DIPEPTIDE STRUCTURE AND DYNAMICS

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Classical mechanical calculations have been performed to minimize the structure of alanine dipeptide using different Amber force fields. The minimization process leads to the second lowest conformer, the C5 conformer, compared to *ab initio* methods and experiment which identify the C7eq conformer as the lowest energy conformer. Then, a classical molecular dynamics calculation was done to search for the structure of the C7eq conformer. Starting from the C5 conformer, the structure of the C7eq conformer was identified after 28 ps of the simulation process. Possible reasons for this behavior are discussed.

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PREFACE

I'd like to thank my thesis committee with whom I have had experiences before. I took an enjoyable group theory course with Prof. Petoud. I learned an amazing way of teaching chemistry (science, history, and demonstrations) from Prof. Siska. I thank Prof. Pratt for his continuous help and support since I meet him in Egypt. I thank the Pratt group (Leo, Jessica, Phil, Diane, Justin, Ryan, Casey, and AJ) for the time I spent with them specifically Leo with whom I have had several scientific discussions. I am grateful to graduate student Sebnem Essiz for help in getting started using the Amber program and two undergraduate students, Mike Yazvac and Courtney McGuire who helped me in the early stages of this project. I appreciate also the helpful discussions with Prof. Daniel Crawford (Virginia Tech) about *ab initio* methods. Thanks to the ACS for allowing me to use figures from reference [6].

Finally, it was not a random decision I took to leave the Ph.D. program at the University of Pittsburgh and terminate my study with a master's degree. But I have decided to continue my Ph.D. journey at Virginia Tech following my research interests, and look forward to the challenges that lie ahead.

1.0 INTRODUCTION

1.1 MOLECULAR STRUCTURE AND CONFORMATIONS

Through the history of human achievements, we come to the conclusion that nature can exists into two forms, matter and radiation. In chemistry we are interested in the structure and transformation of matter, sometimes in the presence of radiation. Moving from the macroscopic view to the microscopic view of matter, we arrive at the world of molecules. We define the molecule as being formed from atoms connected by chemical bonds.

The chemical bond was defined by G. N. Lewis in 1916 as being formed from a shared pair of electrons. In molecules containing more than two atoms, there is more than one bond. Since these bonds point in different directions, molecules have a three-dimensional shape. Simple rules like those provided by valence-shell electron-pair repulsion theory help us visualize the 3D shapes of molecules.

The 3D shape of a molecule represents a specific energetic state. It is in fact, not a static entity but undergoes dynamic fluctuations due to the possibility of absorption of radiation of different frequencies. At room temperature, the molecules can experience internal rotation about their chemical bonds which leads to different energetic states and different 3D shapes. We can correlate those states by defining the potential energy

surface (PES) of the molecule under investigation. A PES helps us to understand the relationship between the structure and dynamics at the atomic level of the molecules and therefore how molecules function, interact, and react.

1.2 POTENTIAL ENERGY SURFACES.

The energy of the molecule is a function of its atomic coordinates (no electrons in the case of the classical mechanical treatment), and other variables like bond distances, bond angles, and bond rotations. We cannot visualize this function beyond two variables. If we restrict our variables to only two (surface), we can plot the so-called potential energy surface (PES).

The PES is a very useful and central concept in chemistry because many ideas which appear very hard to understand mathematically can be illustrated by it. An example of 1D PES is provided by a diatomic molecule which can be compressed or stretched like a spring. The PES will be simply a plot of energy against bond length.

PES can be constructed using classical mechanics using empirical potential energy functions implemented in computer software; the form of these functions is explained in the next section.

1.3 BIO-MOLECULES CONFORMATIONS.

What shapes do proteins have? The 3D shape of proteins determine how they function. So it is essential to know the atomic positions and the connectivity between them in this bio-machine. The determination of the structures of proteins is not trivial.

Proteins have three levels of structure; primary, secondary, and tertiary. The primary structure of a protein is determined by the sequence of amino acids from which it is made. The secondary structure of a protein is determined by interactions of different segments of the amino acid chain with each other, resulting in relatively simple coiled or parallel sheet shapes. The tertiary structure of a protein is determined by the ways in which different coils and/or sheets interact. There is an astronomical number of conformations a protein can achieve. Thus, it is remarkable that proteins can pick the right conformation in a relatively short time. Thus, we can ask, is it possible to predict the 3D structure of a protein from the sequence of its amino acids?

