Predicting Conformations of Biomolecules: Application to a Noradrenaline Analogue

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A new theoretical method is shown to overcome major obstacles in the calculation of biomolecular conformation. The torsional path integral Monte Carlo (TPIMC) technique is applied to the free jet expansion of a noradrenaline structural analogue (2-amino-1-phenylethanol) and is shown to predict the experimentally observed conformer populations accurately. Standard theoretical techniques employing a harmonic approximation in the evaluation of thermodynamic quantities such as free energy are shown to be unreliable for the analysis of conformer populations in flexible molecules. The TPIMC method is a general approach to the thermodynamic simulation of large molecules that treats anharmonicity and quantum effects within the torsional degrees of freedom.

1. Introduction

Molecular conformation is a central question in molecular biology. The structural conformation adopted by a biomolecule is a determining factor in its capacity to moderate and facilitate biochemical and pharmacological processes.[1,2] Both experimental and theoretical techniques have been developed to investigate the relative population of molecular conformations.[3−10] Theory requires treatment of the dynamics involved in conformational change on a potential energy surface. Here, we introduce a new quantum-mechanical technique for studying equilibrium conformational populations in molecules. The method is used to explain recent experimental observations of an analogue of a noradrenaline neurotransmitter for which conventional theoretical techniques, such as those that employ harmonic approximations, are inadequate.

The theoretical prediction of experimentally observed conformers relies, at least in part, on an accurate description of the thermally equilibrated conformational populations. The relative equilibrium populations of two conformers depends on their free-energy difference according to the familiar relationship $K_{eq} = e^{-\Delta G^\circ/kT}$, where K_{eq} is the ratio of conformation populations, k is the Boltzmann constant, T is temperature, and ΔG° is the difference in standard Gibbs free energy between the two conformations. Most techniques attempt to obtain the relative populations from free-energy calculations based on harmonic corrections to the minimized potential energy, but this approach is fundamentally limited.[9,10] The largest contributions to the harmonic correction arise from the lowest vibrational frequencies. However, these lowest-frequency vibrations can correspond to motions such as intramolecular torsions about single bonds with low barriers for which the harmonic approximation is very poor.

A general and accurate technique for the evaluation of equilibrium conformational populations must provide an anharmonic description of the low-frequency torsions within the molecule and should describe quantum-mechanical effects such as zero-point energy accurately. The newly developed torsional path integral Monte Carlo (TPIMC) technique satisfies these criteria and can be used for calculating conformational populations.[11,12] Previously implemented for the evaluation of general thermodynamic averages, TPIMC provides a quantum-mechanically accurate treatment of the intramolecular dihedral or torsional modes of nuclear motion. These torsional modes typically range from a few cm^{-1} to a few hundred cm^{-1} in frequency, and they largely control motion across the conformational free-energy landscape of biomolecules at biochemically relevant temperatures.[13] TPIMC has the further advantage of requiring neither multiple geometry optimizations nor the evaluation of vibrational frequencies during the actual simulation, although structural information is generally needed for input and analysis.[14]

The 2-amino-1-phenylethanol (APE) molecule (Figure 1A) is a structural analogue of both the noradrenaline neurotransmitter molecule and the ephedra class of pharmaceutical molecules. The receptor-specific binding of these molecules determines their function and depends largely on the their conformational preference.[15] A previously published investigation of the APE potential energy surface has revealed that the "tail-down" AG1 structure and "tail-up" GG1 structure shown in Figure 1B are the most stable conformations of the molecule.[16] They were found to be isoenergetic within the accuracy of ab initio electronic structure techniques. Several other related conformations were found with higher energy by 5−11 kJ/mol (Figure 1C). Although these relative conformational energies provide no indication that either of the two most stable conformers would be substantially preferred, recent ion dip spectroscopy experiments by Graham et al. indicate that the AG1 conformation is populated in favor of the GG1 conformation by a ratio of nearly 3:1.[16] Similar inconsistencies between theoretical predictions and experimentally observed populations have been reported for a variety of neurotransmitters, amino acids, and other biomolecules.[17−21] Here we see that the TPIMC technique gives a new explanation for the experimental finding of conformational preference.

