distribution. The expressions for the calculations of the rotational, vibrational, and electronic partition functions can be found in ref 12.

Using the respective ab initio data for the various conformers, that is, inertia moments, vibrational frequencies, and ground-state electronic energies, the gas-phase tryptophan partition functions can be calculated for a set temperature. Consequently, the equilibrium distribution of various conformers at the given temperature can be determined. The electronic partition function has been calculated using the conformational energies obtained at the MP2/6-311++G** level. The vibrational and rotation partition functions have been computed using the date obtain at the B3LYP/6-311G* level, and the vibrational frequencies have been scaled by a factor of 0.96.[11] The conformational distributions at the various temperatures are shown in Table 2. At 85 K, conformer 1 has a concentration of 99.8%. Further tests with bigger basis sets, aug-cc-pVDZ, aug-cc-pVTZ, and 6-311G(2df,p), and different methods for the exchange-correlation energy calculation (B3LYP, B3PW91, and MP2) were performed to verify the results (see Table S1 of the Supporting Information). Although these calculations gave somewhat different relative energies of the conformers, and significant

changes were observed sometimes for some relatively high energy conformers, all results show that conformer 1 is the dominant isomer in the gas phase with more than 99% concentration at 85 K, which strongly supports the conclusion drawn by the experimental result of Compagnon et al.[4] As we have located all of the possible conformers, and all calculations with DFT and MP2 with different basis sets give the consistent result, that is, virtually only conformer 1 is present at the temperature of 85 K or less if equilibrium is attained, it can be concluded that the supersonic jet expansion[2] as well as the embedding cold helium droplets[3] did not produce an equilibrium distribution. This conclusion is consistent with the observation made in ref 13 for the glycerol results.

As shown in Table 2, the concentration of the most stable conformer decreases rapidly with increasing temperature. At room temperature, the concentration of the conformer 1 is about 50%. The five lowest energy conformers are the primary isomers, accounting for about 80% of the distribution. The concentrations of conformers 4 and 5 are close to that of conformer 2 and higher than that of conformer 3, mainly due to favorable vibrational contribution. At 398 K, their concentrations even exceed that of conformer 2. At the source temperature of the supersonic jet-cooled tryptophan experiment (230 °C),[2] tryptophan molecules exist as many diverse conformers in the gas phase. It appears that some of the conformers freeze out by the very rapid cooling in the supersonic jet expansion or due to collision with the He droplet surface, resulting in a rich featured spectrum in the respective experiments.

4. Summary

With a total of 648 exhaustive trial structures determined by all combinations of internal single-bond rotamers of the gas-phase tryptophan molecule, we in all found 45 local minima unique conformers based on geometry optimization by B3LYP/6-311G*. The 45 conformers can be classified with eight types of hydrogen bonds between the amino group and the carboxyl. The conformational distributions by various exchange-correlation methodologies and different basis sets all indicate a single dominant conformer at low temperature and agree with the conclusion drawn by the work of Compagnon and co-workers. We concluded that the supersonic jet expansion reported by Levy and collaborators and the helium droplet embedment by Lindinger and co-workers did not produce equilibrium distributions.

Supporting Information Available: A listing of the relative energies of the 10 lowest lying tryptophan conformers obtained by the MP2 method with the aug-cc-pVDZ and 6-311G(2df,p) basis sets and by the B3LYP and B3PW91 methods with the 6-311G(2df,p) and aug-cc-pVTZ basis sets. This material is available free of charge via the Internet at http://pubs.acs.org.

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