Protein folding processes may occur on time scales varing from microseconds to few minutes. [1] The folding process is not random; otherwise, the protein might take years searching all the possible confirmations. There must a specified path the protein follows. [2]

The current state-of-the-art of experiments is not able to provide enough resolution at the atomic scale to solve the protein folding problem. [3] Computational methods are complementary tools for experiment. They can guide, interpret, confirm, and/or predict experimental results.

Molecular dynamics (MD) simulations can provide atomic details about the folding process, but due to the large number of atoms in a protein (thousands) and the

time required for each step (1ps), it is still beyond computational resources to model the complete folding process exactly. [1] In this thesis, I will model the folding process by using approximations, neglecting any long range interactions and assuming a hard sphere flexible chain with its degrees of freedom described by two torsional angles (Φ, Ψ) around C(alpha); see Figure 1. Observed values of (Φ, Ψ) in a protein are, in general, complicated functions of the interactions between atoms in the chain.

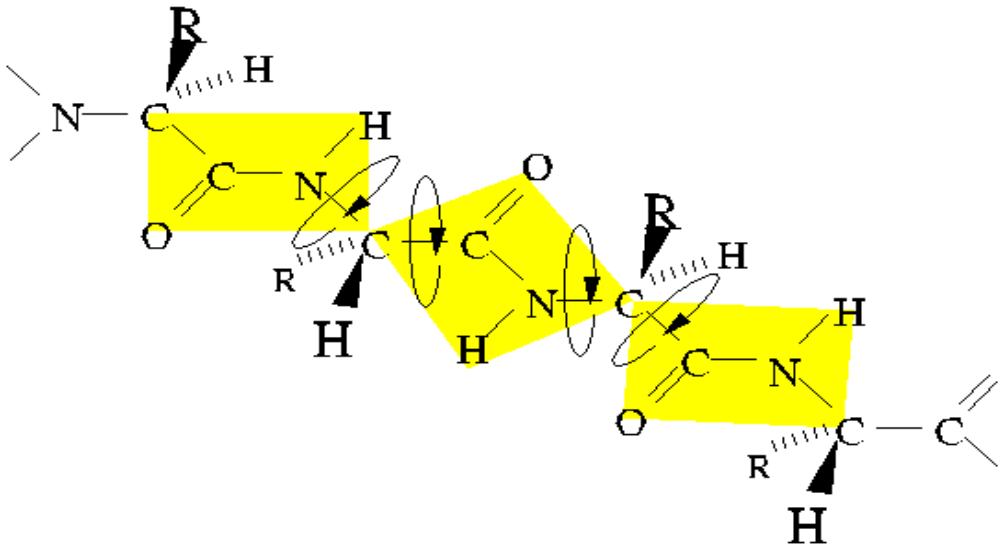

Figure 1. Polypeptide chain [4]

Studying a whole protein will require a hypersurface and the energy of our system will be multivariable function of each angle.

$$E(\Phi 1, \Psi 1, \Phi 2, \Psi 2, ..., \Phi n, \Psi n)$$

The hypersurface can be divided into n surfaces, where n is very large. Thus, to simplify the problem, we focus on a dipeptide. The PES of a dipeptide system will have minimum points (or valleys) corresponding to stable structures (conformers). The change in the side chain angles (w1 and w2) do not affect the topology of the E (Φ, Ψ) surface. Thus, the backbone of the

dipeptide is characterized by the two dihedral angles, Φ and Ψ. These angles are called Ramachandran angles.

A widely studied dipeptide is alanine dipeptide (AD) and its analog, 2-(formylamino)-propanamide, obtained by replacing the terminal methyl groups by hydrogen atoms. [5] These are shown in Figure 2.