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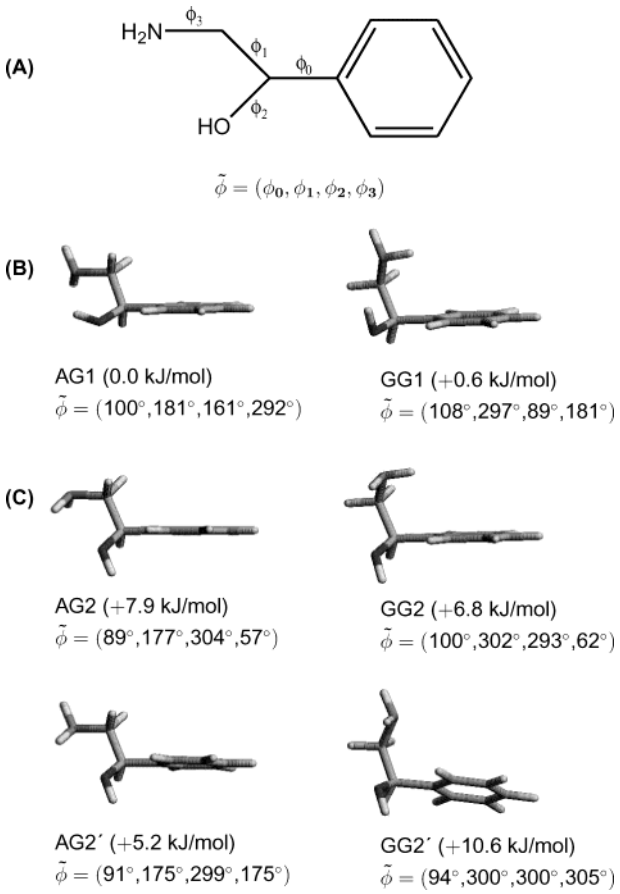

Figure 1. APE and its lowest-energy conformations. **(**A) Molecular schematic labeling the torsional angles. (B) Most stable and effectively isoenergetic AG1 and GG1 conformers. (C) Other low-energy conformers. MM3 relative energies are reported.

2. Methodology

The path integral Monte Carlo (PIMC) method is a general quantum technique for calculating equilibrium thermodynamic quantities at finite temperature.[22−24] The conceptual approach of PIMC is to perform a classical Monte Carlo simulation on a model system. The model is obtained by simultaneously replacing each atom in the original molecular system with a cyclic polymer called a Trotter ring.[23] The number of monomers, or Trotter beads, in each Trotter ring determines the accuracy of the PIMC simulation. With one Trotter bead, the classical mechanical result is obtained. The exact quantum result is obtained in the limit of infinite Trotter beads.

For the torsional PIMC (TPIMC) technique, the model system is obtained by replacing only internal molecular torsions with Trotter rings, which enables the quantum simulation of larger molecular systems.[11,12] The details of the TPIMC technique have been explained elsewhere, so we include only the major features of the reported calculations.[11,12] The TPIMC program developed in our research group uses several subroutines from the TINKER software package.[25]

Simulations were performed at two temperatures: 363 K (90 °C), to compare with the oven temperature of the experiment, and 100 K, to explore low-temperature behavior. Classical TPIMC results were obtained using one Trotter bead, and quantum TPIMC results were obtained with five Trotter beads. Previous applications of quantum TPIMC to molecular torsions suggest that five Trotter beads is sufficient to recover the bulk of quantum behavior.[11,12]

Torsional sampling was performed using the free-rotor (FR-TPIMC) algorithm to ensure that the entire configuration space was explored.[12] For the TPIMC calculations, total enthalpies and free energies were calculated with estimated standard deviations of less than 0.063 and 0.021 kJ/mol, respectively. At 363 K, the standard deviations of the AG1/GG1 population ratio determined using classical and quantum TPIMC are 0.007 and 0.004, respectively. At 100 K, the standard deviations are 0.06 and 0.29. Differences between all reported population ratios are therefore significant with respect to their calculated standard deviations. For all calculations, the estimator bias was found to be within the standard deviation of the reported result.