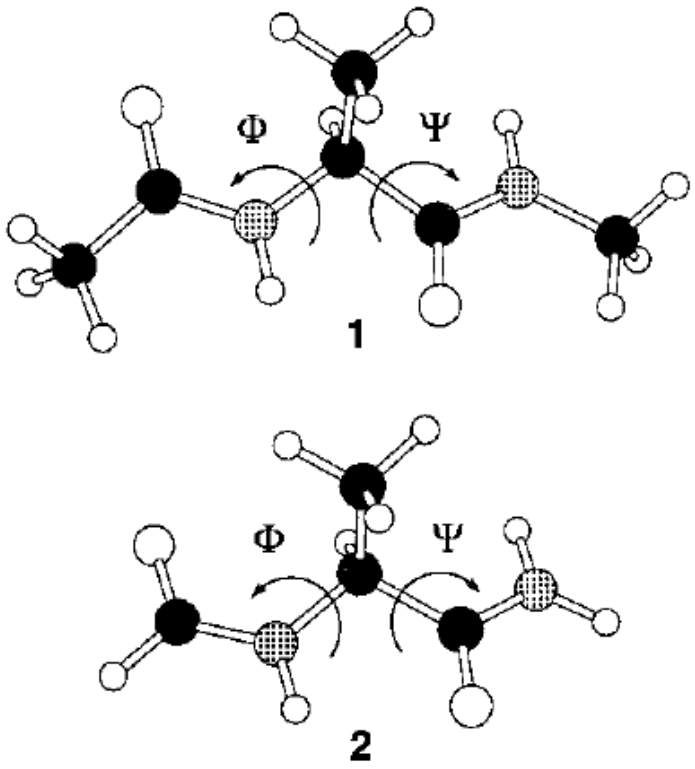

Figure 2. Structure of alanine dipeptide (**1**) and 2-(formylamino)-propanamide (**2**) with the Ramachandran angles Φ and Ψ indicated. The dihedral angle Φ is defined as C(O)-N-C_α-C(O) and the angle Ψ is defined as N-C_α-C(O)-N for both **1** and **2** (from Ref. [6]).

The torsional motion around Φ and Ψ mimics the folding process of a protein. The rotation will produce different conformers with different energies. Conformational analysis by *ab initio*

methods showed there exist nine conformers [6,7]. The lowest two conformers in energy are C7eq and C5 with approximate angles ((Φ, Ψ) of (-80°, 80°) and (-160°,160°) respectively. The relative energy difference between C7eq and C5 is 1.6 kcal/mol and the barrier height is 2.5 kcal/mol [8]. See Figure 3 and Table 1.

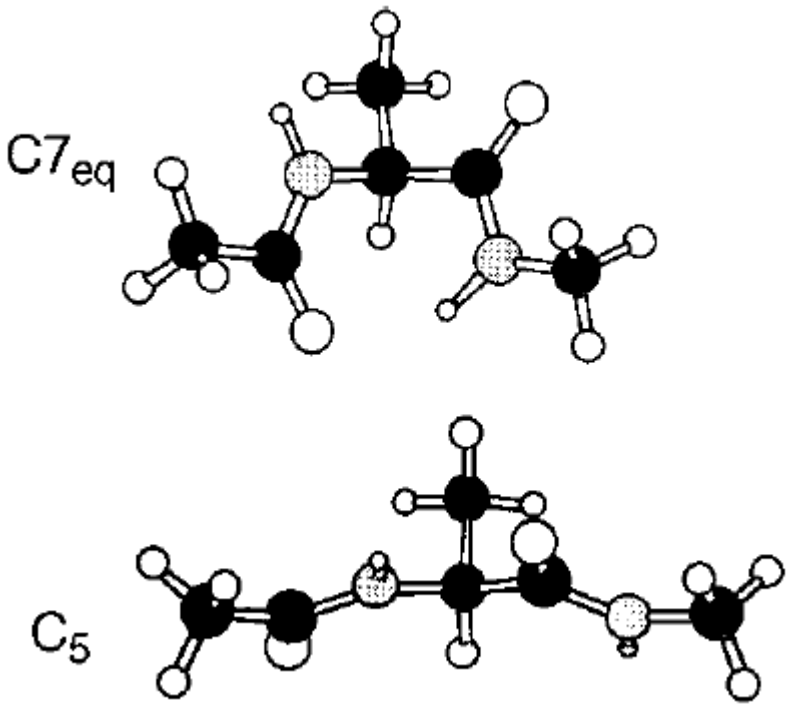

Figure 3. Stereoviews of the C7eq and C5 conformers of alanine dipeptide after optimization at the MP2/aug-cc-pVDZ level of theory (from Ref. [6]).

Table 1. Relative energies (in kcal/mol) for alanine dipeptide conformers at different levels of theory [6].

Conformer	HF/6-31G**	MP2/6-31G*	B3LYP/6-31G*
C7eq	0.00	0.00	0.00
C5	0.40	1.76	1.43

1.4 C7EQ CONFORMER BY EXPERIMENT.

Rotational spectroscopy uses microwave radiation to study the rotations of molecules in the gas phase. The rotational motion is quantized and when the molecule absorbs microwave light, the rotational state of the molecule changes. The frequencies at which these changes occur depend upon the moments of inertia of the molecule about each of its three axes, which in turn depend upon its moments of inertia. And if we can determine the moments of inertia of a molecule, we can know the bond lengths and angles and the shape of the molecule and distinguish between conformational isomers, in the absence of surroundings solvent molecules.

Previously, the C7eq and C5 conformers of alanine dipeptide have been identified from electron diffraction experiments. [9] More recently, the pure rotational spectrum of alanine dipeptide has been observed by Tubergen, *et al*. [10] using a pulsed-molecular-beam Fourier transform microwave spectrometer. The results are in good agreement with prior geometry optimizations and with current V3 barrier calculations which predict the C7eq conformation to be the lowest energy form in the gas phase. Up to now, the next lowest energy conformer C5 in the gas phase has not been detected experimentally.

In summary, proteins consist of amino acids units linked together by peptide bonds. From this primary structure and with flexibility around the single bonds, proteins start folding into more complex structures in order to perform their functions. Studying small peptide systems (like dipeptides) can be useful to understand the complex process of protein folding. Since quantum mechanical modeling of the folding process of proteins is beyond the current state-of-the-art, we choose to use classical force fields to study this process.

2.0 METHODOLOGY.

2.1 MOLECULAR MECHANICS.

Computer software based on physical laws and mathematical models can help us gain information about molecular structure and energy. Our work here is based on the classical mechanical model of the molecule. We are safe to use this model to some point as no breaking or forming of the chemical bonds are included in this study which will require quantum mechanical treatments. Only the fluctuations of molecular structure at room temperature will be investigated. In general, we can write

$$E = E(\Phi, \Psi) \tag{1}$$

The classical mechanical model views the molecule as a collection of balls and springs and divides the molecular energy into:

Energy = Stretching **Energy** + Bending **Energy** + Torsional **Energy** + Non-Bonded Interaction **Energy**

$$\text{Stretching Energy} = \sum_{bonds} k_{(\text{stretch})} (r - r_e)^2 \tag{2}$$

The stretching energy describes the interaction between two atomic pairs connected by a chemical bond where $k_{(stretch)}$ is the proportionality constant, the bigger value of $k_{(stretch)}$ the stiffer the bond; r_e is the equilibrium bond distance; and r is the length of the bond when stretched . The sum is over all two-center bonds in the molecule.

$$\text{Bending energy} = \sum_{angles} k_{(bend)} \, (\theta - \theta_e)^2 \qquad (3)$$

$k_{(bend)}$ is the bending proportionality constant, θ_e is the equilibrium angle in the triatomic unit of the molecule, and θ is the distortion angle from θ_e . The sum is over all three-center angles in the molecule.

An example of applying the last two equations to a triatomic molecule like water would result in two terms in Eq. (2) for each bond length and only one term in Eq. (3) for the bond angle. Molecular mechanics give 0.969 Å and 104° for the bond length and bond angle of the water molecule, compared to the experimental results of 0.958 Å and 104.5°.

$$\text{Torsional energy} = \sum_{dihedrals} k_{(torsion)} \, (B + \cos(n\phi)) \qquad (4)$$

The torsional energy involving tetraatomic units of the molecule can be best described by a trigonometric function because of the periodic motion of the dihedral angle ϕ. n is the foldedness of the barrier, $k_{(torsion)}$ is the proportionality constant, and B = ± 1. B = +1 when the staggered form of the dihedral angle is preferred and B= -1 when the staggered angle is preferred, as in the case of a double bond. The sum is over all dihedral angles in the molecule.

$$\text{Non-bonded interaction} = \sum_{i,j}\left\{\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12}-\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right]+\frac{q_i q_j}{r_{ij}}\right\} \qquad (5)$$

The first term in this expression is the Lennard-Jones potential, where $\boldsymbol{\varepsilon}$ and $\boldsymbol{\sigma}$ are parameters that depend on the types of atoms involved and r is the distance between the non-bonded atoms. The second term accounts for the electrostatic interaction where the q's are the charges and r is the distance between the two charges. The sum is over all atom pairs, i and j.