Although the TPIMC technique can be employed using any potential energy surface, the MM3 force field was selected for this study because of its good agreement with ab initio calculations reported at high levels of theory for this type of molecule.[26] In a previous study, relative APE potential energies were calculated at the MP2/6-311G** level of theory using geometries optimized at the MP2/6-31G* level.[16,27] The selection of other potential functions may slightly alter specific numerical results, but the general issues at hand and the conclusions reached in this paper are likely to remain unchanged.

The bond lengths and angles used to define the structure of the APE molecule during TPIMC simulation were chosen to be the average of the values obtained for the AG1 and GG1 conformations minimized on the MM3 potential. In all cases, bond lengths differed by less than 0.002 Å, and angles differed by less than 1.1°. These structural parameters changed the relative energy of dominant AG1 and GG1 conformers by less than 0.04 kJ/mol. The longest TPIMC calculations reported in this study were completed within 2 days on a single Intel Pentium III processor (1.26 GHz). Results reported in this paper for the harmonic approximation were calculated using the MM3 harmonic vibrational frequencies corresponding to the torsional degrees of freedom. Harmonic thermodynamic quantities were obtained from the standard statistical mechanical expressions for the harmonic oscillator.[28]

3. Results

The APE molecule has four torsional degrees of freedom that are all active during the TPIMC simulations (Figure 1A). To calculate the conformational populations at a given temperature, a 3-D torsional distribution function was stored during the calculation. This function describes the equilibrium population distribution of the molecule with respect to the three torsional angles (ϕ_1, ϕ_2, ϕ_3) that control structural conversion between the dominant conformations (Figure 1). Integrating this function over torsional ranges enables the evaluation of either conformational populations or reduced probability distribution functions. Because the torsional angles in the relevant conformations of the APE molecule correspond neatly to anti or gauche geometries, the integration of conformer populations is simply performed over 120° about the idealized torsional minima. For example, the GG1 conformation has minimized torsional coordinates of (ϕ_1, ϕ_2, ϕ_3) = (297°, 89°, 181°), so the relative population of the GG1 conformer was obtained by integrating the probability distribution function over the range of angles ϕ_1 = [240°, 360°], ϕ_2 = [0°, 120°], and ϕ_3 = [120°, 240°]. (The GG1 conformer is unique in that ϕ_2 is distorted substantially from the idealized gauche angle of 60°. However, post-calculation analysis of neighboring integration ranges verifies that the employed parameters satisfactorily encompass the GG1

TABLE 1: MM3 Harmonic Frequencies for Torsional Modes[a]

	AG1	AG2	AG2′	GG1	GG2	GG2′
ϕ_0	43.8	50.3	50.0	45.0	55.0	48.0
ϕ_1	136.9	115.5	124.2	101.8	98.9	100.6
ϕ_3	259.6	292.5	281.0	279.7	316.7	270.4
ϕ_2	413.2	325.9	322.9	447.7	328.5	330.2

[a] Frequencies reported in cm^{-1}.