The parameters ($k_{(stretch)}$, r_e, $k_{(bend)}$, …etc) in the above equations can be obtained from experiments like microwave or NMR spectroscopy, high-level *ab initio* calculations, or a combination of both.

2.2 MOLECULAR DYNAMICS.

Molecular dynamics (MD) simulations can provide information about the time dependent behavior of a molecular system such as molecular conformation. We can start from one conformer and then search for other low energy conformers. The technique of MD depends on Newton's second law of motion

$$F_i = m_i a_i \tag{6}$$

where **F** is the force exerted on atom *i*, **m** is the mass of the atom, and **a** is its acceleration. Given **m** and if we can determine **F,** it is possible to follow the change in position with time of each atom in the molecule ($a = d^2x_i / dt^2$). The motion of each atom is not free but restricted by the force exerted on each; the atoms move in the field of the force, thereby the name force fields.

How do we determine **F**? It is the gradient of the energy which we can know from MM methods as discussed

$$F = - \frac{dV}{dr_i} \tag{7}$$

Here, **V** is the potential energy as expressed in Eq. (1). If we insert Eq. (7) into Eq. (6) and use the definition of the acceleration, we get

$$-\frac{dV}{dr_i} = m\frac{d^2 r_i}{dt^2} \tag{8}$$

Equation (8) represents the relation between position **r**, energy **V**, and time **t**. Now, we need an equation which predicts the position of each atom as function of time which is easily derived as

$$a = \frac{dv}{dt}$$

After integration,

$$v = at + v_0$$

$$\frac{dr}{dt} = at + v_0$$

and integrating again, we obtain

$$r = at^2 + v_0 t + r_0 \tag{9}$$

Here, $\mathbf{r_0}$ is the initial position of the atom (known from x-ray or NMR), $\mathbf{v_0}$ is the initial velocity (assigned by the program), and **a** is the acceleration, calculated from Eq. (8) after rearrangement

$$a = -\frac{1}{m}\frac{dV}{dr}$$

To know (predict) how the position of atom **i** change with time **t**, we use the equation

$$r_i(t) = \left(-\frac{1}{m_i} \frac{dV}{dr_i} \right) t^2 + v_{i0} t + r_{i0} \qquad (10)$$

Eq. (10) is valid only for very short time steps (fs) because its derivation assumes that the acceleration is constant in time.

2.3 COMPUTATIONAL DETAILS [11].

Amber is the collective name for a suite of programs that carry out molecular simulations, specifically for biomolecules. **Sander** is an Amber program which carries out energy minimization and molecular dynamics.

2.3.1 Generating the model structure.

We need to create the necessary input files for **Sander** to run minimization and molecular dynamics. **Leap** is the program designed to prepare the "inpcrd" and "prmtop" files required by **Sander.**

`inpcrd`**:** The coordinates file which contain initial set of coordinates. The data inside the file is not static and changes during simulations.

prmtop: The parameter/topology file. The file defines the connectivity and parameters for our chosen force field. This information is static (doesn't change during simulations).

2.3.2 Relaxing (minimizing) the system before MD.

Minimization with the **Sander** program will remove any strain in the initial structure.

To run energy minimization with **Sander** we need the following command:

```
sander –i min.in –p *.prmtop –c *.inpcrd –r *.restrt –o *.out
```

Here is the input file "min.in" with explanation between brackets:

```
Initial minimization
 &cntrl
  imin=1  (turn on minimization)
  maxcyc=1000  (1000 steps of minimization)
  ncyc=500  (500 minimization steps by steepest descent algorithm)
  ntb=0  (the system is not periodic)
  ntr=0  (no generalized born salvation model)
  cut=10  (10 angstroms for the non-bonded cutoff)
 /
```

2.3.3 Running MD in-vacuo.

We will run *in-vacuo* simulations assuming the system is in the gas phase with no long range cutoff and use the new coordinate file (*.restrt) created from the minimization step as the input coordination file for the MD run.

To run a molecular dynamics simulation with **Sander** we use the command:

```
sander -i md.in -p *.prmtop -c *.restrt -r *md.restrt
-x *md.mdcrd -e *md.en -o *md.out
```

Here is the input file (md.in):

```
IMIN=0 (turn off minimization)
NTB=0 (disable periodicity)
IGB=0 (we are not using implicit solvent)
NTPR=100 (write information to the output file every 100 steps)
NTWX=100 (write information to the trajectory coordinates file every 100 steps
)
CUT=999 (no cut off range for the long range non-bonded interactions)
NTT=3 (maintain the temperature of our system at 300 K)
```

GAMMA_LN=1(temperature control method uses Langevin dynamics with a collision

frequency given by GAMMA_LN, The Langevin system is much more efficient, however, at equilibrating the temperature)

TEMPI= 0.0 (initial temperature)

TEMP0=300.0 (final temperature)

NSTLIM=100000 (we will run a total of 100,000 steps)

DT=0.001 (1 fs time step)

3.0 RESULTS AND DISCUSSIONS.

Minimization of alanine dipeptide has been performed using the five different force fields (ff94, ff96, ff98, ff99, ff03) that are available in the AMBER program. As shown in Table 2, the different force fields predict different energies for the most stable conformer, More importantly, in every case this conformer is predicted to be the C5 conformer, not the C7eq conformer consistently found by *ab initio* methods [6] as the global minimum and observed by experiment. [10]

Table 2. Absolute energy of alanine dipeptide by different force fields

Method	Stable conformer	E (kcal/mol)
ff94	C5	-26.737
ff96	C5	-28.727
ff98	C5	-26.737
ff99	C5	-21.706
ff03	C5	-19.282

To address this problem, classical MD simulations in the gas phase were then performed to search for the C7eq conformer from the C5 structure. The calculations were done according to Newton's laws of motion; and the MD simulation used the canonical ensemble. A canonical ensemble is characterized by a collection of all microstates with a fixed number of atoms, constant volume, and fixed temperature. We start heating our system computationally from 0 K to room temperature to help the C5 conformer to surmount possible energy barriers. According to the calculations, the energy barrier between C5 and C7eq is estimated to be 2.5 kcal/mol [8] and the transformation should occur within a few ps.

We used the program VMD (Visual Molecular Dynamics) [12] to examine the change of the alanine dipeptide structure with time. Upon simulation using the ff03 force field model, MD methods succeeded in locating the potential well of the structure of C7eq conformer from the C5 conformer. This is shown in Figure 4 and is indicated by the sharp drops in the potential energy at specific times, notably between 27.4 and 28.1 ps.

Table 3 shows the specific values of the hydrogen bond lengths and Ramachandran angles as a function of time during this time "window", as determined from the results of the VMD program. Now, Vargas, *et al*. [6] have determined these quantities using MP2 and DFT methods; they found Φ and Ψ lying in the ranges Φ = (-85.8°) – (-81.9°) and Ψ = (72.7°) – (78.5°) for the C7eq structure and Φ = (-161.1°) – (-155.0°) and Ψ = (155.5°) – (165.3) for the C5 structure. Comparing of these values with those shown in Table 3 shows clearly that the C5 structure exists at 27.5 ps, and is converted to the C7eq structure at 28.0 ps. The hydrogen bond lengths in these two structures are predicted to be the same, 2.2 Å.