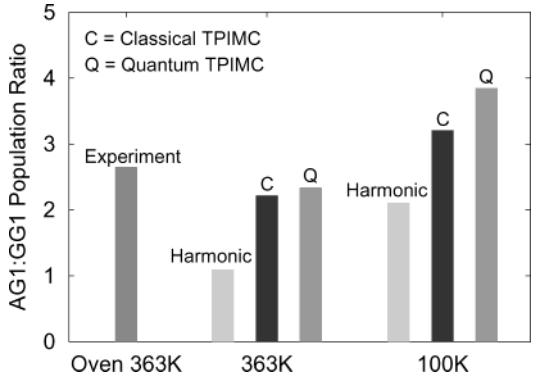

Figure 2. Comparison of the AG1/GG1 conformational population ratios obtained from experiment[16] and with theoretical techniques.

probability distribution.) A similar 3-D energy distribution function was also stored for the calculation of conformational enthalpies.

Table 1 presents the calculated torsional vibrational frequencies used to obtain the harmonic thermodynamic quantities. It is noted that the frequency splitting between the ϕ_2 and ϕ_3 torsional vibrations is greatest in the AG1 and GG1 conformers. In these most stable structures, the intramolecular hydrogen bonding of the $-$OH hydrogen atom with the $-NH_2$ lone pair gives rise to the stronger coupling of the torsional modes.

Figure 2 displays the relative populations of the AG1 and GG1 conformers as determined by experiment and theory. Experimental results were obtained using IR ion dip spectroscopy in which APE molecules were thermally equilibrated in an oven at 363 K before undergoing free jet expansion in a He carrier gas.[16] The ratio of AG1 conformers to GG1 conformers was found to be 2.65.[29] To compare with this experimental result, calculations were performed at the 363 K oven temperature. The harmonic approximation result of 1.10 substantially underestimates the AG1/GG1 population ratio, but the anharmonic treatment of the reported TPIMC calculations reproduces the experimental finding. At 363 K, the classical TPIMC ratio of 2.22 largely accounts for the preference of the APE molecule for the AG1 conformation. The effect of quantum mechanics further shifts the conformational preference in favor of the AG1 structure, resulting in a quantum TPIMC population ratio of 2.34.

To explore the equilibrium distribution of the APE molecules at lower temperature, Figure 2 also includes the conformational population ratios theoretically predicted at 100 K. Both the harmonic and TPIMC results predict a shift in the conformer populations toward the AG1 geometry, partly because of its slightly lower energy predicted by our potential energy surface. At this lower temperature, the free-energy changes due to anharmonicity and quantum effects are enhanced and cause substantial changes in the population ratios. It should be noted that the apparent agreement of the low-temperature harmonic results with experiment is coincidental. Including the effects of anharmonicity would bring these harmonic results into closer agreement with the low-temperature TPIMC calculations.

TABLE 2: Relative Conformational Energy[a] and Standard Free Energy[b] at 363 K Using the Harmonic (HO), Classical TPIMC (C), and Quantum TPIMC (Q) Techniques

	E_{ZP} [c]	G°_{HO}	G°_C	G°_Q
AG1	0.0	0.0	0.0	0.0
GG1	0.7	0.3	2.4	2.6
AG2	7.4	7.3	7.7	7.5
AG2′	4.7	4.7	4.7	4.6
GG2	6.4	6.3	8.4	8.3
GG2′	10.0	9.3	11.4	11.3

[a] E_{ZP} in kJ/mol. [b] G° in kJ/mol. The relative free energy of the GG1 conformer at 363K determined from experimental populations is 2.9 kJ/mol. [c] MM3 relative electronic energies corrected with MM3 zero-point harmonic vibrational energies.

TABLE 3: Relative Standard Conformational Enthalpy[a] and Entropy[b] at 363 K Using the Harmonic (HO), Classical TPIMC (C), and Quantum TPIMC (Q) Techniques

	H°_{HO}	H°_C	H°_Q	S°_{HO}	S°_C	S°_Q
AG1	0.0	0.0	0.0	0.0	0.0	0.0
GG1	0.7	0.3	0.5	1.1	−5.8	−5.8
AG2	7.7	8.9	8.5	1.0	3.2	2.6
AG2′	5.0	5.6	5.3	0.8	2.3	2.1
GG2	6.6	7.4	7.1	0.9	−2.7	−3.3
GG2′	10.4	11.5	11.2	3.0	0.4	−0.1

[a] H° in kJ/mol. [b] S° in J/mol/K.