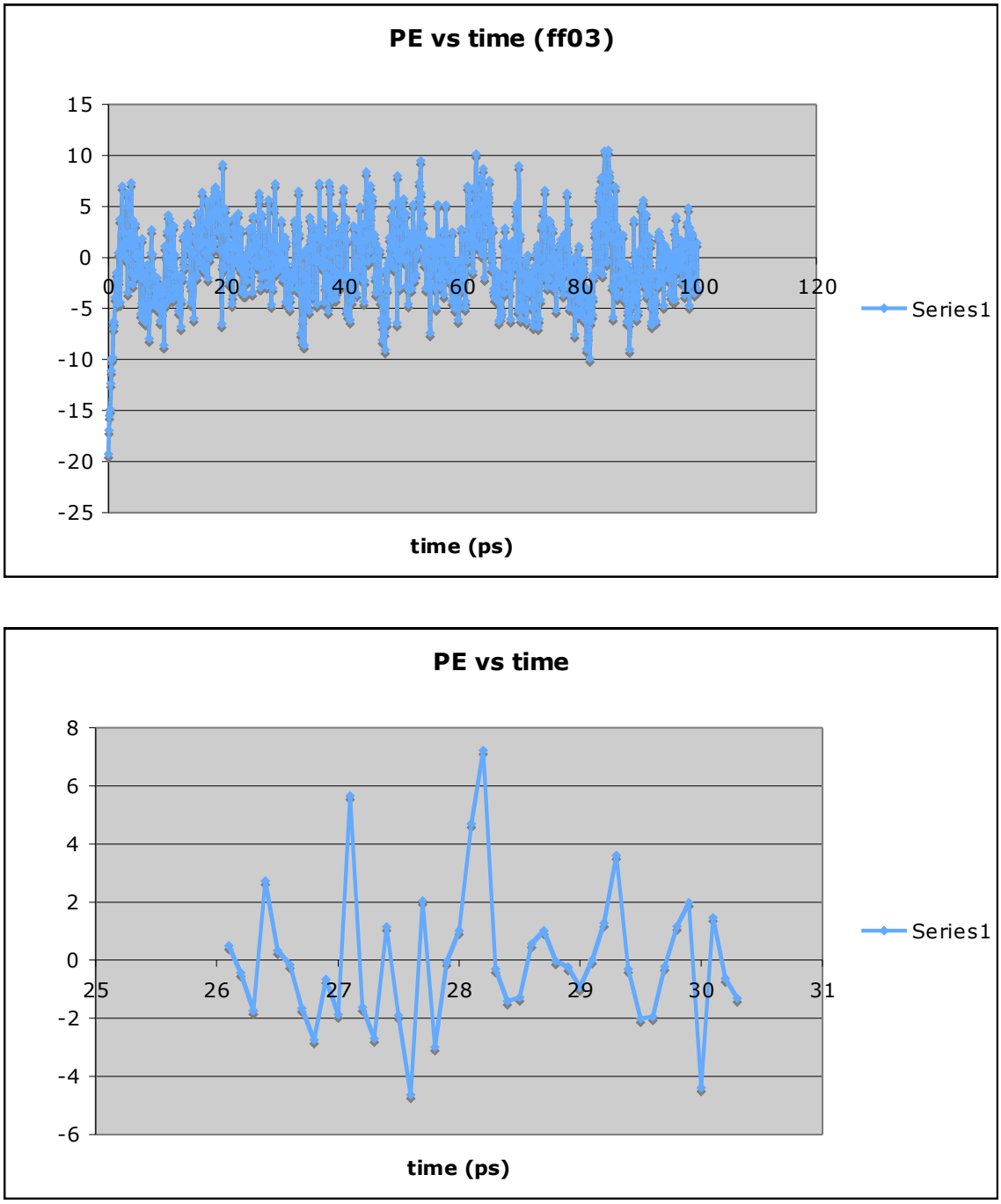

Figure 4. Variation of the potential energy of alanine dipeptide with time during MD simulation starting from structure optimized by force field ff03 method. The top figure spans 100 ps; the bottom figure shows the region between 25 and 30 ps in more detail. As seen, the conformational isomerization occurs from C5 to C7eq conformer around 28.1 ps.

Table 3. Variation of hydrogen bond lengths and dihedral angles in alanine dipeptide with time. At 27.5 ps, the dipeptide has the structure of the C5 conformer; at 28.0 ps, the C7eq conformer appears.

Time (ps)	H-bond (Å) C7eq	H-bond (Å) C5	ϕ angle	ψ angle
27.4	5.5	2.0	-166.3	166.0
27.5	4.9	2.2	-169.4	155.8
27.6	4.6	2.6	-159.2	134.2
27.7	4.1	2.6	-128.4	126.8
27.8	3.5	2.6	-104.7	111.5
27.9	3.1	2.9	-101.8	98.7
28.0	2.2	3.7	-69.1	84.1
28.1	2.5	3.5	-74.2	95.4
28.2	2.3	3.4	-83.9	80.9
28.3	2.5	3.3	-92.7	76.5
28.4	1.9	3.9	-71.1	53.0
28.5	1.9	4.2	-70.2	29.3
28.6	2.8	4.0	-94.5	13.5
28.7	3.1	3.9	-107.5	23.4
28.8	3.4	3.7	-123.6	30.4