The relative free energies at 363 K of the major APE conformations are displayed in Table 2. We see that the harmonic approximation incorrectly predicts a decrease in the free-energy difference between the AG1 and GG1 conformers. A comparison of the relative free energies predicted by the quantum harmonic oscillator approximation and the quantum TPIMC technique reveals that the anharmonic correction is responsible for almost 90% of the 2.6 kJ/mol energy difference between the dominant conformers found using quantum TPIMC.

The calculated conformational enthalpies and entropies in Table 3 reveal the flaw in the harmonic approximation. At both temperatures, the harmonic approximation is fairly accurate at recovering the enthalpy that depends heavily on the region of the potential energy surface local to the conformational minimum. The primary source of anharmonic error clearly arises in the calculated entropies. In every case, the harmonic entropies compare poorly with the TPIMC result. For the difference in AG1 and GG1 conformational entropies, the comparison is particularly poor, which leads to the inaccurate harmonic estimation of the relative conformational populations.

Figure 3A displays the 2-D torsional distribution of the AG family of structural conformers at 363 K calculated using quantum TPIMC. This function is obtained from the 3-D torsional distribution by integrating over $\phi_1 = [120°, 240°]$. A related GG torsional distribution plot could be obtained by integrating over $\phi_1 = [240°, 360°]$. Although a large peak in the probability distribution is observed in the AG1 conformation around $(\phi_2, \phi_3) = (180°, 300°)$, the AG2 conformation around (300°, 180°) and others are partially populated at this higher temperature. Upon reduction of the temperature to 100 K, Figure 3B shows that quantum TPIMC predicts the equilibrated AG conformational population to settle into the deepest AG1 free-energy well. The effect of quantum mechanics on structure is observed by comparing Figure 3A and B with the corresponding classical TPIMC distributions in Figure 3C and D. At both temperatures, quantum effects cause the height of the peaks to diminish as the population distribution tunnels into the neighboring configuration space.

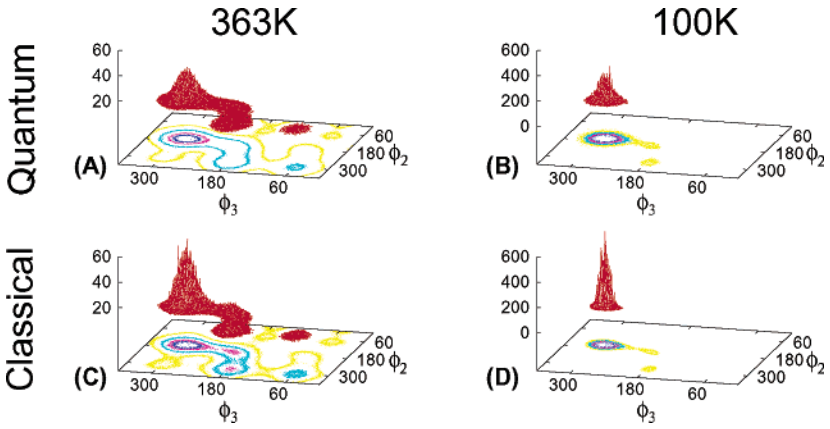

Figure 3. Torsional population distributions for the AG structural family. Quantum-mechanical distributions in A and B reveal that several AG conformers are populated at 363 K, whereas only the AG1 conformer at (ϕ_2, ϕ_3) ≈ (180°, 300°) is found at the lower temperature. Comparison to the corresponding classical distributions in C and D reveals the structural influence of quantum mechanics to be a spreading of the torsional distribution.

As it is implemented in the current study, the TPIMC technique provides an accurate description of the intramolecular torsional modes and holds all other degrees of freedom fixed. We therefore assume that the torsional motions dominate the conformational thermodynamics and that the thermodynamic contributions corresponding to nontorsional degrees of freedom are the same for all conformers. Although the good agreement between the TPIMC and experimental results for the APE molecule supports this assumption, the possibility of a cancellation of errors must be recognized. Some nontorsional vibrations, such as bending modes associated with intramolecular hydrogen bonds, can vary between conformers and are potentially anharmonic in character. Inexplicit treatment of the nontorsional modes, along with the shortcomings of the potential energy surface employed in this study, gives rise to the possibility of fortuitous agreement between the TPIMC and experimental results. A subsequent TPIMC study employing explicit treatment of the nontorsional modes and a more accurate potential energy surface is in progress.

The kinetic mechanism by which molecules collapse from higher-energy conformations to lower-energy conformations during the cooling process of free-jet expansion is of major experimental and theoretical interest.[6−9,30,31] Previous studies of molecules in supersonic expansion suggest that conformer populations observed experimentally are nonequilibrium distributions that have pseudo-vertically collapsed from the high-temperature equilibrium distribution achieved prior to expansion.[30] That is, molecules in a high-energy conformation are expected to collapse only if they are connected to a more stable conformation by a sufficiently low-energy transition pathway.[6−8]

In the APE molecule, the higher-energy members of the AG and GG structural families are connected to their respective AG1 and GG1 minima by transition barriers of only a few kJ/mol. The two structural families, however, are divided by a transition barrier on the order of 30 kJ/mol. It is therefore possible that in the population analysis of the spectroscopy experiment all of the conformations in the AG and GG structural families had collapsed into their respective minima. The appropriate comparison between theory and experiment may be to relate the experimentally observed population ratio to the calculated ratio of the total AG and GG family population weights.

Figure 4 shows the AG/GG family population ratios determined with the various techniques. A comparison of the ratios in Figure 4 with the AG1/GG1 conformational population ratios in Figure 2 reveals that the alternative analysis yields nearly

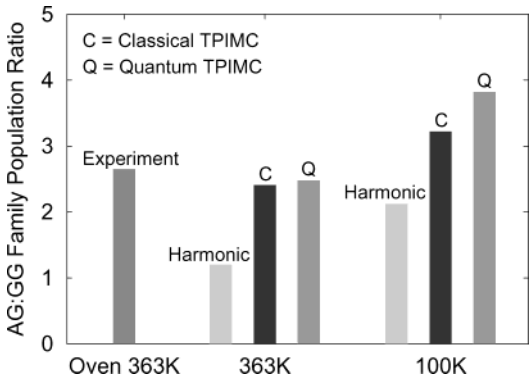

Figure 4. AG/GG conformational family population ratios obtained from experiment[16] and with theoretical techniques.

identical results. The TPIMC results calculated at 363 K remain in agreement with experiment.

It is interesting that although the TPIMC technique succeeds in reproducing the experimentally observed conformational populations a thermodynamic analysis is unable, in this case, to resolve whether the less stable structures in each conformational family have collapsed in the experiment to their respective minima. We point out, however, that equilibrium thermodynamics can resolve this issue for other molecular applications and that the TPIMC technique is the ideal method for performing such analysis and relating to experiment.[32] A thorough quantum-dynamical study of the collisional cooling of the APE molecule during free-jet expansion is of interest.

4. Conclusions

The TPIMC technique is found to be an effective new approach to calculating molecular conformation. The method has been used to explain recent experimental observations that theory was previously unable to reproduce. The TPIMC results presented in this study illustrate that free-energy corrections arising from anharmonicity and quantum mechanics are substantial enough to potentially reorder the relative energies of conformers and markedly alter theoretical predictions. TPIMC can be applied to molecules much larger than that considered here[11,12] and is expected to find useful application in a broad range of chemical and biological problems.

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Supporting Information Available: MM3 optimized geometries of the APE conformations. This material is available free of charge via the Internet at http://pubs.acs.org.

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