Salahub, *et al.* [8] also studied this problem using the alanine dipeptide analog. The analog has the same structure as alanine dipeptide with the exception that the two terminal methyl groups are replaced with hydrogen atoms as shown before in Figure 2. A previous study concluded that the effect on the Ramachandran energy map by using the

alanine dipeptide analog is small.[13] They also start the MD simulation with the C5 conformer of alanine dipeptide analog with the result that classical MD methods do not yield transition between C5 and C7eq conformer even after nanoseconds, but using *ab initio* molecular dynamics (AIMD) methods they found the gas-phase conformational dynamics occurs on the picoecond time scale.

In our study we succeeded in observing the transition after 28 ps. The transition should occur faster but many factors may come to explain the delay. The performance of classical molecular dynamics is related to the design of the force fields as the forces we used in MD to move atoms are the gradients of the potential energy expressed by molecular mechanics. So the quality of these potentials will affect the MD simulations. The barrier height between C5 and C7eq may be unrealistically high in the force field design. Again, we need to remember how we move the atoms in our simulation; we use Newton's law while at the atomic level our system follows quantum mechanics. Also, we start our simulations at 0 K and then start heating but if we are expecting the transition from C5 conformer to C7eq conformer to occur within 1 ps as predicted by Arrhenius equation and the relationship between and the rate constant and time for unimolecular reactions, the temperature does not reach the 298 K within this time frame as shown in Figure 5.

Recently, Hornak, et al. [14] have reported improvements of the Φ /Ψ dihedral terms in the ff99 energy function. These new parameters are based on fitting the energies of multiple conformations of glycine and alanine tetrapeptide from high level *ab initio* calculations.

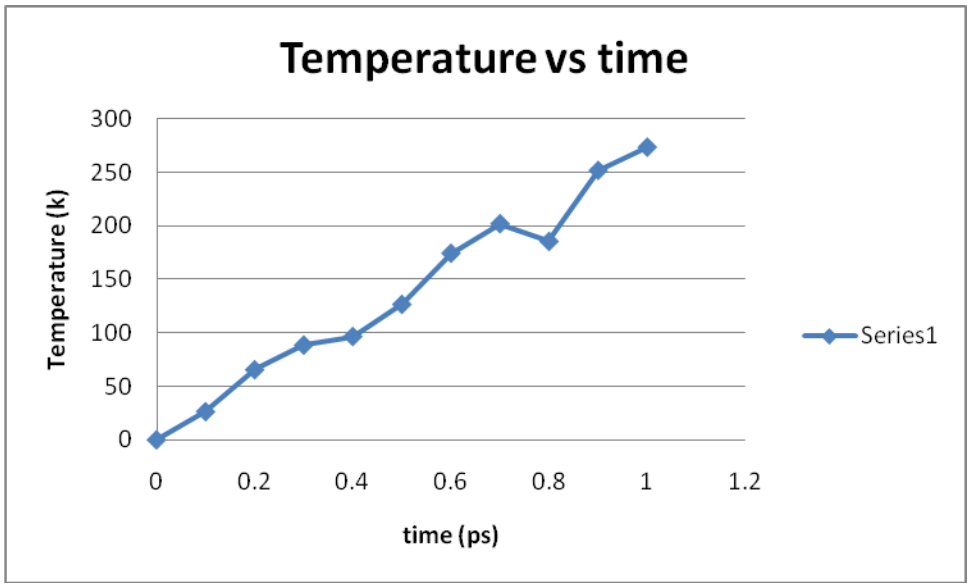

Figure 5. Variation of temperature with time at the beginning of the simulation from 0 K until it reaches room temperature at 1 ps.

With these new parameters, the authors found that ff99SB (the modified program) accomplishes improved agreement with published experimental data for the conformational preferences of short alanine peptides. According to Chong [15], the most accurate results are obtained when one uses an explicit water model.

CONCLUSIONS.

We minimized the structure of alanine dipetide using different force field models. The minimization process leads to the second lowest energy conformer C5. After using classical mechanics (specifically the ff03 force field) for minimization, molecular dynamics simulations succeeded in locating the potential well of the structure of the C7eq conformer observed by experiment and predicted by *ab initio* methods. This supports MD simulations as a successful tool in searching for other conformers of molecules.

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